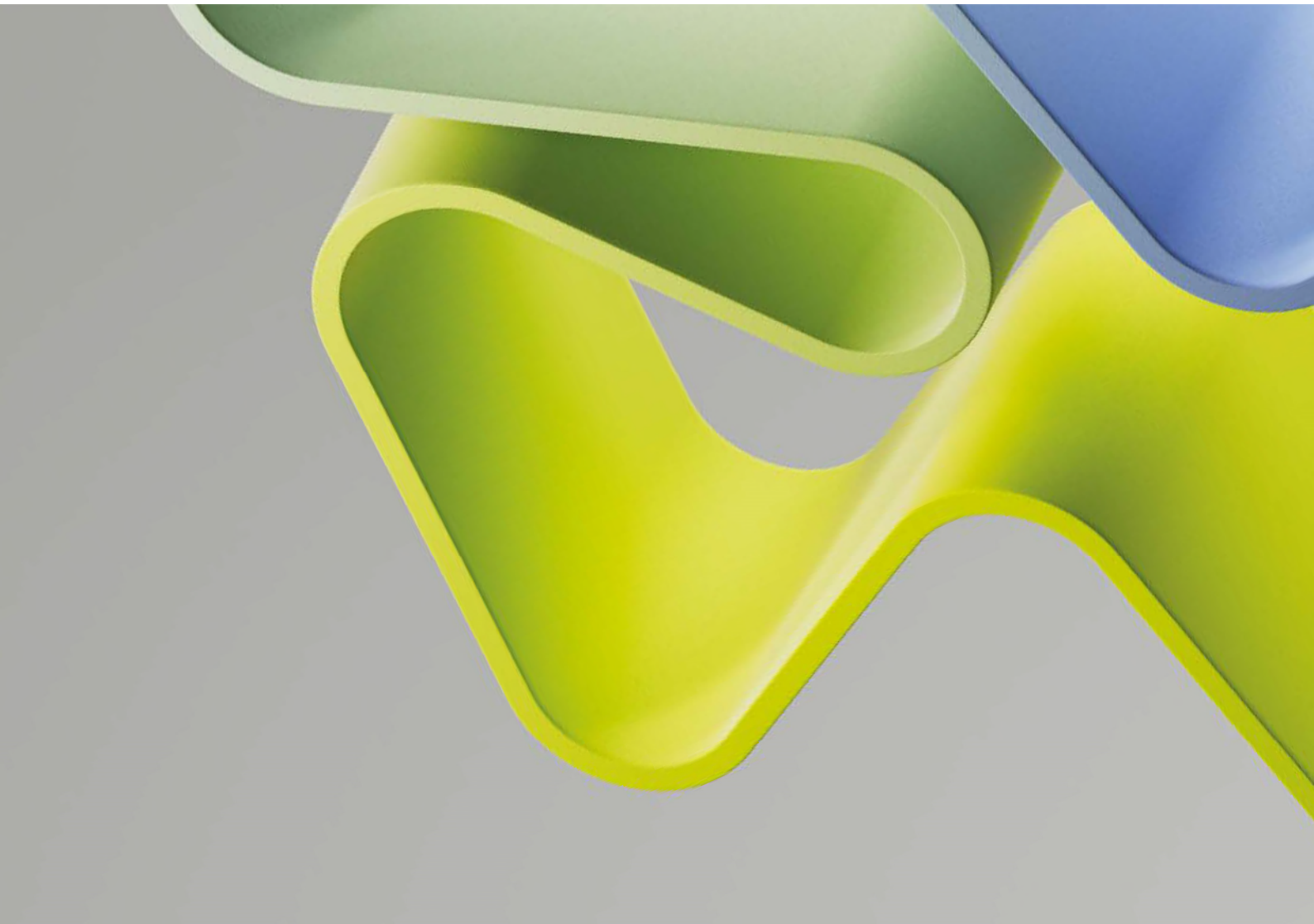
The Research Council of Norway

Evaluation of Biosciences 2022–2023

Impact cases

April 2024



Introduction

Administrative units participating in the Evaluation of Biosciences Research in Norway 2022-2023 were invited to submit case studies documenting the societal impact of their research. In this report the impact cases will be presented in the way they were submitted by the institutions using the template for impact cases (attachment) which were sent to the 22 enrolled administrative units 15. September 2022 with a deadline of 16. January 2023. Impact cases from the administrative units are presented here. Some of the cases are also presented in the National report for Biosciences research.

Definition

The definition of, and model for, societal impact was derived from the 2021 Research Excellence Framework (REF) in the United Kingdom:

> Definition of Societal impact: an effect on, change or benefit to the economy, society, culture, public policy or services, health, the environment or quality of life, beyond academia.
>
> Impact includes the reduction or prevention of harm, risk, cost or other negative effects.
>
> Academic impacts on research or the advancement of academic knowledge are excluded. Impacts on students, teaching or other activities both within and/or beyond the submitting institution are included.
>
> Impact includes, but is not limited to, an effect on, change or benefit to:
> - the activity, attitude, awareness, behaviour, capacity, opportunity, performance, policy, practice, process or understanding
> - of an audience, beneficiary, community, constituency, organisation or individuals
> - in any geographic location whether locally, regionally, nationally or internationally

Impact case guidelines

Each case study should include sufficiently clear and detailed information to enable the evaluation committee to make judgements based on the information it contains, without making inferences, gathering additional material, following up references or relying on members' prior knowledge.

Timeframes

- The impact must have occurred between 2011 and 2021
- Some of the underpinning research should have been published in 2010 or later
- The administrative units were encouraged to prioritise recent cases

Maximum number of cases permitted per administrative unit

For up to 10 researchers: one case; for 10 to 30 researchers: two cases; for 30-50 researchers: three cases; for 50-100 researchers: four cases, and up to five cases for units exceeding 100 researchers.

CBU impact case number 1

Institution: University of Bergen
Administrative unit: Computational Biology Unit
Title of case study: ELIXIR Norway
Period when the underpinning research was undertaken: 2012-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2012-2021
Period when the impact occurred: 2021

1. Summary of the impact *(indicative maximum 100 words)*
This section should briefly state what specific impact is being described in the case study.

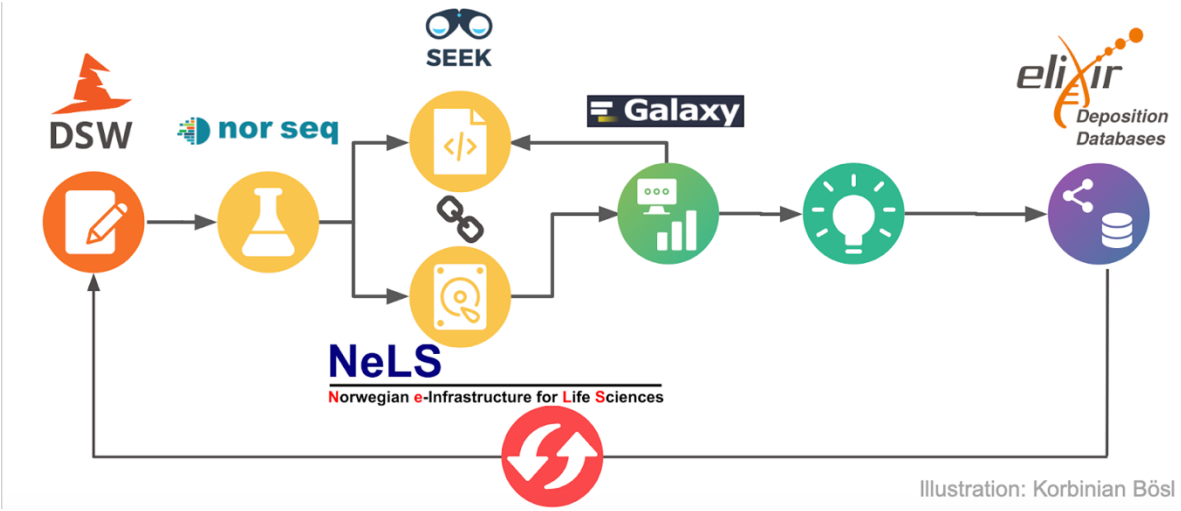
ELIXIR Norway has been coordinated by CBU and its infrastructure group since 2012, and its predecessor, the FUGE technology platform for bioinformatics, since the initiation of CBU in 2002. This case study highlights scientific and societal impact of selected services provided by ELIXIR Norway, the national research infrastructure for bioinformatics, including the Norwegian tool assembly for research data management, the Norwegian node in the federated EGA and the efforts towards Covid-19 data mobilisation. In summary, each of these three services greatly contribute to an increased level of FAIRness of Norwegian life science research data, by enabling life scientists to better manage and share their data.

2. Underpinning research *(indicative maximum 500 words)*
This section should outline the key research insights or findings that underpinned the impact, and provide details of what research was undertaken, when, and by whom. This research may be a body of work produced over a number of years or may be the output(s) of a particular project. References to specific research outputs that embody the research described in this section, and evidence of its quality, should be provided in the next section. Details of the following should be provided in this section:
 The nature of the research insights or findings which relate to the impact claimed in the case study.
 An outline of what the underpinning research produced by the submitted unit was (this may relate to one or more research outputs, projects or programmes).
 Dates of when it was carried out.
 Names of the key researchers and what positions they held at the administrative unit at the time of the research (where researchers joined or left the administrative unit during this time, these dates must also be stated).
 Any relevant key contextual information about this area of research.

Since we are here highlighting the impact of a research infrastructure hosted by CBU, we will outline two key services and activities of ELIXIR Norway, rather than research insights and findings, all being carried out during the period 2012-2021.

1. The Norwegian research data management tool assembly (2015 to date)

The Norwegian tool assembly for research data management is an integrated e-infrastructure platform supporting life science researchers throughout all steps of the research data management life cycle, from data management planning, throughout analysis to deposition of data in FAIR databases, as illustrated below.



The platform is built around the Norwegian e-infrastructure for Life Sciences, an e-infrastructure platform that allows easy upload, sharing and short-term storage of data. NeLS is further integrated with UseGalaxy, that allows computing power and pre-installed workflows. Gradually, more services have been added to complete the cycle, notably a national instance of the Data Stewardship Wizard, to enable data management planning integrated with NeLS, and integration with the SEEK platform, to enable metadata capture along the process. Ultimately, ELIXIR Norway provides a brokering service, to help researchers deposit their data in FAIR databases. This tool assembly is recommended by both the Research Council of Norway and the European Commission.

2. Mobilisation of Covid-19 data (2020 to date)

Through its engagement in ELIXIR Norway, CBU worked to enable Norwegian users utilize available data through ELIXIR and other databases, and we worked to make it easier for Norwegian government institutions and researchers to share data generated linked with the pandemic. In particular we worked with the National Institute of Public Health (Folkehelseinstituttet, FHI) to facilitate sharing of Norwegian viral sequence data in the open repository ENA, European Nucleotide Archive, and not only in the GISAID databases traditionally used. We also worked with the regional hospitals to enable data flow from their sequencers to FHI and further to ENA. We also took part in Monthly meetings with the European Commission and other European research infrastructures and stakeholders to facilitate sharing of molecular level Covid-19 data.

3. References to the research *(indicative maximum of six references)*

This section should provide references to key outputs from the research described in the previous section, and evidence about the quality of the research. All forms of output cited as underpinning research will be considered equitably, with no distinction being made between the types of output referenced. Include the following details for each cited output:

- *Author(s)*
- *Title*
- *Year of publication*
- *Type of output and other relevant details required to identify the output (for example, DOI, journal title and issue)*
- *Details to enable the panel to gain access to the output, if required (for example, a DOI or URL).*

All outputs cited in this section must be capable of being made available to panels. If they are not available in the public domain, the administrative unit must be able to provide them if requested by RCN or the evaluation secretariate.

NeLS paper:

- Tekle et al 2018: Norwegian e-Infrastructure for Life Sciences (NeLS) F1000Research https://doi.org/10.12688/f1000research.15119.1

link to NeLS platform
- https://nels.bioinfo.no/

DS Wizard ELIXIR Norway
- https://elixir-no.ds-wizard.org/

SEEK platform for metadata
- https://seek4science.org/

UseGalaxy Norway
- https://usegalaxy.no/

Link to Norwegian tool assembly entry in RDMkit
- https://rdmkit.elixir-europe.org/nels_assembly.html

opinion piece on the efforts that go into making life science data FAIR
- https://khrono.no/det-er-arbeidskrevende-a-gjore-data-delbare/590972

FEGA Norway launched
- https://elixir.no/news/89/63/ELIXIR-Norway-has-signed-the-Federated-EGA-Collaboration-Agreement
- https://elixir-europe.org/news/federated_ega

Launch of GDI project
- https://elixir.no/news/95/63/Genomic-Data-Infrastructure-is-launched-a-new-EU-project-to-unlock-the-potential-of-human-genomics-for-healthcare-research-and-innovation

Announcement og ELIXIR Norway - NIPH collaboration
- https://elixir.no/news/68/63/Norwegian-SARS-CoV-2-sequences-now-openly-available-in-ENA

Link to Covid-19 workflows in GitLab:
- Metadata conversion: https://gitlab.com/uit-sfb/gisaid2ena
- De-sensitisation: https://gitlab.com/uit-sfb/fhi-desensitize

On the importance of open data in overcoming Covid
- https://forskersonen.no/kronikk-meninger-om-forskning/kampen-mot-korona-er-avhengig-av-apne-data/1674226
- https://elixir.no/news/46/63/How-open-databases-turn-out-to-be-crucial-in-the-fight-against-Covid-19

Impact of ELIXIR in general:
Martin CS et al. 2021: Demonstrating public value to funders and other stakeholders—the journey of ELIXIR, a virtual and distributed research infrastructure for life science data APCE, https://doi.org/10.1111/apce.12328

4. Details of the impact *(indicative maximum 750 words)*
This section should provide a narrative, with supporting evidence, to explain:
How the research underpinned (made a distinct and material contribution to) the impact;
The nature and extent of the impact.
The following should be provided:
A clear explanation of the process or means through which the research led to, underpinned or made a contribution to the impact (for example, how it was disseminated, how it came to

influence users or beneficiaries, or how it came to be exploited, taken up or applied).
 Where the submitted administrative unit's research was part of a wider body of research that contributed to the impact (for example, where there has been research collaboration with other institutions), the case study should specify the particular contribution of the submitted administrative unit's research and acknowledge other key research contributions.
 Details of the beneficiaries – who or what community, constituency or organisation has benefitted, been affected or impacted on.
 Details of the nature of the impact – how they have benefitted, been affected or impacted on.
 Evidence or indicators of the extent of the impact described, as appropriate to the case being made.
 Dates of when these impacts occurred.

1. The Norwegian research data management tool assembly
By developing and offering an end-to-end tool assembly for life science research data analysis and management, accompanied with a training programme, ELIXIR Norway has contributed greatly to the FAIRification of Norwegian life science data.

ELIXIR Norway is a consortium between UiB, as the coordinating unit, and UiO, NTNU, NMBU and UiT. All partners are involved in developing the joint services, but UiB/CBU personnel have a key role in each of the services highlighted here. ELIXIR Norway further constitutes the Norwegian node of ELIXIR, the European research infrastructure for life science data. ELIXIR Norway is an active partner in this network and most activities are aligned with ongoing initiatives there. Hence, the services developed benefit life science researchers on all levels, locally, nationally and internationally.

As a result of this work in ELIXIR Norway, about 1500 researchers are currently using NeLS to manage and share data, a number that is increasing every year. About 300 researchers are using DSW, and about 130 000 computing jobs were sent to UseGalaxy during 2021. In addition to the Covid-19 sequences mentioned in the section below, ELIXIR Norway has brokered about 300 sequences for other Norwegian researchers. ELIXIR personnel were further involved in the organisation of more than 40 training events nationally and internationally during 2021.

2. Mobilisation of Covid-19 data
As a result of our collaboration with NIPH, about 23 000 Norwegian SARS-CoV2 sequences have now been published into the open repository European Nucleotide Archive, ENA. The importance of having such related data openly available for overcoming this (and other) pandemic has been highlighted several times, including by ELIXIR norway here https://forskersonen.no/kronikk-meninger-om-forskning/kampen-mot-korona-er-avhengig-av-apne-data/1674226 and here https://elixir.no/news/46/63/How-open-databases-turn-out-to-be-crucial-in-the-fight-against-Covid-19. Furthermore, the benefit to society of having such data openly available was outlined in the report from a national committee advising the ministry of research on investments into infrastructures for FAIR research data in 2022, view report here

The collaboration with NIPH is further described here: https://elixir.no/news/68/63/Norwegian-SARS-CoV-2-sequences-now-openly-available-in-ENA, and the workflows available from GitLab here

- Metadata conversion: https://gitlab.com/uit-sfb/gisaid2ena
- De-sensitisation: https://gitlab.com/uit-sfb/fhi-desensitize

Covid-19 related efforts are funded through the ELIXIR2- and BioMdData projects funded by RCN, through the PaRI project by NeIC, and by up-lifts in Evuropean Commision grants

ELIXIR-CONVERGE and EOSC Life, as well as the new grant BY-COVID, funded by the European Commission.
5. Sources to corroborate the impact (indicative maximum of ten references)

IMR Advisory and Research Program unit case no 1

Institution: Institute of Marine Research
Administrative unit: IMR Advisory and Research Program unit
Title of case study: Securing the pelagic large fish populations in the Northeast Atlantic - the mackerel story
Period when the underpinning research was undertaken: 2010-2020
Period when staff involved in the underpinning research were employed by the submitting institution: 2010-2020
Period when the impact occurred: 2010-current

1. Summary of the impact (maximum 100 words)

The pelagic fish stocks in the Northeast Atlantic are vast resources, represent some of the largest fish stocks globally, and create societal impacts through employment, industrial and rural development and, most importantly, healthy nutrition for millions of people in Norway and internationally. The mackerel stock dramatically changed its spatial distribution, migration and aggregation patterns from the mid-2000's – profoundly challenging assessments. Dedicated international research efforts were then undertaken, initiated and lead by IMR scientists, including a new pelagic trawl survey and modern tagging-recapture method for quantitative abundance estimation and to understand major drivers for continued sustainable management of this valuable population.

2. Underpinning research (indicative maximum 500 words)

The Northeast Atlantic mackerel is a widely distributed pelagic fish species that plays a key role in the marine ecosystem. In recent years, there has been a large fishery targeting mackerel in the NEA. At the same time as the geographic range of the mackerel fishery has expanded and the spatial distribution of the stock been defectively determined, the stock assessment was considered highly uncertain (Simmonds et al., 2010) by the International Council for the Exploration of the Sea (ICES). This was the starting point for a dedicated project strengthening of the stock assessment and quota advice for the mackerel, which is most valuable fish stock in the North Atlantic. The project focused on the considerable monitoring efforts required, their utility and need for development, and took a broad cross-sectoral approach spanning vessel management and operations, trawl technology and methodology, biology, oceanography numerical modelling, mathematics and applied statistical analyses. Such a broad and holistic approach is possible for a research institution like the IMR and enabled the complex challenges in the stock assessment to be successfully addressed. IMR has had a leading role in here and notable innovations has been establishing a new cost-effective tagging programme [RFID] supplementing the preceding steel-tag series [1980-2006] and providing the basis for state-space modelling supplementing capture data and the tri-annual mackerel egg surveys (Tenningen et al., 2011; Ono et al., 2022); introducing a novel trawl-based survey (Nøttestad et al., 2016); and a new survey estimation tool (Johnson et al., 2019). Methodological assessment questions involved the evaluation of the ICA model (ageing of fish [catch-at-age] to estimate stock size), particularly in terms of effects of released fish affecting mortalities without being reflected in catches. Alternative methods using different methods and data indicated considerably larger abundance estimates than the official ICES estimates, in particular Simmonds et al. (2010) and Tenningen et al. (2011). From 2006 onwards it became increasingly evident that there were considerable changes in the respective significance of the mackerel fishery for different coastal states were taking place, especially regarding Iceland, Russia and Greenland. Key studies included analysing agreement between data sources using Bayesian state-space models (Simmonds et al., 2010) and abundance estimation of Northeast Atlantic Mackerel based on tag-recapture data (Tenningen et al., 2011). Changes in abundance, biomass, and spatial distribution of northeast Atlantic mackerel were quantified in Nøttestad et al. (2016). Uncertainty and consistency for the ecosystem survey are calculated using the model StoX (Johnsen et al., 2019). This was developed at the Norwegian Institute of Marine Research and has found international use in handling data and calculations of stocks and uncertainty based on acoustic or swept area surveys. Predictions of

near-future impacts of climate change on the productivity of key stocks in the Northeast Atlantic including the mackerel stock, were described in Kjesbu et al. (2021). Their assessment is that the mackerel stock will cope well under expected climate change towards 2050 as it is a warm water adapted species, and it is also able to respond quickly to changes in distribution of water masses and prey.

Key IMR personnel
Leif Nøttestad, Principal scientist
Aril Slotte, Principal scientist, Research group leader
Sindre Vatnehol, Researcher
Are Salthaug, Senior researcher
Espen Johnsen, Principal scientist
Dankert Skagen, Emeritus researcher
Eilert Hermansen, Chief technician
Ørjan Sørensen, Research technician
Bjørn Erik Axelsen, Programme director
Geir Huse, Research director

3. References to the research (indicative maximum of six references) (IMR staff in **bold**)

Simmonds, E.J., E. Portilla, **D. Skagen**, D. Beare, and D.G. Reid (2010): Investigating agreement between different data sources using Bayesian state-space models: an application to estimating NE Atlantic Mackerel catch and stock abundance. ICES Journal of Marine Science, 67: 138-1153.

Tenningen, M., **A. Slotte**, and **D. Skagen** (2011): Abundance estimation of Northeast Atlantic Mackerel based on tag recapture data – A useful tool for stock assessment? Fisheries Research, 107 (2011), pp. 68-74.

Johnsen E, T**otland A, Skålevik Å**, et al. StoX: An open-source software for marine survey analyses. Methods Ecol Evol. 2019; 10:1523–1528. https://doi.org/10.1111/2041-210X.13250
Ono, K; Slotte, A; Hølleland, S; Mackinson, S; Jonsson, SP; Jacobsen, JA; Olafsdottir, AH. 2022. Space-time recapture dynamics of PIT-tagged Northeast Atlantic mackerel (Scomber scombrus) reveal size-dependent migratory behaviour. Frontiers in Marine Science. Volume 9, Article Number 983962, DOI10.3389/fmars.2022.983962

Nøttestad L., Utne K. R., Óskarsson G. J., Jónsson S. Þ., Jacobsen J. A., **Tangen Ø**., et al. (2016). Quantifying changes in abundance, biomass, and spatial distribution of northeast Atlantic mackerel (*Scomber scombrus*) in the Nordic seas from 2007 to 2014. ICES J. Mar. Sci. 73, 359–373. doi: 10.1093/icesjms/fsv218

Kjesbu et al., 2021. Highly mixed impacts of near-future climate change on stock productivity proxies in the Northeast Atlantic. Fish and Fisheries 00, 1–15 15 doi 10.1111/faf.12635

4. Details of the impact (indicative maximum 750 words)
Norway has harvested marine living resources for millennia. In the past, marine living resources were thought to be so large that they could not be overexploited, but history taught us differently. The pelagic fish stocks in the northeastern Atlantic represent vast resources. In 2021, the combined export values of mackerel (5.9 b NOK) and herring (4.2 b NOK) alone exceeded 10 billion NOK, creating vast societal impacts through employment, industrial and rural development and, perhaps most importantly, safe nutrition for millions of people in Norway and internationally. Not only the mackerel has been affected. The Norwegian Spring Spawning (NSS) herring stock collapsed in the late 1960s. In response a moratorium banning all fishing was proposed, implemented, and enforced. This was followed by a comprehensive monitoring and an assessment scheme that was put in place under the auspice of the International Council for the Exploration of the Sea (ICES). In close coordination with adjacent coastal states, this is continuously being developed to date, thus securing further sustainable

uses of these vast resources once they recovered in the 1980s to date, and potentially far into the future. The total value of the NSS herring catches for all the coastal states amounts to about 7.5 billion NOK (assuming equal value as for the Norwegian part of the harvest of 57%).

While the mackerel in the northeast Atlantic did not collapse, the case of the NSS herring stock collapse, and its rebuilding, vividly illustrated the value of having thriving fish stocks that can, and should, be sustainably exploited. A similar calculation as for the NSS herring (assuming that 25% of the catch was Norwegian in 2021) gives a total value of mackerel fishery for all the coastal states of almost 24 bill NOK. The work done by IMR, ICES and our research partners to strengthen the mackerel assessment is vital to achieve a near optimal exploitation of these stocks. For the mackerel, however, the challenges in fisheries-independent monitoring include e.g. acoustics not being applicable, tagging and recapture involving errors/ biases caused by unaccounted mortalities, vast and ever-expanding distribution areas, changed migration patterns and aggregation dynamics, likely including influences of climate change etc. This, along with emerging inadequacies of the historic assessment approaches, represented great challenges in terms of e.g. discrepancies between indices used in the assessment, and considerable annual fluctuations in biomass and spatial distribution estimates. Addressing broadly the drivers of such change, the need for revision of methods and scientific approach, along with a broader understanding of the research-industry contact interface, research-societal impact relations, a broader understanding of stakeholder contexts and international relations and differences, all contributed to re-establishing a challenging, but nevertheless robust assessment and management scheme – all under the auspices of ICES - and thereby bringing the stakeholders together for balanced debates and discussions on appropriate and acceptable solutions. While the research basis for the assessments and the resulting advice today is effectively unanimously agreed within ICES and between the coastal states, and other stakeholders, the challenge of distribution of national quota (TACs) within the total available TAC remains unresolved. Lack of agreement thus still leads to consistent overfishing over time, despite the knowledge basis for achieving this is already in place. These challenges are, however, political of nature rather than scientific. The mackerel stock has been particularly productive in the recent decade compared to previous periods, and this has contributed to the current strong state of the stock - despite its high exploitation rate. It is essential to achieve an agreement between involved coastal states to avoid a collapse in the stock, given that the productivity could drop. While the monitoring and assessment of the stock has been considerably strengthened, it is possible to further reduce uncertainties with methods improvements and extended time series. These efforts are indeed maintained, and will continue to be so moving forwards.

5. Sources to corroborate the impact (indicative maximum of ten references)

Nøttestad, L; Utne, KR; Oskarsson, GJ; Jonsson, ST; Jacobsen, JA; Tangen, O; Anthonypillai, V; **Aanes, S**; **Volstad, JH**; Bernasconi, M; Debes, H; Smith, L; Sveinbornsson, S; Holst, JC; Jansen, T; **Slotte, A** (2016): Quantifying changes in abundance, biomass, and spatial distribution of Northeast Atlantic mackerel (*Scomber* scombrus) in the Nordic seas from 2007 to 2014. ICES Journal of Marine Science 73(2): 359-373. doi 10.1093/icesjms/fsv218

Nøttestad, L; **Diaz, J**; **Pena, H**; **Søiland, H**; **Huse, G**; Fernö, A (2016): Feeding strategy of mackerel in the Norwegian Sea relative to currents, temperature, and prey. ICES Journal of Marine Science 73(4): 1127-1137. doi 10.1093/icesjms/fsv239

Hansen, C; **Drinkwater, KF**; Jahkel, A; Fulton, EA; Gorton, R; **Skern-Mauritzen, M** (2019): Sensitivity of the Norwegian and Barents Sea Atlantis end-to-end ecosystem model to parameter perturbations of key species. PLOS ONE 14(2). doi 10.1371/journal.pone.0210419

Skogen, MD; **Hjøllo, SS**; Sando, AB; Tjiputra, J (2018): Future ecosystem changes in the Northeast Atlantic: a comparison between a global and a regional model system. ICES Journal of Marine Science 75(7): 2355-2369. doi 10.1093/icesjms/fsy088

Jansen, T; **Slotte, A**; Schmidt, TCD; Sparrevohn, CR; Jacobsen, JA; **Kjesbu, OS** (2021): Bioenergetics of egg production in Northeast Atlantic mackerel changes the perception of fecundity type and annual trends in spawning stock biomass. Progress in Oceanography 198. doi 10.1016/j.pocean.2021.102658

Tenningen, M; Pobitzer, A; **Handegard**, **NO; de Jong, K** (2019): Estimating purse seine volume during capture: implications for fish densities and survival of released unwanted catches. ICES Journal of Marine Science 76(7): 2481-2488. doi 10.1093/icesjms/fsz119

Holmin, AJ; **Mousing, EA**; **Hjøllo, SS**; **Skogen, MD**; **Huse, G**; **Handegard, NO** (2020): Evaluating acoustic-trawl survey strategies using an end-to-end ecosystem model. ICES Journal of Marine Science 77(7-8): 2590-2599. doi 10.1093/icesjms/fsaa120

Planque, B; Favreau, A; Husson, B; **Mousing, EA**; **Hansen, C**; **Broms, C**; **Lindstrom, U**; Sivel, E (2022): Quantification of trophic interactions in the Norwegian Sea pelagic food-web over multiple decades. ICES Journal of Marine Science 79(6): 1815-1830. doi 10.1093/icesjms/fsac111

Simmonds, E.J., E. Portilla, D**. Skagen, D**. Beare, and D.G. Reid (2010): Investigating agreement between different data sources using Bayesian state-space models: an application to estimating NE Atlantic Mackerel catch and stock abundance. ICES Journal of Marine Science, 67: 138-1153

Meld. St. 32 (2018–2019) Et kvotesystem for økt verdiskaping En fremtidsrettet fiskerinæring.

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

ICES Advice 2022 – mac.27.nea – https://doi.org/10.17895/ices.advice.19772392
ICES Advice 2022 – her.27.1-24a514a – https://doi.org/10.17895/ices.advice.19772380.

More information about mackerel: Mackerel | Institute of Marine Research (hi.no)

IMR Advisory and Research Program unit case no 2

Institution: Institute of Marine Research (IMR)
Administrative unit: IMR (Advisory and Research program unit)
Title of case study: From risk assessment to a system for regulation of salmon farming in Norway (the Traffic Light System)
Period when the underpinning research was undertaken: 2011 and onwards
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 and onwards
Period when the impact occurred: 2014 and onwards

1. Summary of the impact

The "Traffic Light System" (TLS) was developed to regulate an environmentally sustainable growth in the Norwegian salmonid industry, approved by the Norwegian parliament in 2016 and implemented by the Ministry of Trade, Industry and Fisheries (NFD) in 2017. The TLS is based on yearly scientific evaluations of the impact of the parasitic salmon louse from farmed salmon on wild salmonids along the Norwegian coast. IMR is a major contributor to TLS performing yearly monitoring of environmental status, suggesting indicators and acceptance criteria, and by development of impact models to complement field observations supported by peer reviewed scientific publications

2. Underpinning research

The spillover of sea lice from farmed to wild salmonids has long been considered an environmental issue of concern, recognized for instance in the yearly risk assessments of Norwegian aquaculture conducted by IMR since 2011 (Taranger et al. 2015). IMR has also developed biophysical models, where the drift of the pelagic stages of the salmon louse is simulated using hydrodynamic models (Asplin et al. 2013). These models show that drift is significant, but there also appears to be natural barriers to this drift. Implementation of protected areas as the National salmon fjords have shown that fallowing even on a small scale can have an effect (Serra-Llinares et al. 2014), but only if the drift of the salmon louse is taken into consideration. IMR was asked to evaluate if the coast could be divided into zones which were as separate as possible. This was done using IMRs salmon lice model (Ådlandsvik 2015). Also, prior to the implementation of the Traffic Light System (TLS), IMR chaired a working group that was asked to evaluate the models used to calculate infection pressure and lice infestation on salmonids (Karlsen et al. 2016). In this evaluation, IMRs collection of data through the national monitoring program of lice on wild salmonids (NALO), and the newly documented ability of the lice model to predict lice on fish in net-pens and sentinel cages (Sandvik et al., 2016), and the later published virtual smolt model (Johnsen et a., 2021) was presented. In addition, research aiming at understanding the effect of salmon lice on wild salmonids is performed (e.g. Fjelldal et al. 2020). TLS was implemented in 2017, but the government had prior to this established a steering group, which appointed an expert group to evaluate the status in each of the 13 production zones defined in 2015. IMR personnel are participating in both the steering group and the expert group. The expert group relies on data (NALO) and model results to evaluate the status in each production zone. IMR delivers the bulk of the data and analysis used by the expert group (environmental data, results from the lice model, the virtual smolt model, NALO data (sentinel cages, nets and trap, and trawl data where the smolt is assigned to home river). As part of this system, IMR supplies a salmon lice density map that is updated weekly throughout the year (www.lakselus.no).

Key scientist/research leaders
(listed chronologically/alphabetically, and position 31.12.2021/end of period)

Jon Albretsen (2011-), Principal scientist

Lars Asplin (2011-), Principal scientist
Pål Arne Bjørn (2011-), Principal scientist (Project leader 2011-2014)
Karin K. Boxaspen (2011-), Research director
Sussie Dalvin (2011-), Principal scientist
Bjørn Olav Kvamme (2011-), Research group leader
Mari Myksvoll (2011-), Senior scientist
Rune Nilsen (2011 -), Senior engineer
Anne Sandvik (2011-), Principal scientist
Rosa Maria Serra Llinares (2011 -), Scientist
Jofrid Skarhammar (2011-), Senior scientist
Ove Skilbrei (2011- 2018), Principal scientist
Terje Svåsand (2011-), Program director
Geir Lasse Taranger (2011-), Research director
Bjørn Ådlandsvik (2011-), Principal scientist
Ingrid A. Johnsen (2014-), Senior scientist
Ørjan Karlsen (2015 -), Principal scientist (Project leader from 2015)
Per Gunnar Fjelldal, (2017-), Principal scientist
Tom Hansen, (2017-), Principal scientist
Alison Harvey (2017-), Scientist
Vidar Wennevik (2017-), Principal scientist
Thomas Bøhn (2018-), Principal scientist
Pål Næverlid Sævik (2020-), Scientist

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4. Details of the impact

The present regulation of the growth of the Norwegian open net salmonid farming industry through the Traffic Light System (TLS) is based mainly on the risk assessment and research of IMR. Since 2011, IMR has produced a yearly risk assessment of the environmental impacts of Norwegian fish farming, including the effect of salmon lice on wild salmonids (Taranger et al. 2011, 2012, 2015), and further expansion of the salmonid farming industry was more or less put on a hold from 2012 due to documented environmental impacts. The Norwegian government decided in 2014 to initiate work on new regulations enabling growth in the aquaculture industry ending up with a White paper to the Norwegian Parliament in 2015, suggesting that the capacity for production of Atlantic salmon, rainbow trout and trout should be regulated according to impact from salmon lice from fish farm on wild salmonids (Ministry of Trade, Industry and Fisheries 2015).

IMR was the major contributor of scientific knowledge in this process, including assessment of indicators and acceptance thresholds for key environmental impact of fish farming proposed together with the Norwegian Veterinary Institute (Taranger et al. 2012), coordinated the national salmon lice monitoring program (NALO) since 2010 (e.g. Nilsen et al. 2021) and developed and combined hydrodynamic coastal models with biological models, to create a salmon lice model for the entire Norwegian coast (See 2. Underpinning research).

Further, IMR was requested by the NFD to propose a set of production zones covering the Norwegian coast to function as management areas where the cross infestation of salmon lice should be kept at a minimum. In this way the lice situation in a zone depends on the activity in the zone itself and is independent of the other zones. The proposal was based on connectivity analysis on results from the IMR salmon lice model system (Ådlandsvik 2015). The fish farms were divided into clusters and geographical boundaries were drawn enclosing the clusters. A system with 13 production zones were chosen, and a new regulation on production areas for aquaculture of seafood in salmon, sea trout and rainbow trout (TLS) was implemented in 2017 (FOR-2017-01-16-61).

IMR has also been a major contributor of scientific knowledge after the implementation of TLS. In the first two years after implementation, IMR had the leadership of the Steering group being responsible for appointment of the expert group and reporting to the Ministry of Trade, Industry and Fisheries (Boxaspen, Hjeltnes & Næsje 2017)

The expert group uses observations from the monitoring program and combined with model from IMR and other Norwegian scientific partner to assess to what degree salmon lice from fish farming inflicts mortality on wild salmon above the governmental set thresholds. They report to the steering group for TLS (e.g. Næsje, Boxaspen & Hjeltnes, 2019.) which again reports to the NFD for a final decision (e.g. Ministry of Trade, Industry and Fisheries, 2020). If the condition is judged acceptable in a production zone, the salmon farms can increase the production capacity by 6% every 2nd year, if they are judged to have an unacceptable state production capacity is decreased by 6%, and if the impact is moderate production capacity are set to be constant.

Due to the role of salmon farming in Norway, decision in TLS has large economic and societal impacts. This is not limited to the direct implications for export income and job creation on the coast in the salmon farming industry and in the large associated supply chain in term of goods and services, but also concerns farmers wanting to buy new licences or extension of existing licences for salmon farming at a relative high cost to the government. This represents a large income at both local (municipality and county) and national level. The large economic impact of the decision in the TLS resulted in the salmon farmers in one of the production zones to bring both the science behind and the TLS as such into court in 2021 and 2022. The farmers in that region had to reduce their production capacity with 6% after the 2018-2019 assessment (Ministry of Trade, Industry and Fisheries, 2020) and disagreed on the verdict. Scientists from IMR and other research institutions had a role as expert witnesses in both trials, and the result was that the farmers lost again the government in both cases.

5. Sources to corroborate the impact

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IMR Advisory and Research Program unit Case number 3

Institution: IMR
Administrative unit: IMR Advisory and Research Program unit
Title of case study: Safe and healthy seafood
Period when the underpinning research was undertaken: 1993-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2006-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact

IMR provide data for science-based advice on safe and healthy seafood in the marine food-value chain in production of farmed fish including wild catches, with focus on contaminants, biohazards and nutrients in relation to international food regulations and environmental status. The impact falls in the categories 1) Transparent database documenting undesirables and nutrients in seafood, 2) Enabling risk-benefit assessments on Norwegian seafood by expert agencies that lays the foundation for national and international recommendations for seafood intake, 3) Contributing to obtain the SDGs addressing feed, food security and nutrition.

2. Underpinning research

Generation of data on nutrient content of seafood has been ongoing at the IMR since 1947 while the focus on contaminants increased after the adoption of a White Paper on Food Safety by the European Commission in January 2000. The European Union aimed to ensure the highest standard of food safety in Europe as a key policy, and subsequently a major programme of legislative reform was proposed to complete the EU's food safety priority in the whole food chain from "farm to fork". This led to an increasing demand for reliable data collection regarding the levels of both new and existing undesirable substances in seafood and fish feed in order to contribute to the establishment of regulatory limits and science-based risk assessments.

The process of generating high quality, reliable data is a research-driven undertaking involving researchers, technical staff and investment in laboratory infrastructure. The interplay between researcher and technical staff includes improvements of existing methodology to achieve both lower quantification levels, higher throughput of samples in the laboratory and updating of existing methods to fulfil changes in regulatory demands for the determinations of certain substances, as well as developing new methods for emerging contaminants. Development of methodology for the determination of both nutrients and contaminants involves investing in research infrastructure such as HPLC-ICPMS, GC-ICPMS, GC-MSMS and LC-MSMS. These analytical instrumentation platforms enable analyses of a host of nutrients including vitamins, minerals, protein, amino acids, lipid classes and fatty acids, as well as contaminants including metals, metal speciation, dioxins, PCBs, pesticides, PBDEs, HBCD, PFOS and drug residues. In addition, over the years, IMR has provided the scientific foundation on parasites (anisakis) which has resulted in regulatory changes and for the exemption of freezing treatment of cultured salmon and rainbow trout intended for raw or semi-raw consumption. The method is now recognized as one of two international standards for the detection of parasitic nematodes in fishery products (ISO 23036-1:2021).

Over the years 2000-2022, a considerable database on both nutrients and contaminants has been built for seafood from Norwegian waters (https://sjomatdata.hi.no/#search/), along with many peer-reviewed articles (Julshamn *et al.* 2013 and Nøstbakken *et al.* 2015). In addition to documenting presence of contaminants and nutrients in seafood, the presence of such compounds in fish feed has been documented since the late 1990' (Sissener *et al.* 2013). By studying the transfer of contaminants from feed to fillet, the IMR has been able to provide

mathematical models as a tool that can integrate and harmonise legislation on feed and food by showing which concentrations in feed that will lead to compliant concentrations of the same contaminant in food (Berntssen *et al.* 2016).

There is an increasing awareness on the need to address the interaction of contaminants and nutrients when performing risk assessment on food, i.e. risk-benefit assessments. Our data on both contaminants and nutrients in Norwegian seafood (and fish feed) as well as human epidemiological studies (such as Øyen *et al.* 2021) provide unique datasets enabling risk-benefit assessments on Norwegian seafood (wild and farmed) both nationally and internationally. These risk-benefit assessments lay the foundation for the health authorities to provide dietary recommendations to the various groups of the population.

Professor Kåre Julshamn (department leader in the years 1990 to 1998) contributed greatly to the institute's cornucopia of methods, in particular metal analysis, and to the high standard of laboratory proficiency (Julshamn et al 2007).
Professor Øyvind Lie (department leader in the years 1998-2017) contributed greatly to the investment in laboratory infrastructure for contaminant analysis and establishment of the Seafood database.
Gro-Ingunn Hemre, 2001-2011 Head of Research, Principal Scientist 1999-, Director of Research 2011-dd, and responsible for safe and healthy seafood overall activity.
Professor Livar Frøyland, Programme Director 2018-, Research Director 2011-2017, Head of Research 2004-2010, Researcher 1997-2003
Amund Måge (unit leader in the years 1990 to 1998 and 2011 to 2017)
Professor Anne-Katrine Lundebye (2000-2017) - leader of the Seafood safety research program (2003-2011), served as a seafood expert in many risk assessments at national, regional and international level.
Professor Rune Waagbø, Principal scientist since 1999, Director of Research 2011-2017, Program Director 2018-2022, leading development of methods within nutrition.
Robin Ørnsrud - Principal scientist since 2013, leader of the Feed safety research group (2011-2022)
Monica Sanden – Principal scientist since 2013, leader of Contaminants and Biohazard research group (2017-)
Lisbeth Dahl – Principal scientist and member of the National Council on Nutrition and nutrition expert in VKM, since 2017 -
Marian Kjellevold, Principal scientist 2019-, Head of research 2013-2019, Researcher 2003-2013.

Arne Levsen, Principal scientist 2005- , research and advisory activities related to fish-borne parasites and seafood hazardous parasites.

The roles of these lead IMR scientists include serving as experts to several national and international risk assessment and advisory bodies such as the Norwegian Scientific Committee for Food and Environment (VKM), Nasjonalt råd for ernæring, The Norwegian Food safety Authority, The Norwegian Research Council, COST, ICES, NMKL, European Food Safety Authority (EFSA), European Union Reference Laboratory (EURL), Codex Alimentarius, Food and Agriculture Organisation, World Health Organisation of the United Nation (FAO/WHO), CFS-HLPE.

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Øyen J, Brantsæter AL, Nøstbakken OJ et al. 2021. Intakes of Fish and Long-Chain n-3 Polyunsaturated Fatty Acid Supplements During Pregnancy and Subsequent Risk of Type 2 Diabetes in a Large Prospective Cohort Study of Norwegian Women. Diabetes Care 2021;44(10):2337–2345. DOI: 10.2337/dc21-0447

4. Details of the impact

Knowledge about the whole aquatic food chain from healthy waters through the value chain to healthy people is a prerequisite for sustainable food from the oceans today and in the future. Marine pollution has been a national and international challenge for decades and do not only affect ecosystem health and environmental status, but also the marine food web and safe and healthy seafood. The IMR has documented the nutrient and contaminant content in both farmed fish, such as salmon, and feral fish since the early 1990's and provided a substantial body of data. The data have been made publicly accessible in a seafood database (https://sjomatdata.hi.no/#search/), integrated in The Norwegian Food Composition Table which provides data on the content of nutrients and energy in the most commonly consumed foods in Norway. Data on chemical hazards in seafood has been delivered annually to the Norwegian Food Safety Authority and delivered to the European Food Safety Authority (EFSA) as a part of their Chemical Hazards Database (OpenFoodTox). Moreover, data on nutrients and contaminants have been shared with different international agencies such as the Food and Agriculture organisation (FAO) or the World Health Organisation (WHO) of the UN through several "Calls for data" over the last 20 years.

There is an increasing awareness on the need to address the interaction of contaminants and nutrients when performing risk assessment on seafood, i.e., risk-benefit assessments. Several scientific opinions on national, regional and global levels have performed risk-benefit assessments where data on both contaminants and nutrients from seafood are pivotal and where the IMR has been a major contributor. These assessments have resulted in recommendations for seafood intake on a national, regional and international level.

IMR provides, through the above-mentioned research and monitoring tasks, the analytical data of safe and healthy seafood (wild and farmed) in Norway based on annual monitoring

programs on a range of components such as contaminants and biohazards, medicinal residues (in farmed fish) as well as the nutritional composition. Data are made available to the public through an open Seafood database where you can search for and compare the contents of contaminants and nutrients in fish and other seafood https://sjomatdata.hi.no/#search and open access peer reviewed scientific articles. The total amount and broad aspect of analyzes of Norwegian seafood make these datasets unique.

The impact relates to our scientific based advice to governmental bodies from monitoring and research and is effectuated when regulatory systems are defining new maximum levels and setting consumer advice on seafood or is using our data in risk-benefit analysis. The societal impact is safer and healthier sustainable seafood to consumers. This must be done as open and scientifically based documentation of food safety and nutritional quality in alignment with amending food legislations in the global market. To support this endeavor, the IMR has been given the role as National Reference Laboratory for several methods analyzing nutrients, contaminants, parasites, and microorganisms in seafood, with around 70 methods accredited in accordance with the standard NS-EN ISO/IEC 17025.

A sustainable growth in aquaculture with the production of safe and healthy seafood depends on novel, nutritious suitable and safe ingredients with a low footprint. Knowledge about the whole aquatic food chain from healthy waters through the value chain to healthy people is a prerequisite for sustainable food from the oceans today and in the future. As an example, from aquaculture, we have proven that insect larvae, produced on substrates based on terrestrial and marine waste streams are resources of a very high potential to fill the protein and lipid gaps for animal feed. In collaboration with an insect producing industry, IMR early showed the nutrient suitability of insect products in salmon feed, and later projects (Research Council of Norway projects AquaFly and Entofôr) confirmed suitability, safety, edible quality, as well as sustainability in the salmon production chain. This included use of seaweed in the substrate to tailor a marine profile of the larvae meal. IMR led the Nordic project Insects in a circular economy, has participated as scientific partner in various RFF funded projects with insect start-ups, and participates in the ongoing EU SUSINCHAIN project validating aquafeed insect inclusion at a commercially relevant scale. A FORSTERK funded project reaches out to 9thgrade students and teaches about the circular insect economy. The impact has been at a societal level (information and public accept), change in the legal use of insects in feed in EU and Norway, and assisting national insect industries. The legal support included demonstration of safety (fish health and seafood safety), insect species authentication, and now, in the latest FHF funded project SecureFeed research on potential use of wastes from aquaculture as substrate. Last years, the confirmation of suitability, safety, edible quality and sustainability in the whole production chain is investigated for new low trophic feed resources in the ongoing NRC Oven to Plate project for blue mussels, mesopelagic species and seaweed (eg Berntssen et al 2021). Micronutrients must be bioavailable in new feed resources, and this has been investigated in several projects targeting speciation of e.g., zinc and selenium. These endeavors show a clear commitment contributing to obtain the SDGs addressing food security and nutrition for a growing world population.

5. Sources to corroborate the impact

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Report from the Norwegian Scientific Committee for Food Safety (VKM) 2014: Benefit-risk assessment of fish and fish products in the Norwegian diet – an update Opinion of the Scientific Steering Committee of the Norwegian Scientific Committee for Food Safety ISBN: 978-82-8259-159-1

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Commission Regulation (EU) No 1276/2011 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the treatment to kill viable parasites in fishery products for human consumption. https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32011R1276&from=EN

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§ 40 in FOR-2021-12-23-3910: Forskrift om gjennomføring av fiske, fangst og høsting av viltlevende marine ressurser (høstingsforskriften) - Lovdata

IMR Advisory and Research Program unit case no 4

Institution: Institute of Marine Research
Administrative unit: IMR Advisoryand Research program unit
Title of case study: Cumulative Anthropogenic Pressures on Coastal and Oceanic Ecosystems – Avenues to Sustainable Blue Growth
Period when the underpinning research was undertaken: from 2011 and onwards
Period when staff involved in the underpinning research were employed by the submitting institution:
Period when the impact occurred: Since the inception of Management plans in 2001

1. Summary of the impact (indicative maximum 100 words)

To achieve the political ambitions of a sustainable blue growth and a green transition, despite pressing issues of marine ecosystem footprints from various sectors, require data and knowledge. IMR supports the management and development of marine industries across all Norwegian EEZ with data, knowledge and advice while at the same developing the basis for sustainable ecosystem-based management for open ocean and coastal seas. Specifically for the BS; 1) monitoring, mapping and modelling (joint Norwegian-Russian annual monitoring of Barents Sea (BS) ecosystem, mapping of seabed together with Geological Survey of Norway and the Norwegian Mapping Authority, and complementary ecosystem modelling, 2) QC, processing, communication and analysis of data for implementation in peer-review publications and key white papers, 3) support to national and international commitments for sustainable use of ecosystem services such as the Norwegian cross sector management for the oceans, SDGs, and sustainable ocean economy under the High Level Panel.

2. Underpinning research (indicative maximum 500 words)

In the continuous process of developing holistic ecosystem management plans (MPs) for the Norwegian seas, IMR has contributed significantly to develop the framework including ecosystem indicators while at the same time continuously monitoring and mapping the marine environment and its inhabitants – in particular key ecological and commercial species and stages and nature types as well as biodiversity (e.g. benthos, fish communities) (see e.g. BESS, 2022). Similar surveys covering multiple trophic levels are carried out in the Barents Sea, Norwegian Sea, North Sea and along the Norwegian coast in a total of 2000 ship days annually. Data are made available through national and international portals to a diverse group of users including scientists, management and industries.

The BS has been monitored together with Russia for the last 65 years supporting joint management of fisheries resources. This scientific collaboration has led to the development of the most comprehensive marine ecosystem survey in the world, providing the basis for understanding the recent changes and interactions throughout the BS ecosystem (Fossheim et al., 2015). The survey data comprise physical, chemical and biological data collected near synoptical on multiple Norwegian and Russian research vessels jointly covering the entire Barents Sea across species ranging from bacteria to whales (Planque et al., 2014). Together with ecosystem models of various complexity this allows the understanding of drivers and processes that underpin changes in structure and function of the BS ecosystem under a changing climate and implications for key ecological and commercial species (Ingvaldsen et al., 2021) and advice on management adaptations (Kjesbu et al., 2014).

The outcome of the surveys and the knowledge developed found the basis for stock advice and environmental status reports through the ICES to several national and international advisory organizations based on consistent and efficient data handling (Johnsen et al. 2019). Joint monitoring, stock and integrated ecosystem assessments found the basis for ecosystem-based management (Skern-Mauritzen et al., 2016) – rarely included globally - that have

resulted in sustainable living marine resource harvesting valued between 15 and 20 billion Norwegian kroners (first-hand values) annually.

In total, these survey data are key to develop the much-needed scientific basis for MPs initiated in 2001, merged across all seas in the Norwegian EEZ in 2021 with an upcoming revision due 2024, led by two expert groups (IMR leading one of these) answering to a steering group with representatives from all Norwegian ministries. A recent key contribution to the MPs is the IMR led national revision of 19 particularly ecological and biological significant areas (SVO) including 74 experts from 8 institutions assessing environmental value, vulnerability and risks from cumulative anthropogenic pressures. The MPs are holistic frameworks providing scientific approaches to disentangle pressures from natural variability, understand the diverse causes and risks (SVO, 2022b), and allow exploration of management options and trade-offs where the interaction with stakeholders including managers, industries and NGOs secure the inclusion of sector specific knowledge on both impacting activities, emerging issues, future perspectives, relevant management interventions and potential consequences of these.

Key personell

Mette Skern-Mauritzen, Research group leader, IMR, 2011 - ongoing
Maria Fossheim, Programme director, IMR, 2011 - ongoing
Elena Eriksen, Senior scientist and programme director, IMR, 2011 - ongoing
Olav Kjesbu, Senior scientist, IMR, 2011 - ongoing
Randi Ingvaldsen, Senior scientist, IMR, 2011 - ongoing
Lis Jørgensen, Senior scientist, IMR, 2011 - ongoing
Espen Johnsen, Sensior scientist, IMR, 2011 - ongoing
Cecilie Hansen, Senior scientist, IMR, 2011 - ongoing
Benjamin Planque, Senior scientist, IMR, 2011 - ongoing

3. References to the research (indicative maximum of six references) – note that bold refs are the once used in section 2 while the others are used in section 5

BESS 2022 https://www.hi.no/resources/IMR-PINRO-report-2021-survey-3.pdf

Buhl-Mortensen et al. (2015) The MAREANO programme – a full coverage mapping of the Norwegian off-shore benthic environment and fauna. Journal of Marine Biology Research 11 (1). https://doi.org/10.1080/17451000.2014.952312

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Jørgensen et al. (2015) Distribution of benthic megafauna in the Barents Sea: baseline for an ecosystem approach to management. ICES Journal of Marine Science 72. https://doi.org/10.1093/icesjms/fsu106

Kjesbu et al. (2014) Synergies between climate and management for Atlantic cod fisheries at high latitudes. PNAS 111 (9). https://doi.org/10.1073/pnas.1316342111

Kjesbu et al. (2021) Highly mixed impacts of near-future climate change on stock productivity proxies in the North East Atlantic. Fish and Fisheries. https://doi.org/10.111/faf.1263

Lind et al. (2018) Arctic warming hotspot in the northern Barents Sea linked to declining sea-ice import. Nature Climate Change 8 p. 634-639

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Skern-Mauritzen et al. (2022) Marine mammal consumption and fisheries removals in the Nordic and Barents Sea. ICES JMS 79 (5). https://doi.org/10.1093/icesjms/fsac096
SVO (2021) Particularly valuable and vulnerable areas in Norwegian Seas - Environmental values / Særlig verdifulle og sårbare områder (SVO) i norske havområder – miljøverdier (In Norwegian with English summary). Rapport fra havforskningen 2021-26. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2021-26
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SVO (2022b) Cumulative pressures in particularly valuable and vulnerable areas in Norwegian marine waters / Samlet påvirkning i foreslåtte særlig verdifulle og sårbare områder i norske havområder (In Norwegian). Rapport fra havforskningen 2022-46. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2022-46

4. Details of the impact (indicative maximum 750 words)

Since 2018, 17 countries has committed to the High-Level Panel for a Sustainable Ocean Economy initiative for 100 % sustainable management of ocean areas under national jurisdiction by 2025 accounting for both effective protection, sustainable production and equity. This complies with many of the SDGs, the CBD, recommendations by the IPCC and the ICES strategy. A fundamental basis for reaching these goals are data, knowledge and frameworks for linking knowledge to management support. Accounting for natural variability also requires timeseries enabling investigation of how ecosystem structure and function vary and change over time.

IMR receives an annual mission letter with funds from the Ministry of Trade, Industry and Fisheries (MTIF) in addition to the main purpose letter as well as funds obtained through competition from various external sources. The assignment from the MTIF states explicitly that IMR shall develop further an ecosystem-based approach to knowledge development and advice on human use of the marine environment and ecosystem services. To meet this requirement IMR has over time developed ship-based surveys towards holistic monitoring assessing not only commercially harvested species for stock assessment and quota advice but concurrently also key ecologically species and nature types for assessing ecosystem integrity, structure and function as well as anthropogenic impacts through harvesting, pollution and climate change.

In the Barents Sea (BS) the ecosystem survey is carried out jointly with the Russian VNIRO in the western and eastern parts of the BS ecosystem, respectively, on multiple research vessels to secure near synoptic in situ measurements, is the backbone of BS ecosystem-based management (Haug et al., 2008). It allows investigation of physical changes either link to natural variability or climate change (Lind et al., 2018; Skagseth et al., 2020) and how this act as drivers for ecosystem components (Huserbråten et al., 2019; Ingvaldsen et al., 2021) through combination with ecosystem models of various complexity. Mapping the structure and function of the ecosystem is also a key to developed the basis for this (Jørgensen et al., 2015; Skern-Mauritzen et al., 2022; Planque et al., 2014).

We are heavily involved in developing new scientific assessment methods for commercial resources, and among the few that use multispecies information as a basis for advice on catch quotas – for fish and marine mammals (Johnsen et al., 2019). The management has been very

successful and has for example resulted in a record high abundance of Northeast Arctic cod despite ongoing climate change (Kjesbu et al., 2014; 2021). The total first hand value of the Arctic stocks is close to 20 billion NOK where Northeast Arctic cod alone is 10 billion NOK.

Furthermore, IMR is project lead for the Mareano project mapping continental shelf depth, topography, sediment composition and pollutant content, biodiversity, habitats and biotopes annually since 2006 together with the Geological Survey of Norway and the Norwegian Mapping Authority (Buhl-Mortensen et al., 2015). This work-intensive mapping has sparked IMR method developments (ML & AI) and implementation of disruptive technologies (AUVs, USVs) and models to ensure effective, high-qualitive processed seabed data fit for MPs.

Similar approaches are followed in the other ocean and coastal areas within the Norwegian EEZ such as the North Sea (Olsen et al., 2022).

The combined seabed and water column marine data are utilized to identify particularly ecological and biological significant areas with respect to environmental values (SVO, 2021), vulnerability (SVO, 2022a) and risks from cumulative anthropogenic pressures (SVO, 2022b) that are key to the revision of the Norwegian MPs, consideration of what areas to protect (30 % by 2030) and considerations of areas to open for new industries such as offshore winds and deep sea mining. The Norwegian MPs for the North Sea, Norwegian Sea and the Barents Sea was initiated in 2001 and merged in 2021 with a revision due 2024. The steering group of the MPs are inter-ministerial and use the MPs for guiding revisions of long-term research priorities, monitoring, area-planning, industry development and mitigation actions.

Our work is carried out in collaboration with national and international institutes and organizations (such as The Nansen Legacy – https://arvenetternansen.com; The Bjerknes Centre for Climate Research - https://www.uib.no/en/bjerknes; the Fram Centre - https://framsenteret.no, the ICES - https://www.ices.dk, and the IPCC - https://www.ipcc.ch) through projects or co-authoring reports. Joint integrated ecosystem assessments for ocean areas under the ICES are formerly chaired by Mette Skern-Mauritzen (IMR) with current co-chairs from IMR for the North Sea (WGINOS) and Norwegian Sea (WGINOR), and chair for the Barents Sea (WGIBAR) by Elena Eriksen (IMR).

5. Sources to corroborate the impact (indicative maximum of ten references) - note that bold refs are the once used in section 2 while the others are used in section 5

BESS 2022 https://www.hi.no/resources/IMR-PINRO-report-2021-survey-3.pdf

Buhl-Mortensen et al. (2015) The MAREANO programme – a full coverage mapping of the Norwegian off-shore benthic environment and fauna. Journal of Marine Biology Research 11 (1). https://doi.org/10.1080/17451000.2014.952312

Fossheim et al. (2015) Recent warming leads to a rapid borealization of fish communities in the Arctic. Nature Climate Change 5 (7). https://doi.org/10.1038/nclimate2647

Haug et al. (2008) https://www.tandfonline.com/doi/full/10.1080/17451000802512747

Huserbråten et al. (2019) Polar cod eggs in jeopardy under the retreating Arctic sea ice. Communication Biology 2.

Ingvaldsen et al. (2021) Physical manifestations and ecological implications of Arctic Atlantification. Nature reviews earth & environment 2. https://doi.org/10.1038/s43017-021-00288-x

Johnsen et al. (2019). StoX: An open source software for marine survey analyses. Methods in Ecology and Evolution 10. https://doi.org/10.1111/2041-210X.13250

Jørgensen et al. (2015) Distribution of benthic megafauna in the Barents Sea: baseline for an ecosystem approach to management. ICES Journal of Marine Science 72. https://doi.org/10.1093/icesjms/fsu106

Kjesbu et al. (2014) Synergies between climate and management for Atlantic cod fisheries at high latitudes. PNAS 111 (9). https://doi.org/10.1073/pnas.1316342111

Kjesbu et al. (2021) Highly mixed impacts of near-future climate change on stock productivity proxies in the North East Atlantic. Fish and Fisheries. https://doi.org/10.111/faf.1263

Lind et al. (2018) Arctic warming hotspot in the northern Barents Sea linked to declining sea-ice import. Nature Climate Change 8 p. 634-639

Olsen et al. (2022) Testing management scenarios for the North Sea ecosystem using qualitative and quantitative models. ICES JMS. https://doi.org/10.1093/icesjms/fsac231

Planque et al. (2014) Who eats whom in the Barents Sea: a foodweb topology from plankton to whales. Ecology 95. https://doi.org/10.1890/13-1062.1

Skagseth et al. (2020) Reduced efficiency of the Barents Sea cooling machine. Nature Climate Change 10. https://doi.org/10.1038/s41558-020-0772-6

Skern-Mauritzen et al. (2015) Ecosystem processes are rarely included in tactical fisheries management. Fish and Fisheries. https://doi.org/10.1111/faf.12111

Skern-Mauritzen et al. (2022) Marine mammal consumption and fisheries removals in the Nordic and Barents Sea. ICES JMS 79 (5). https://doi.org/10.1093/icesjms/fsac096

SVO (2021) Particularly valuable and vulnerable areas in Norwegian Seas - Environmental values / Særlig verdifulle og sårbare områder (SVO) i norske havområder – miljøverdier (In Norwegian with English summary). Rapport fra havforskningen 2021-26. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2021-26

SVO (2022a) Vulnerability of ecosystem components in Norwegian marine waters / Miljøverdiers sårbarhet i Norske havområder (In Norwegian with English summary). Rapport fra havforskningen 2022-33. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2022-33

SVO (2022b) Cumulative pressures in particularly valuable and vulnerable areas in Norwegian marine waters / Samlet påvirkning i foreslåtte særlig verdifulle og sårbare områder i norske havområder (In Norwegian). Rapport fra havforskningen 2022-46. https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2022-46

IMR Advisory and Research Program unit Case number 5

Institution: IMR
Administrative unit: IMR Research and Advice Program unit
Title of case study: Global sustainable ocean management
Period when the underpinning research was undertaken:1975-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 1975-2021
Period when the impact occurred: 2012-2021

1. Summary of the impact

In addition to providing science based advice on fisheries, aquaculture, seafood and the ocean environment in ocean areas under Norwegian jurisdiction, IMR research has had significant impact on the development of sustainable ocean management globally. The impact falls in the categories 1) Manage seafood production sustainably, 2) Mitigate climate change, 3) Stem biodiversity loss, 4) Seize opportunities for economic development, 5) Manage the ocean holistically (Lubchenco et al., 2020). Modalities of the impact include capacity and institution building in partner countries, science support to international ocean management bodies and agenda setting for global ocean science and ocean policy.

2. Underpinning research

The area of research has gradually developed and expanded over the years from focus on science support to fisheries management for Maximum Sustainable Yield (MSY) and analyses of nutrition and contaminants in seafood since the start in the 1970ies, to include aquaculture including its environmental impacts from the 1980ies, environmental impact assessments for other ocean economy sectors from the 1990ies, integrated ocean management from the 2000s, and finally broader sustainability concerns from the 2010s.

While IMRs involvement in seafood safety and nutrition started in 1947 and projects in developing countries started in 1965, we have chosen the starting time of the Nansen programme in 1975 as the starting date of the research for this impact case. The present phase of the Ecosystem Approach to Fisheries (EAF) Nansen programme runs from 2017 through 2023 and has 32 partner countries in Africa and the Bay of Bengal. The Nansen Programme is the longest running development collaboration programme in the world and has received several prizes. It is executed by FAO with funding from Norad and with IMR providing the research vessel services and scientific services. Research on food security and nutrition (Golden et al., 2021) is included in the Nansen Programme as well as in a series of bilateral projects.

While aquaculture in Norway is dominated by salmon farming, IMRs international involvement in aquaculture includes low trophic aquaqulture and projects in Asian countries including China (Asplin et al., 2021). Different environmental impacts of fisheries and aquaculture and other human activities including oil and gas, shipping and offshore wind are addressed in series of studies (See de Jong et al., 2020, for the case of anthropogenic noise). Actions that can be taken to restore impacted ecosystems (Moland et al., 2013) are receiving increasing attention. Involvement in the Antarctic include work to support the establishment of Marine Protected Areas (MPAs) as well as sustainable harvesting (Krafft et al., 2021).

Several aspects of the output of the research and related scientific services including capacity development activities have directly addressed tangible impacts which are documented primarily in technical reports under the auspices of the Food and Agriculture Organization (FAO), IMR and others. Some of these reports including all expert reports from the High Level Panel for a Sustainable Ocean Economy (Ocean Panel) published by World Resources Institute (WRI) are peer reviewed, but not all are in journals. Here we concentrate on peer review journal papers from the latter part of the period of the research, global high level achievements, and researchers who have been involved in the recent synthesis and science-

policy processes rather than the equally important enabling and specific research activities from the earlier periods.

Peter M. Haugan, Programme Director 2019-, Research Director II, 2015-2018.
Marian Kjellevold, Principal scientist 2019-, Head of research 2013-2019, Researcher 2003-2013
Gro-Ingunn Hemre, Research Director 2013-, 2001-2013 Head of Research, Senior Scientist 1996-2001
Livar Frøyland Programme Director 2018-, Research Director 2011-2017, Head of Research 2004-2010, Researcher 1997-2003,
Øivind Strand Principal scientist 2009-, Researcher 1994-2009
Ann-Lisbeth Agnalt, Programme Director 2021-, Principal scientist 2014-, Researcher 1996-2014
Even Moland Assistant Programme Director 2021-, Principal Scientist 2019- , Senior Scientist 2010-2019
Bjørn Krafft 2009-2011 Principal scientist 2017-, Senior scientist 2011-2017, Post Doc 2009-2011
Mette Skern-Mauritzen Head of research 2014 –, Principal scientist 2013-2014, Senior scientist 2002 – 2013
Erik Olsen Head of research 2015-, Visiting scientist NOAA NMFS 2013-2015, Programme director 2007-2013

The roles of these lead IMR scientists cover different aspects including Food Security and Nutrition (FSN) in developing countries (Kjellevold), aquatic food for global food security (Hemre and Frøyland), low trophic aquaculture (Strand), lead in aquaculture in ICES and developing countries (Agnalt), fisheries conservation science (Moland), protection and sustainable use (Krafft), lead in Integrated Assessments in ICES (Olsen and Skern-Mauritzen) and sustainable ocean management (Skern-Mauritzen, Moland and Olsen). Peter M Haugan has contributed to taking these and other inputs forward to impacts via the Ocean Decade and the Ocean Panel (see section 4).

3. References to the research

Lubchenco, J., Haugan, P.M. and Pangestu, M.E. 2020. Five priorities for a sustainable ocean economy. *Nature* **588**, 30-32 (2020) doi: https://doi.org/10.1038/d41586-020-03303-3

Golden CD, JC Koehn, …, M KJELLEVOLD, et al. Aquatic Foods for Nourishing Nations. 2021. Nature URL/DOI: https://doi.org/10.1038/s41586-021-03917-1.

Moland, E., Olsen, E.M., Knutsen, H., Garrigou, P., Espeland, S.H., Kleiven, A.R., André, C. and Knutsen, J.A. 2013. Lobster and cod benefit from small scale northern MPAs: inference from an empirical before-after control-impact (BACI) study. Proceedings of the Royal Society B 280: 2012269. https://royalsocietypublishing.org/doi/10.1098/rspb.2012.2679

ASPLIN L, F Lin, P BUDGELL, Ø STRAND. 2021. Rapid temperature variations of the water at the Northern Shelf of the Yellow Sea and implications for sea ranching. Aquaculture Environment Interactions 13: 111–119, https://doi.org/10.3354/aei00394

KRAFFT, BA, GJ MACAULAY, G SKARET et al. Standing stock of Antarctic krill (Euphausia superba Dana 1850 (Euphausiacea)) in the Southwest Atlantic sector of the Southern Ocean, 2019. 2021. Journal of Crustacean Biology. 41:1-17. https://doi.org/10.1093/jcbiol/ruab046.

de Jong, K., Forland, T.N., Amorim, M.C.P. et al., 2020. Predicting the effects of anthropogenic noise on fish reproduction. Rev Fish Biol Fisheries 30, 245–268 (2020). https://doi.org/10.1007/s11160-020-09598-9.

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4. Details of the impact

In 2012 the United Nations Conference on Sustainable Development (Rio+20) started the process to develop a set of Sustainable Development Goals (SDGs). Based on IMR participation and competence, the Committee on World Food Security in 2014 included in its 7th report fish as a part of the solution to solve nutritional challenges based on input from a High Level Panel of Experts. In 2017 the first UN Ocean Conference was held in New York and

the UN Decade of Ocean Science for Sustainable Development (Ocean Decade) was proclaimed by the UN General Assembly. In 2020 the High Level Panel for a Sustainable Ocean Economy (Ocean Panel) launched their political commitment “Transformations” and the underpinning science reports. In 2021 the UN Food Summit and the UNFCCC COP initiated a growing emphasis on aquatic food for global food security and a suite of ocean-based climate solutions, respectively.

IMR contributed significantly to all of these developments. Building upon prior work from a range of scientists, Peter M Haugan of IMR, serving as elected chair of the Intergovernmental Oceanographic Commission (UNESCO/IOC) 2015-2019 (and vice chair 2011-2015) designing the UN Decade and as co-chair of the Expert Group of the Ocean Panel (2018-…) providing recommendations (Lubchenco et al., 2020) was able to capitalize on and represent the insights and experience from the relevant scientific achievements in these processes.

There is now a commitment by all countries affiliated with the Ocean Panel to 100% sustainable management of ocean areas under national jurisdiction by 2025 and a call from the Ocean Panel (now representing 17 countries including US, France and UK) on all other countries to do the same by 2030. The Ocean Decade, UNESCO/IOC and the Ocean Action 2030 coalition initiated by the Ocean Panel and coordinated by WRI now all join forces to support the governmental commitments to managing the ocean sustainably.

There is now a growing understanding that Sustainable Ocean Planning, based on adequate science, is both feasible and a major opportunity for humankind, both within Exclusive Economic Zones and in the high seas, to develop a sustainable ocean economy, contribute to fight against poverty, and address climate change, while at the same time preserving and restoring the ocean health. The Convention on Biological Diversity and the process towards a legal instrument on conservation and sustainable use of marine biodiversity of areas beyond national jurisdiction (BBNJ) present complementary avenues for supporting sustainable ocean management and receive increasing attention and scientific support, also from IMR.

The global food system is dominated by products from agriculture with a high climatic and environmental footprint. Scaling up aquatic food will contribute significantly to FSN and to the solution of our climate crises. Aquatic food is a diverse source of micronutrients, high quality protein and long chain omega-3 fatty acids and can be developed further and used better for human nutrition. Aspects of the nexus between the food system, climate and nature has become clearer in recent years.

IMR has come to the issues of climate solutions, biodiversity conservation and sustainable ocean management from decades of work on seafood harvesting and production. The gradually expanded scope towards an integrated approach has dominated Norwegian domestic ocean management with the development of integrated management plans during the past 20 years, with IMR leading the related monitoring and assessment. The International Council for the Exploration of the Seas (ICES) has seen a similar expansion of tasks and responsibilities. IMR researchers contribute to and since 2010 chair several working groups of ICES addressing Integrated Assessments of different international sea basins and for Aquaculture. Ecosystem Based Management (EBM) is a common key feature of these as well as for work supporting the Arctic Council and the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) where IMR scientists play leading roles in scientific committees.

The key here is integration, agenda setting and the science-policy interface, but specifically within the 5 categories identified by Lubchenco et al (2020):

1) Manage seafood production sustainably: IMR has contributed with development of sustainable fisheries management capabilities through the Nansen programme and bilateral projects in a series of partner countries.

2) Mitigate climate change: Support to ocean-based renewable energy, to the global action network for the role of aquatic food in the global food system, to coastal management including conservation of blue carbon through the Norwegian blue forest network 3) Stem biodiversity loss: Science support to MPAs in international and remote waters 4) Seize opportunities for economic development: Science support to fisheries, aquaculture, ocean-based renewable energy and coastal management 5) Manage the ocean holistically: Impact and risk assessments, identification of vulnerable areas.
5. Sources to corroborate the impact Vladimir Ryabinin, Executive Secretary UNESCO/IOC Manuel Barange, Director Fisheries and Aquaculture, FAO Craig McLean, Director of NOAA Research (retired) Peter Thomson, UN SG Special Envoy for the Ocean Vidar Helgesen, Executive Director of the Nobel Foundation Kristian Teleki, Global Director, Ocean Program, World Resources Institute Henrik Harboe, Special Envoy for the Ocean Panel, Norwegian Ministry of Foreign Affairs. Shakuntala Thilsted, WorldFish

NINA_NINA 1

Institution: Norwegian Institute for Nature Research (NINA)
Administrative unit: Norwegian Institute for Nature Research (NINA)
Title of case study: Handbook for environmental design in regulated salmon rivers
Period when the underpinning research was undertaken: 2009-2013
Period when staff involved in the underpinning research were employed by the submitting institution: The whole period
Period when the impact occurred: From 2013 and onwards

1. Summary of the impact

The handbook strongly impacted practises in both public management (the Norwegian Environment Agency and the Norwegian Water Resources and Energy Directorate) and in the hydropower industry. Today, most of the environmental impact studies in regulated rivers are based on the methodology developed in the diagnoses part of the handbook and win-win (for hydropower and fish) design solutions have been or are under implementation in several rivers. University continuing education courses on environmental design have been arranged as well as courses for hydropower companies. A Chinese translation have been published after initiative from hydropower organisations in China. Research continues to expand the concept of environmental design to other fish species and ecosystem components.

2. Underpinning research

While the handbook is based on accumulated knowledge from the more than 50 years of extensive environmental research on Atlantic salmon in Norway and internationally, the book and its major methodologies was developed in the EnviDORR project (2007-2013, 24.5 mill NOK) and the CEDREN research centre (2009-2016, c. 300 mill NOK, www.cedren.no) both funded by RCN and industry and management partners. For the NINA scientists the main research contributions relates to the fundamentals of population regulation mechanisms for juvenile Atlantic salmon and how habitat and hydrological variables influence population bottlenecks and dynamics. Laboratory and field studies on the importance of shelter availability (and methods to quantify) and the spatial distribution of both shelter and spawning habitat was essential for the development of the diagnostic tools in the handbook. Also, several environmental impact studies in regulated rivers (some published internationally, other in Norwegian reports) provided information on hydrological bottlenecks and links between flow patterns and population regulation. The EnviDORR project was initiated and managed by NINA and NINA was central in the development and application for CEDREN, and part of its leader group.

Key researchers:

Torbjørn Forseth, senior researcher NINA, project manager EnviDORR, main author of the biological part and editor of the handbook. At NINA for the whole period.

Ola Ugedal, senior researcher NINA for the whole period. *Eli Kvingedal*, researcher at NINA for the whole period. *Line Sundt-Hansen*, post doc in EnviDORR from 2008 and researcher at NINA from 2009 until present. *Anders G. Finstad*, PhD position at NINA from 2003, researcher at NINA from 2006 to September 2014. *Sigurd Einum*, researcher at NINA 2001-2007. *Maxim Teichert*, PhD position at NINA funded by EnviDORR 2008-2011.

3. References to the research

Forseth, T. & Harby, A. (eds.). 2014. Handbook for environmental design in regulated salmon rivers. - NINA Special Report 53. 90 pp. (English version). https://www.nina.no/archive/nina/pppbasepdf/temahefte/053.pdf

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Skoglund, H., S. Einum, T. Forseth, and B. T. Barlaup. 2012. The penalty for arriving late in emerging salmonid juveniles: differences between species correspond to their interspecific competitive ability. Functional Ecology 26:104-111.

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Teichert, M.A.K., Foldvik, A., Forseth, T., Ugedal, O., Einum, S., Finstad, A.G., Hedger, R.D. & Bellier, E. (2011). Effects of spawning distribution on juvenile Atlantic salmon (*Salmo salar*) density and growth. *Canadian Journal of Fisheries and Aquatic Sciences*, **68**, 43-50.

4. Details of the impact

The “Handbook for environmental design in regulated salmon river” describes how to evaluate, develop and implement measures to improve living conditions for salmon in regulated river systems, while taking hydropower generation into account. Rather than mimicking natural conditions in regulated rivers, the environmental design concept is based on utilising the fact that environmental conditions (flow and temperature) to some extent can be controlled in regulated rivers, allowing conditions to be adapted to the needs of salmon. To the extent possible, the aim is to arrive at solutions that increases both salmonid and hydropower production, but in some cases increasing salmon production while maintaining hydropower production or minimizing loss may be the goal. Norway depends heavily on hydropower. There are 1739 hydropower stations in Norway producing nearly 90% of the country’s electric energy. At the same time, a large proportion of the healthy populations of Atlantic salmon in the world are found in Norway, making Norway important for the protection of salmon. Nearly 30% of the salmon populations are to different degrees negatively affected by hydropower and the trade-off between hydropower production and protection of salmon is very important.

The handbook was a direct and planned major delivery of the EnviDORR project and the work within the CEDREN research centre. The EnviDORR project filled major knowledge gaps, particularly through the research on Atlantic salmon population regulation outlined above, that was used alongside the extensive existing knowledge on salmon ecology in an innovation process to develop the concept of environmental design. It was a multidisciplinary cooperation between experts on fish ecology (NINA, UiO, NTNU and LFI Bergen) and hydrology and hydropower experts (SINTEF Energy and NTNU).

The handbook is a printed and online book, available in Norwegian, English and Chinese. It is structured into diagnosis and design solution parts. The diagnosis part aims at understanding the hydropower system and its environmental effect and identifying the habitat- and hydrological bottlenecks for salmon production and includes a toolbox of methods to arrive at the diagnosis. Part two present design solutions, how to reduce the effects of the major bottlenecks for salmon smolt production, involving both habitat measures and hydrological (use of water) measures, and combinations of the two. It also involves detailed description on design criteria for the different measures.

The impact of the handbook comes partly from the methodological toolbox for identifying habitat- and hydrological bottlenecks in regulated rivers, and partly from application of the concept in environmental design projects in several regulated river systems.

The methodology rapidly became the standard of effects assessments in regulated rivers, commissioned by the Norwegian Environment Agency as well as for the hydropower companies in their efforts to improve conditions for salmonids in regulated rivers. The Norwegian Water Resources and Energy Directorate have embraced the concept and use in their assessment of new hydropower developments and in revision of concessions. It is used in research as well as in consultancies. Three large scale environmental design projects (the rivers Mandalselva, Kvina & Aurland) and several smaller ones (the rivers Kåfjordelva, Stjørdalselva, Røssåga and more) have been performed, resulting in large habitat restoration projects as well as changes in operation of hydropower facilities to improve hydrological condition. The River Kvina case is particularly interesting because fully developed the suggested measures would double the salmon production in this strongly impacted river while also proving an additional 130 GWh of hydropower production. The hydropower expansion and environmental measures have been assessed and recommended by the Norwegian Water Resources and Energy Directorate and are currently considered by the Ministry of Petroleum and Energy. The River Mandalselva case is the largest environmental design project so far (c. 13 mill NOK) resulting in large scale physical habitat restoration and major changes in the operation of hydropower station. The estimated outcome is return of the salmon production to pre hydropower development levels and a small increase in the hydropower production. Effects on the salmon population are currently evaluation, showing very promising results. The third large case is the River Aurland. Here the methodology was mainly used to improve conditions for sea trout rather than salmon and shows that the concept can also be adapted to other salmonid species. The diagnosis identified major habitat bottlenecks and extensive habitat restoration measures were implemented.

Another illustration of the impact is the interest in education on the concept and methodology. The Norwegian University of Technology and Science (NTNU) arranges a university course at master level in environmental design (VM6006 - Environmental Design), where the main textbook is the handbook. The course is part of NTNU's continuing education portfolio. The course gives 7.5 SP and has been arranged twice, in 2019 and 2021, respectively. The course is planned to run for a third time in May 2023. Course participants ranges from public managers, via consultants and hydropower operators to local fishery managers. International seminars and shorter courses in environmental design have also been arranged - in Beijing, China in 2015 (RCN Future Hydro project) and in Iasi, Romania in 2017. In Romania the course was a three-day seminar with participants from NGO's, nature management and hydropower industry. In 2022 a 4-day course was arranged for the largest hydropower company in Norway (Statkraft), also based on the handbook.

Through a new research centre, the HydroCen (Hydrocen - NTNU), the environmental design concept is under further development to involve inland fish species, biodiversity and other user interests.

"Environmental design" (in Norwegian: Miljødesign) has become a widespread phrase and concept known to most people interested in protection of salmonids in regulated rivers, ranging from parliament politicians to local river managers. A google search on the Norwegian title of the handbook ("Håndbok for miljødesign i regulerte laksevassdrag") return more than 1000 hits.

5. Sources to corroborate the impact

Large scale environmental design studies resulting in major measures:

Forseth, T., Robertsen, G., Gabrielsen , S.E., Sundt , H., Skår , B. & Ugedal, O. (2012). Back to historic smolt production in River Kvina – an exploration of the possibilities. NINA Rapport 847, 60 s. In Norwegian. https://www.nina.no/archive/nina/PppBasePdf/rapport/2012/847.pdf

Ugedal, O., Pulg, U., Skoglund, H., Charmasson, J., Espedal, E.O., Jensås, J.G., Stranzl, S., Harby, A. & Forseth, T. 2020. Sea trout and Atlantic salmon in the Aurland watercourse 2009-2018. Effects of regulation, environmental design and habitat measures. NINA rapport

1716. Norwegian Institute for Nature Research. In Norwegian. https://brage.nina.no/nina-xmlui/handle/11250/2621914

Forseth, T., Fjeldstad, H-P., Gabrielsen, S.E., Skår, B., Lamberg, A., Hedger, R.D., Kvingedal, E. & Havn, T.B. 2020. Environmental design in River Mandalselva. Action plan and summary of results. NINA rapport 1691. Norwegian Institute for Nature research. In Norwegian. https://brage.nina.no/nina-xmlui/handle/11250/2634396

Smaller inventory studies using environmental design:

Arnekleiv, J.V., Bergan, P.I., Sundt-Hansen, L.E., Kielland, Ø.N., Foldvik, A., Davidsen, J.G., Først, M. & Vaskinn, K.A. 2020. Environmental design in regulated salmon watercourses: The River Stjørdalselva in Meråker County – NTNU Vitenskapsmuseet naturhistorisk rapport 2019-4: 1- 105. In Norwegian with English summary. https://www.ntnu.no/documents/10476/1292086791/2020-4+Rapport+Milj%C3%B8design+Stj%C3%B8rdalselva.pdf/de9deee0-adbb-dafe-d423-1139fbea30ac?t=1620809096478

Bremset, G., Holthe, E., Kanstad-Hanssen, Ø., Lo, H., Jensås, J.G., Karlsson, S., Museth, J., Tønder, T.S. & Ulvan, E.M. 2022. Fiskebiologiske undersøkelser i Røssågavassdraget. Årsrapport for 2021. NINA Rapport 2036. Norsk institutt for naturforskning. https://brage.nina.no/nina-xmlui/bitstream/handle/11250/3000478/ninarapport2036.pdf?sequence=1&isAllowed=y

Kvingedal, E., Bremset, G., Sundt-Hansen, L.E.B., Ugedal, O. & Forseth, T. (2017) Guolasjohka hydropower station in River Kåfjordelva. Assessment of status for anadromous salmonids and predicted effects of different habitat and hydrological measures. NINA rapport 1338. Norwegian Institute for Nature Research. 64 s. In Norwegian. https://brage.nina.no/nina-xmlui/handle/11250/2437080

Environmental design courses:

https://www.ntnu.edu/studies/courses/VM6006#tab=omEmnet

https://www.cedren.no/english/Projects/FutureHydro

NINA_NINA 2

Institution: Norwegian Institute for Nature Research (NINA)
Administrative unit: Norwegian Institute for Nature Research (NINA)
Title of case study: Large carnivore monitoring
Period when the underpinning research was undertaken: 2003–2021
Period when staff involved in the underpinning research were employed by the submitting institution: 1999 t.d.
Period when the impact occurred: 2011–ongoing

1. Summary of the impact (indicative maximum 100 words)

Large carnivores live at low population densities and roam widely, traversing international borders. For sustainable conservation management, detailed population monitoring is crucial, albeit challenging. The Norwegian Monitoring Program for Large Carnivores was established at NINA already in 2000, with Rovdata as the responsible unit from 2010 onwards. Research at NINA, and a broad network of collaborators, have contributed to unprecedented carnivore monitoring in Scandinavia (Norway and Sweden), strongly influencing management strategies and decisions. Important impacts are: (1) Improvement of monitoring methods and approaches (2) Increased focus on collaboration across national borders, with several signed MOUs at the management and ministry level on transboundary carnivore management, (3) A comprehensive toolbox for outreach and dissemination (4) Facilitation of public participation in monitoring activities.

2. Underpinning research (indicative maximum 500 words)

In Scandinavia, the implementation of large carnivore monitoring was preceded by, and is still guided by, research across a broad range of topics. These include aspects related to life history strategies and population dynamics, pedigree assessments, inbreeding issues, as well as questions related to dispersal, connectivity, migration, and genetic structure. In addition, there is a continuous refinement of monitoring techniques such as non-invasive genetic sampling (NGS) and camera trapping. Several NINA researchers and other NINA staff have been central in this research and the associated development and improvement of the monitoring program.

At the turn of the century, when large carnivore monitoring in Scandinavia was based on traditional approaches such as snow tracking and den registration, a pioneering PhD project was initiated in Uppsala, Sweden, paving the way for the inclusion of NGS as a fundamental part of the monitoring program. The PhD student (Øystein Flagstad, now a senior researcher at NINA) carried out his research in close collaboration with NINA researchers and the Norwegian Environmental Agency. To our knowledge, Flagstad's work on DNA-based wolverine monitoring (**R1**) was the first scientifically published example of NGS applied at a larger spatial scale, where hundreds of scat samples were collected and genotyped from a study area of several thousand square kilometers in Southern Norway. At the same time, the first NGS trials from Swedish localities were undertaken, which enabled a joint and harmonized Scandinavian effort towards DNA-based monitoring of the Scandinavian wolf, wolverine, and brown bear populations, including estimates of population size and vital rates (**R2**).

For the Eurasian lynx, where NGS is hampered by species-specific behavior (hidden scats and urine markings contaminated by several individuals), data on population dynamics and life history strategies have been obtained from other sources (snow tracking, camera trapping and dead recoveries). An important achievement regarding the lynx was the development of a model to forecast lynx population dynamics (**R3**). Four senior researchers at NINA co-authored the paper (Henrik Brøseth, John Odden, John DC Linnell, and Erlend B Nilsen). The model predicts the probability that the forecasted population size will be below or above a specified

management goal when subjected to different harvest quotas. This model has become an important applied tool for adaptive management at both regional and national scales.

The Scandinavian carnivores live at the periphery of their Eurasian distribution, which implies that connectivity and gene flow is pivotal for long-term population viability. The extreme inbreeding and importance of immigrants into the Scandinavian wolf population have been addressed in several of Flagstad's papers (e.g., **R4**). Connectivity in the other Scandinavian carnivores has been addressed in more recent papers. **R5**, co-authored by four NINA researchers (Alexander Kopatz, Oddmund Kleven, Jonas Kindberg and Øystein Flagstad), provides an example where restored transborder connectivity for Fennoscandian brown bears were documented.

Close collaboration across national borders is of vital importance for sustainable management of transboundary carnivore populations. This was profoundly unveiled in a paper assessing wolverine management regimes in Norway and Sweden. The paper, co-authored by two NINA researchers (Henrik Brøseth and John DC Linnell), demonstrated that the failure to coordinate management across national borders may hinder the achievement of national management goals (**R6**)

3. References to the research (indicative maximum of six references)

(**R1**) Flagstad, Ø., E. Hedmark, A. Landa, H. Brøseth, J. Persson, R. Andersen, P. Segerström and H. Ellegren (2004). "Colonization history and noninvasive monitoring of a reestablished wolverine population." Conservation Biology 18: 676-688. https://doi.org/10.1111/j.1523-1739.2004.00328.x-i1

(**R2**) Bischof, R., C. Milleret, P. Dupont, J. Chipperfield, M. Tourani, A. Ordiz, P. de Valpine, D. Turek, J. A. Royle, O. Gimenez, Ø. Flagstad, M. Åkesson, L. Svensson, H. Brøseth and J. Kindberg (2020). "Estimating and forecasting spatial population dynamics of apex predators using transnational genetic monitoring." Proceedings of the National Academy of Sciences of the United States of America 117: 30531-30538. https://doi.org/10.1073/pnas.2011383117

(**R3**) Andrén, H., N. T. Hobbs, M. Aronsson, H. Brøseth, G. Chapron, J. D. C. Linnell, J. Odden, J. Persson and E. B. Nilsen (2020). "Harvest models of small populations of a large carnivore using Bayesian forecasting." Ecological Applications 30:e02063. https://doi.org/10.1002/eap.2063

(**R4**) Åkesson, M., O. Liberg, H. Sand, P. Wabakken, S. Bensch and Ø. Flagstad (2016). "Genetic rescue in a severely inbred wolf population." Molecular Ecology 25: 4745-4756. https://doi.org/10.1111/mec.13797

(**R5**) Kopatz, A., O. Kleven, I. Kojola, J. Aspi, A. J. Norman, G. Spong, N. Gyllenstrand, L. Dalen, I. Fløystad, S. B. Hagen, J. Kindberg and Ø. Flagstad (2021). "Restoration of transborder connectivity for Fennoscandian brown bears (*Ursus arctos*)." Biological Conservation 253: 108936. https://doi.org/10.1016/j.biocon.2020.108936

(**R6**) Gervasi, V., J. D. C. Linnell, H. Brøseth and O. Gimenez (2019). "Failure to coordinate management in transboundary populations hinders the achievement of national management goals: The case of wolverines in Scandinavia." Journal of Applied Ecology 56: 1905-1915. https://doi.org/10.1111/1365-2664.13379

The research listed is published in high-ranking scientific journals and citation rates are high. Their relevance to and impact on management strategies and decisions are indicated from the citation of these papers also in documents from politicians and managers (see **S6** and **S7** in Section 5 below).

4. Details of the impact (indicative maximum 750 words)

Sustainable management of large carnivores requires reliable data on population dynamics and -trends. These kinds of data can be difficult to obtain as large carnivores typically live at

low densities while they simultaneously roam over large areas, often traversing national borders. In addition, their elusive nature makes it difficult to observe them directly. As such, monitoring efforts often rely on advanced technologies and methods; for example: aerial surveys, camera traps, and genetic analysis of non-invasively collected biological material such as scats, urine, and hair. Combining robust methods, including non-invasive DNA, allows the public to be part of a monitoring program. Indeed, it would be impossible to run a program at the same scale and ambition as in Scandinavia entirely with researchers and wardens.

Pathways to the impacts
Based on previous research of which some is described in Section 2 above, Rovdata was established in 2010 to implement an overarching, transparent and science-based framework for large carnivore monitoring in Norway. Rovdata is an independent unit within NINA and is responsible for operating, communicating and improving the Norwegian Large Carnivore Monitoring Program (**S1**). In addition to the execution of evidence-based monitoring, an integral part of Rovdata is a comprehensive framework for dissemination and outreach to achieve transparency, i.e., communicating results, research and advances to the public, key stakeholders and government authorities through a mix of different media types.

The underlying holistic philosophy of Rovdata is an adaptive management framework, where research precedes the choice of monitoring design, and transparent dissemination of the results may provoke feedback from managers or other stakeholders. Evaluation of the feedback may lead to new research, which in turn might lead to new monitoring methods or approaches. As such, by providing results with strong implications to conservation management as well as new methodological approaches and management tools, the underpinning research have strongly impacted on the monitoring program itself, which in turn has had a substantial impact on management strategies and decisions. For example, in the parliament document on wolf management (**S2**), the severe inbreeding situation in the Scandinavian wolf population is referred to numerous times, and then taken into account when evaluating the political goal on the number of yearly wolf reproductions.

Evidence is given below of specific impacts relating to the monitoring program itself, management strategies and decisions, outreach and dissemination, and finally the facilitation of stakeholder participation and engagement.

1) Improvement of monitoring methods and approaches
The pioneering PhD research in the early 2000s led to the inclusion of NGS as an integrated part of the monitoring program. Since then, more than 127,000 scat, urine, secrete, blood, and hair samples have been analysed within the framework of the Scandinavian monitoring program, all together representing 2,659 individual wolves, 5,278 wolverines and 8,109 brown bears. All monitoring data are registered in a publicly available database, where researchers, managers and other stakeholders can follow monitoring efforts and results (**S3**).

In addition to providing precise data on population dynamics and vital rates (**R2**), data from NGS can be combined with other data sources. For example, NGS data was combined with data on GPS-collared brown bears to develop a tool for estimating the number of yearly brown bear reproductions in Norway (**S4**).

2) Increased focus on transboundary collaboration
As noted above, close collaboration across national borders is pivotal for sustainable management of transboundary carnivore populations. A few years ago, four working groups of researchers, wardens and stakeholders, whose mandate was to evaluate and execute perfectly harmonized monitoring methods in Norway and Sweden (**S5**).

Later, Finland has become an increasingly important partner in a trilateral Fennoscandian network and has adopted several of the same methods as are applied in Scandinavia. The harmonization of methods has been accompanied by Memorandums of understanding

(MOUs), at the governmental level as well as at the level of management authorities (**S6**, **S7**). The basis for these agreements is the research emphasizing the importance of connectivity in the Fennoscandian carnivore populations (**R4**, **R5**) and papers emphasizing the importance of coordinated management efforts (**R6**), papers which often are cited in these agreements. In addition, inspired by the Scandinavian monitoring regime Finland, is increasingly using NGS (**R1**, **R2**) in their monitoring designs.

3) Outreach and dissemination

Rovdata is responsible for disseminating methods and approaches as well as the results from the National Monitoring Program for large carnivores. All annual monitoring reports are openly published on Rovdata's webpage (**S8**). Rovdata has its own communications advisor, who assists in the dissemination work. In the period from 1 January 2011 to 31 December 2021, Rovdata published 389 news stories/press releases on rovdata.no. There were 1.8 million unique visitors to our web site, with 4.5 million page views, and an average visit time of approximately 2 minutes (Source: Plausible.io). In the years from 2013 to 2021, there were about 12,000 media reports in which Rovdata is mentioned, which is a media coverage corresponding to an advertising value of NOK 371,403,379 (Source: Retriever). Gross readership is estimated at 879,697,525 (Source: Retriever/Media companies' National Association and TNS-Gallups Forbruker & Media). Films and interactive webinars are important forms of communication in Rovdata, and we have made both informational and instructional movies for the public (**S9**).

4) Stakeholder engagement

Rovdata works to strengthen local participation in mapping and monitoring the species and has established Skandobs (**S10**) in collaboration with The Norwegian Biodiversity Information Centre in Norway and the Swedish Environmental Protection Agency. It is a public solution for registering carnivore observations. At the end of 2021, there were 49,359 registered users in the system in Norway and Sweden, and 53,789 observations entered.

There is also a strong local engagement for providing DNA samples to the monitoring program. In fact, hunters and other local contributors have provided more than 40,000 DNA samples, which is approximately 30% of all samples that have been analysed since non-invasive genetic sampling was implemented as an integrated part of large carnivore monitoring in Scandinavia in the early 2000s.

5. Sources to corroborate the impact (indicative maximum of ten references)

S1 Norwegian regulations on the management of wildlife and game: https://bit.ly/3GY3ZI7

S2 "The wolf in the Norwegian nature" which states the population targets for wolves and wolf zones and recommendation from the Ministry of Climate and the Environment from 18 March 18, 2016, approved by the government (e.g., issue of inbreeding is mentioned dozens of times): https://bit.ly/3GGPNCU

S3 Rovbase, the management tool to accumulate and verify information on the distribution and occurrence of carnivores, the depredation on livestock of carnivores, and the compensation for depredation of carnivores on livestock: www.rovbase.no

S4 Scientific paper that presents the model to estimate the number of brown bear reproductions: https://doi.org/10.1890/11-0013.1

S5 Example on the numerous projects to harmonize the large carnivore inventory methods in Sweden and Norway: https://bit.ly/3kepVH9 (Report in Norwegian)

S6 MOU at the ministerial level: Agreement between the Ministry of Environment, Sweden, the Ministry of Environment, Norway, and the Ministry of Agriculture and Forestry, Finland, in

developing collaboration on large carnivores – brown bear, wolf, lynx and wolverine. August 12, 2011. https://bit.ly/3ZwKXAT

S7 MOU regarding the establishment and continuance of a monitoring system for large carnivores in Sweden and Norway https://shorturl.at/euEHZ

Other MOUs at the management level: https://bit.ly/3iBl0j4 and https://bit.ly/3ZxrFLG

S8 Website of Rovdata for communication with the public: www.rovdata.no

S9 Outreach examples explaining monitoring of large carnivores and results with a combination of mixed media:

- Movie “DNA-based monitoring of large carnivores”: https://youtu.be/8J_DbzoA50c (in Norwegian with English subtitles)
- Movie "On the trail of wolves with SNO" https://bit.ly/3QBIWPK (in Norwegian)
- Movie “How to recognize bear scats” https://bit.ly/3iE9oMg (in Norwegian)
- Podcast “With the wolf in the test tube”: http://bit.ly/3IK50Wq (in Norwegian)
- StoryMaps “Large carnivore DNA-based monitoring”: www.rovdata.no/DNA (in Norwegian and English)
- Press release “DNA is an ingenious tool in large carnivore monitoring”: http://bit.ly/3w2cZqg (in Norwegian)
- Webinar “Scandinavian Wolf“ https://youtu.be/Ll3-IAb4slw (in Norwegian)
- Webinar “Scandinavian Wolf Report”: https://bit.ly/3X6x3nB (in Norwegian)

S10 Skandobs, the app and website, where everyone can report their sightings of lynx, wolverine, brown bear, and wolf. A tool for citizen science to contribute to knowledge about the distribution and number of carnivores in Scandinavia: www.skandobs.no

NINA_NINA 3

Institution: Norwegian Institute for Nature Research (NINA)
Administrative unit: Norwegian Institute for Nature Research (NINA)
Title of case study: SEATRACK – mapping seabird non-breeding distribution for better management and marine protection in the North Atlantic
Period when the underpinning research was undertaken: 2014–ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: The whole period
Period when the impact occurred: 2016–ongoing

1. Summary of the impact (indicative maximum 100 words)

Sustainable ocean management aims to conserve unique marine biodiversity while facilitating resource acquisition by humans. Such management is challenging and requires extensive knowledge of the distribution of marine organisms which are highly mobile and difficult to study. By using new and appropriate technology and through large-scale international collaboration since 2014, SEATRACK has provided such knowledge for seabirds in the North Atlantic. Societal impacts at international and national levels are: 1) designation of a new large marine protected area (NACES) in the North Atlantic by OSPAR, 2) providing knowledge basis for national policies on marine conservation, 3) mitigation of conflicts caused by transition to renewable energy and sustainable offshore wind developments in Norway

2. Underpinning research (indicative maximum 500 words)

The world's oceans contain unique biodiversity, life forms and genetic resources that provide ecosystem services of enormous value to human societies. At the same time, impacts of human activities on the ocean are substantial, ubiquitous and rapidly growing. The High-level Panel for a Sustainable Ocean Economy consisting of 17 world leaders, have committed to sustainably manage 100% of the ocean areas under their national jurisdictions by 2025 and to protect 30% of the worlds' oceans as Marine Protected Areas (MPAs) by 2030. Maps of the spatial distribution of marine animals, especially those that are endangered and vulnerable, are powerful tools in ecosystem-based marine spatial planning of MPAs and in environmental impact assessments of human activities such as fisheries, shipping, oil and gas exploitation, and offshore wind farms.

Seabirds are one of the most threatened groups of vertebrates, with almost half of all species (47%) experiencing population declines. The North Atlantic supports some of the largest seabird populations in the world. Many seabird species undergo extensive seasonal migrations, often across large marine ecosystems or between marine areas under different national jurisdictions. The advances of electronic tracking, especially of the application of Global Location Sensors (GLS or geolocators), have made it possible to study the seasonal movements of seabirds throughout their entire annual life cycle. To take full advantage of this development, there is a need for large-scale and multi-species programmes. The SEATRACK project started in 2014 and is still ongoing. It is led by the Norwegian Polar Institute (NPI) in collaboration with Norwegian Institute of Nature Research (NINA) and the Norwegian Environmental Agency, with 50 partners from 11 countries (Norway, Russia, Iceland, UK, Ireland, France, Poland, Denmark, Faroes, Greenland, and Canada), and aims to identify the year-round distribution and movements of seabirds breeding in colonies across the northern part of the North Atlantic. Four main research themes have been defined (**R1**): (1) the assessment of variation in migration strategies among individuals, populations and species (e.g. **R2**); (2) the linkage of migration strategies and winter distribution to demography and population dynamics (e.g. **R3**); (3) the linkage of non-breeding distribution to contaminants (e.g. **R4**) and (4) the use of tracking data in marine spatial planning (e.g. **R5**). By 2023, 16 000

loggers were deployed on 11 species in 57 seabird colonies, and data from 8300 retrieved loggers have been analyzed and compiled (**R1**).

Based on the positional (GLS) dataset two spatial datasets were developed: (i) kernel distribution maps for all 11 species and colonies showing the seasonal (autumn, winter, spring) distribution of tracked species and colonies (available at https://seatrack.seapop.no/map/) and (ii) a unique spatial dataset of the predicted monthly distribution of 6 pelagic seabird species, covering 23.5 million adult birds, constituting 87% of their combined breeding populations in the Northeast Atlantic (available at https://seatrack-e9bdc.web.app/). This dataset combines tracking data, data describing the physical environment and data on seabird population sizes. It consists of 4692 map layers, each layer predicting the densities of birds from a given species, colony, and month across the Northeast Atlantic (**R5**).

Both spatial datasets are now used widely for research (40+ peer-reviewed papers produced by 2023) and in management processes, including for example the identification of populations influenced by marine protected areas and human activities.

Key researchers: Senior researcher Per Fauchald, senior researcher Børge Moe and researcher Arnaud Tarroux are part of the project group leading SEATRACK together with NPI and the Norwegian Environmental Agency. Fauchald and Moe has been working in the project group since the start in 2014, while Tarroux started in the group as a post doc in 2017-2019 and got a permanent position as a researcher since 2020. In addition, 9 researchers from NINA are partners/participants in the project, and one technician from NINA is employed full time on SEATRACK since the start of the project.

3. References to the research (indicative maximum of six references)

R1. Strøm H, Descamps S, Ekker M et al. (2021) Tracking the movements of North Atlantic seabirds: Steps towards better understanding of population dynamics and marine ecosystem conservation. *MEPS 676: 97-116, https://doi.org/10.3354/meps13801*

R2. Moe, B., F. Daunt, V. S. Bråthen, et al. (2021) Twilight foraging enables European shags to survive the winter across their latitudinal range. Marine Ecology Progress series 676: 145–157, https://doi.org/10.3354/meps13697

R3. Reiertsen TK, Layton-Matthews K, Erikstad KE (2021) Inter-population synchrony in adult survival and effects of climate and extreme weather in non-breeding areas of Atlantic puffins. Marine Ecology Progress Series 676: 219-231, https://doi.org/10.3354/meps13809

R4. Albert C, Bråthen VS, Descamps S. et al. (2021) Inter-annual variation in winter distribution impacts individual seabird contamination with mercury. Marine Ecology Progress Series 676: 243–254, https://doi.org/10.3354/meps13793

R5. Fauchald P, Tarroux A, Amélineau F et al. (2021). Year-round distribution of Northeast Atlantic seabird populations: applications for population management and marine spatial planning. *MEPS 676: 255-276, https://doi.org/10.3354/meps13854*

4. Details of the impact (indicative maximum 750 words)

Impact 1. OSPAR designation of the North Atlantic Current and Evlanov Sea basin Marine Protected Area (NACES MPA).

OSPAR is the mechanism by which fifteen governments and the EU cooperate to protect the marine environment of the North-East Atlantic. The NACES MPA is a vitally important area for seabirds covering approximately 600 000 km^2. By establishing this MPA in 2021 (**S1**), OSPAR reached the UN Convention for Biodiversity 2020 Aichi target of designating 10% of marine waters as MPAs, and it will be an important step for achieving the global target, recently

adopted by United Nations Convention on Biological Diversity (CBD), of protecting 30 % of the oceans by 2030.

The designation of the NACES MPA was first proposed in 2016, with a workshop led by BirdLife International for seabird experts. This led to an effort to gather all available data, in which SEATRACK contributed significantly with seabird tracking data for the North Atlantic. The collaborative analysis of this dataset identified this seabird hotspot (**S2**) and it was officially designated by the OSPAR Commission on the 1st October 2021 (**S1**), making it the first MPA on the High Seas to be identified from tracking data. The scientific paper by Davis et al (2021, S2) identifying the MPA was co-authored by >20 partners of SEATRACK.

Impact 2. Knowledge basis for national politics on marine conservation
By providing data and results to the Norwegian Environment Agency and the Ministry of Climate and Environment, SEATRACK contributes significantly to the scientific basis for national policies on marine conservation. Specifically, SEATRACK provides annual progress reports and engages annually in progress meetings with the Norwegian Environment Agency and the Ministry of Climate and Environment. Norway has recently, through the membership and lead of the Ocean Panel, as well as the United Nations CBD, given its support to a global target of protecting 30 % of the oceans by 2030 through marine protected areas and other effective area-based conservation measures. In the governmental white paper 29 (2020–2021) to the Norwegian Parliament (**S3**), the Ministry of Climate and Environment presents its national plan for conservation of areas of special importance for marine biodiversity, stating that Norway's goal is to play a leading role in developing an integrated, ecosystem-based marine management regime protecting biodiversity while providing a sound basis for sustainable use of resources. The SEATRACK project is referred to as an important research activity for reaching this goal and for identifying areas of great importance for marine biodiversity in Norwegian waters (page 20-21, **S3**): '*By tracking birds from populations subjected to monitoring of trends, reproduction and survival, new and revolutionary knowledge is provided about the species, e.g. about their habitat use, population origin, migration routes, wintering areas and how vulnerable populations are in Norwegian waters***'.**

Impact 3. Transition to renewable energy and sustainable offshore wind developments in Norway
Development of offshore wind energy is increasingly regarded as a solution to the climate crisis and the energy crisis. However, there is also an ongoing nature/biodiversity crisis, and offshore wind farms may have negative environmental impacts. Seabirds are at risk of colliding with offshore windmills, displacement and habitat loss, and it is vitally important that windfarms are established in areas with the lowest potential for conflicts. In 2018, NINA used data from SEATRACK in an environmental impact assessment and provided suggestions for mitigating actions for Equinor and the Hywind Tampen wind farm (**S4**). Hywind Tampen is the first floating offshore windfarm in Norway, and its electricity production will reduce Norway's CO_2 emission by 200 000 metric tonnes per year. Furthermore, The Norwegian Water Resources and Energy Directorate (NVE) has used NINA as scientific advisors in their assignment to locate new areas for offshore wind developments for the Ministry of Petroleum and Energy (**S5**). For this task in 2022, NINA used SEATRACK data to construct a seabird sensitivity map covering the Norwegian Economic Zone (**S6**). NVE will use this to rank potential areas for new wind development according to sensitivity and potential impact.

5. Sources to corroborate the impact (indicative maximum of ten references)

S1. OSPAR Commission (2021) OSPAR Decision 2021/01 on the establishment of the North Atlantic Current and Evlanov Sea basin Marine Protected Area. OSPAR 21/13/1, Annex 23

S2. Davies, T. E., A. P. B. Carneiro; M. Tarzia et al. (2021) Multi-species tracking reveals a major seabird hotspot in the North Atlantic. Conservation Letters 14: e12824.

S3. Klima- og miljødepartementet (2021). Heilskapleg nasjonal plan for bevaring av viktige område for marin natur. Meld. St. 29 (2020 –2021).

English translation: Ministry of Climate and Environment (2021). Norway's integrated plan for the conservation of areas of special importance for marine biodiversity. Meld. St. 29 (2020–2021) Report to the Storting (white paper).

S4. Moe, B., Christensen-Dalsgaard, S., Follestad, A. et al. 2018. Hywind Tampen vindpark. Vurdering av konsekvenser for sjøfugl. NINA Rapport 1521. Norsk institutt for naturforskning.

English translation: Moe, B., Christensen-Dalsgaard, S., Follestad, A. et al. 2018. Hywind Tampen wind farm. Assessment of potential consequences on seabirds. NINA Report 1521. Norwegian Institute for Nature Research.

S5. Norges vassdrags- og energidirektorat (NVE). Ny fornybar energiproduksjon til havs

English translation: The Norwegian Water Resources and Energy Directorate (NVE). Norwegian New renewable energy production at sea.

S6. Norges vassdrags- og energidirektorat (NVE). Sensitivitetskart for sjøfugl fra NINA

English translation: The Norwegian Water Resources and Energy Directorate (NVE). Seabird sensitivity map by NINA.

NINA_NINA 4

Institution: Norwegian Institute for Nature Research (NINA)

Administrative unit: Norwegian Institute for Nature Research (NINA)

Title of case study: Urban ecosystem accounting in Oslo

Period when the underpinning research was undertaken: 2014–2022

Period when staff involved in the underpinning research were employed by the submitting institution: whole period

Period when the impact occurred: 2020–2022

1. Summary of the impact

The impact concerns the uptake by stakeholders in urban planning of NINA's research in urban ecosystem accounting, and specifically mapping and valuation of trees in Oslo's built zone. Urban ecosystem accounting is a subset of ecosystem accounting and nature valuation methods. In this case we document impact looking specifically at the policy objective of providing benefits to inhabitants from ecosystem services provided by the conservation and restoration of urban tree cover in Oslo. For the summary of impact we divide uptake of our research and impact into three main valuation purposes in a policy cycle, according to the IPBES Values Assessment Summary for Policy Makers typology: to inform, to design policy and to support decisions:

Informative impact

1) Urban ecosystem accounting methodology adopted in UN guidance for Ecosystem Accounting (SEEA EA).
2) Urban tree canopy accounting methods adopted by Oslo Municipality.

Policy design impact

3) Urban tree canopy mapping supporting municipal BGF performance standards for nature-based solutions.
4) Valuation of ecosystem services from urban tree canopy supporting performance standards.

Decision-support impact

5) Research support for pausing urban densification that was leading to loss of large trees in Oslo's Small House Planning Area.
6) Multi-criteria decision support for spatial priority-setting of nature-based solutions in Oslo.

2. Underpinning research

The underpinning research starts can be traced back to the first use of urban ecosystem service valuation in Oslo through a case study by NINA in the EU FP7 project OpenNESS (2012-2016, 11,5 M€)) "Ecosystem services from concepts to real-world applications". In NINAs OSLOpenNESS case study we collaborated with Oslo's Urban Environment Agency, conducting the first economic valuation of urban trees in Norway, showing that they were worth billions of kroner annually. The URBAN EEA Project – "Experimental Urban Ecosystems Accounting - improving the decision-support relevance for municipal planning and policy" (2016-2020; 9.9 MNOK) was funded by the Norwegian Research Council. In collaboration with Statistics Norway, NINA tested urban accounting in Norway, funding the first mapping of urban tree canopy in Oslo using Lidar and satellite data. The project provided support for NINA to participate in a working groups developing the UN SEEA EA statistical standard. The project developed the online Urban Nature Atlas for Oslo. The Biodiversa funded ENABLE project "Enabling Green and Blue Infrastructure Potential in Complex Social-Ecological Regions: A System Approach for Assessing Local Solutions" (2016-2019, 2,54 M€) allowed NINA to i.a. develop applications for integrating ecosystem services from urban green to prioritise nature-based solutions such as green roofs to complement existing green infrastructure. NINA funded the "Urban Nature Values Project" (2018-2022) using Strategic Institute Initiative (SIS) funds to support a Ph.D.grant for Z. Cimburova. The Ph.D. research demonstrated integration of remote sensing data from previous prior projects with advanced valuation models, developed urban ecosystem services theory and two applications for quantifying and valuing tree visibility. Research has also been conducted

using Barton's basic research funding provided through NINA from the Norwegian Research Council and NINA's professional development scheme (FU).

Key researchers: Senior researcher David N. Barton 2008–present; Researcher Zander S. Venter 2018–present. Researcher Megan Nowell 2015–present. Research fellow/PhD candidate Zofie Cimburova 2017–2022; Senior IT technician Frank Hanssen 2006–present.

3. References to the research

1) Cimburova, Z., Barton, D.N., 2020. The potential of geospatial analysis and Bayesian networks to enable i-Tree Eco assessment of existing tree inventories. Urban Forestry & Urban Greening 55, 126801. https://doi.org/10.1016/j.ufug.2020.126801
2) Garnåsjordet, P.A., Steinnes, M., Cimburova, Z., Nowell, M., Barton, D.N., Aslaksen, I., 2021. Urban green. Integrating ecosystem extent and condition data in urban ecosystem accounts. Examples from the Oslo region. SJI 37, 1247–1274. https://doi.org/10.3233/SJI-210834
3) Hanssen, F., Barton, D.N., Venter, Z.S., Nowell, M.S., Cimburova, Z., 2021. Utilizing LiDAR data to map tree canopy for urban ecosystem extent and condition accounts in Oslo. Ecological Indicators 130, 108007. https://doi.org/10.1016/j.ecolind.2021.108007
4) Venter, Z.S., Barton, D.N., Martinez-Izquierdo, L., Langemeyer, J., Baró, F., McPhearson, T., 2021. Interactive spatial planning of urban green infrastructure – Retrofitting green roofs where ecosystem services are most needed in Oslo. Ecosystem Services 50, 101314. https://doi.org/10.1016/j.ecoser.2021.101314
5) Cimburova, Z., Blumentrath, S., 2022. Viewshed-based modelling of visual exposure to urban greenery – An efficient GIS tool for practical planning applications. Landscape and Urban Planning 222, 104395. https://doi.org/10.1016/j.landurbplan.2022.104395
6) Cimburova, Z., 2022. Capturing the context: Developing GIS methods for modelling the ecosystem services of urban trees. PhD thesis. NTNU. https://ntnuopen.ntnu.no/ntnu-xmlui/handle/11250/2998719

4. Details of the impact

NINAs research on urban nature is summarised and referenced here https://www.nina.no/%C3%98kosystemer/Natur-i-by

Informative impact

1) **Urban ecosystem accounting methodology adopted in UN guidance for Ecosystem Accounting (SEEA EA)** (2021; Barton) NINA participated in the development of UN SEEA EA guidance on thematic urban accounts; urban tree canopy mapping is highlighted as a practical measures of urban ecosystem condition, with an example from Oslo. Statistics Norway has integrated urban tree canopy mapping, based on remote sensing data as part of its proposal for regular statistics on built areas.

2) **Urban tree canopy accounting method adopted by Oslo Municipality** (2020-2022, Barton, Hanssen, Cimburova). NINA developed a method for mapping urban tree canopy cover based on widely available remote sensing (Lidar) data collected for urban planning purposes. The method has guided improved contracting of Lidar data quality specifically for tree canopy accounting purposes. NINAs methods are documented as open source which makes them easier to adapt by municipal geodata departments than code used by private consulting firms. The method has been further co-developed with the Oslo Planning and Building Authority and will be adopted by the municipality in the city's future tree mapping and accounting. The report by Hanssen et al.(2019) provided recommendations for Lidar data collection which supported the Planning and Building Agency in quality assuring data deliveries by external consultants and generation of the most current tree cover map for Oslo.

Policy design impact

3) **Urban tree canopy mapping supporting municipal BGF performance standards for nature-based solutions** (2019-2022, Barton, Cimburova). NINA developed a method for calculating the blue-green factor (BGF) performance standard for urban development projects, using aerial photography and a QGIS-app. The methodology was used to account

for before-after impact on BGF of development projects in Bærum municipality. NINA research lead to D.N. Barton participating in Norway's technical committee for Blue-Green Factor. Tree canopy mapping statistics and valuation of tree ecosystem ser ices for Oslo (above) where used to justify increasing the relative weighting of tree canopy relative to the area-based value other nature-based solutions making up the performance criteria. In 2022 Barton participated in Oslo Municipality's technical committee for revision of its BGF norm, also achieving an increase in the relative valuation of tree canopy.

4) **Valuation of ecosystem services from urban tree canopy supporting performance standards**. (2021-2022, Barton, Cimburova) Z. Cimburova's Ph.D. at NINA developed a method to use remotely sensed tree canopy in a well-known method (iTree Eco) for valuing regulating ecosystem services. Cimburova's Ph.D. thesis also developed a method for quantifying tree canopy visibility. Barton participates in Standard Norway's technical committee on a norm for Valuation of Trees (VAT). NINAs research has been the basis for introducing regulating ecosystem services into the proposed VAT norm (expected 2023), and for a quantitative method of assessing visibility values. Standard Norway's VAT norm proposal has not been published, but NINAs research papers can be compared against various reports evaluating the Danish VAT standard and testing it in Oslo. The Danish VAT standard was the starting point for the current development of the Norwegian standard.

Decision-support impact

5) **Support for pausing urban densification leading to loss of large trees in Oslo's Small House Planning Area** (2020, Hanssen, Barton). The Small House Plan is effectively Norway's largest regulation plan by area covering around 29 000 properties. NINAs tree canopy accounting 2011-2017 documented for the first time a significant loss of large trees due to urban infill within the planning area. NINAs tree accounting research was one of the knowledge bases used by the Planning and Building Agency to justify a halt in building permits until the regulation plan could be revised to better guarantee conservation of vegetation. The role of this data in the decision cannot be documented in writing. It is based on personal communications with Oslotree Project staff.

6) **Multi-criteria decision support for spatial priority-setting of nature-based solutions in Oslo** (2020, Venter, Barton, Cimburova). NINA has mapped a series of ecosystem services from urban vegetation in Oslo. These maps were made publicly available in an Urban Nature Atlas. The map layers were used as the basis for two priority-setting applications developed in collaboration with municipal planners: a green roof prioritisation tool which was developed further into an urban tree planting prioritization tool developed in collaboration with the Oslo Trees Project.

5. Sources to corroborate the impact

Informative impact:

1. Urban ecosystem accounting methodology adopted in UN guidance for Ecosystem Accounting (SEEA EA):

United Nations, 2021. System of Environmental-Economic Accounting—Ecosystem Accounting (SEEA EA). White Cover. Available at: https://seea.un.org/ecosystem-accounting.
https://seea.un.org/sites/seea.un.org/files/documents/EA/seea_ea_white_cover_final.pdf
Section 13.6 and Figure 13.4 referring to NINA Oslo's Urban Nature Atlas:
https://nina.earthengine.app/view/urban-nature-atlas

2. Urban tree canopy accounting method adopted by Oslo Municipality

Hanssen, F., Barton, D.N., Nowell, M.S., Cimburova, Z., 2019. Mapping urban tree canopy cover using airborne laser scanning – applications to urban ecosystem accounting for Oslo. NINA Report 1677. Norwegian Institute for Nature Research.

Planning and Building Agency tree cover map for Oslo.
https://experience.arcgis.com/experience/cfda10bc7a8c4649bb8632e38ba4b3d0

Policy design impact:

3. Urban tree canopy mapping supporting municipal BGF performance standards for nature-based solutions

Horvath, P., Barton, D.N., Hauglin, E.A. & Ellefsen, H.W. 2017. Blue-Green Factor (BGF) map-ping in QGIS. User Guide and Documentation - NINA Report 1445. 47 pp.

Standard Norway's Blue Green Factor (2021): https://www.standard.no/fagomrader/bygg-anlegg-og-eiendom/parker-og-grontanlegg/blagronn-faktor/

Oslo's proposed Blue Green Factor norm (2023) (in Norwegian): Saksinnsyn - Plan- og Bygningsetaten, Oslo kommune; in particular:

- Summary of changes to the norm: 2022 12 20 vedlegg 2 - oppsummering av endringer
- Public hearing notes, i.a. provided by NINA: 2021 12 21 vedlegg 5 - høring

4. Valuation of ecosystem services from urban tree canopy supporting performance standards.

Lauwers, L., Barton, D.N., Blumentrath, S., Nowell, M.S., 2017. Accounting for urban trees. Updating the VAT03 Compensation Value Model. NINA Report 1453.

Nollet, A., Barton, D.N., Cimburova, Z., Often, A., 2021. Accounting for amenities and regulating ecosystem services of urban trees. Testing a combined field protocol for VAT19 and i-Tree Eco valuation methods. Report 1948. Norwegian Institute for Nature Research. 86.

Decision-support impact

5. Support for pausing urban densification leading to loss of large trees in Oslo's Small House Planning Area

Oslo trees mapping databases documented in Hanssen et al. (2019, 2021, see above): https://nina.earthengine.app/view/urban-nature-atlas See indicators for >> Tilstand >> Trehøyde(tidsserie)

Oslo municipality revision of the Small House Regulation Plan:
https://www.oslo.kommune.no/slik-bygger-vi-oslo/revisjon-av-smahusplanen/#gref

6. Multi-criteria decision support for spatial priority-setting of nature-based solutions in Oslo

Oslotrees planting strategy MCDA application (beta version) developed together with the Oslo Trees project, based on method in Venter et al. 2021:
tree_planting_prio_beta (earthengine.app).

Personal confirmation of collaboration can be obtained from
hanne.johnsrud@pbe.oslo.kommune.no

NINA_NINA_5

Institution: Norwegian Institute for Nature Research (NINA)
Administrative unit: Norwegian Institute for Nature Research (NINA)
Title of case study: Research on wild goose populations as a knowledge basis for adaptive management
Period when the underpinning research was undertaken: 2013–2022
Period when staff involved in the underpinning research were employed by the submitting institution: 1992–present
Period when the impact occurred: 2014–ongoing

1. Summary of the impact

Most of the European goose populations have increased with corresponding challenges. These extend from (I) agricultural conflicts, (II) impacts on ecosystems, (III) risks to air-safety, to (IV) wildlife management. A knowledge-based management solving these challenges depends on updated information mainly generated from research. An adaptive goose management will hence gain from a close collaboration with researchers. In this respect, NINA's projects have played a significant role not only for goose management, but also for stakeholders and the society. Examples are research on mitigating measures providing knowledge on how to prevent and reduce agriculture damage, both in the short-term (crop protection) and long-term (population regulation, subsidies).

Key researcher NINA: Senior researcher Ingunn M. Tombre (Dr. Scient). Tombre was the project leader of the three Research Council's projects referred to in Box 3 in which the impact is based on.

2. Underpinning research

Case 1. Crop damage and conflict reduction

One of the largest challenges with increasing goose numbers is their preferences for crop and their impact on farmland. Tombre, Prof. J. Elmberg, The University of Kristianstad (S) and Prof. A.D. Fox, Aarhus University (DK), therefore carried out a comprehensive review in 2014–2016 (publised 2017, **Ref.1**) of the scientific basis for how to improve management based on current knowledge, also from "grey" literature. The paper revealed a set of shortcomings in terms of successful effects, and provided further suggestions for improvement of measures implemented on farmland. These were separated in actions at the farmer/farm level and actions at a higher adminstrative level (municipality, region, international). Another paper published the same year (**Ref.2**), in collaboration with Prof. J. Madsen and PhD. C.E. Simonsen (both Aarhus University) demonstrated exactly how difficult it may be, from a single farmer perspective, to solve the goose abundance challenge on cultivated fields (scaring geese off the cropland). This was a case study from Trøndelag conducted in 2012. These papers were a later reference when new mitigation studies were implemented in Norway (testing alternative methods like laser, fences, lethal scaring) all sponsored by the environmental authorities both at the regional and national level. Hence, Ref.1 and Ref.2 were door openers for new and relevant projects.

Case 2. Subsidies as a mitigating measure

A subsidy scheme was established in 2006 in Norway for the Svalbard-breeding goose populations with stopover sites in spring in Mid- and North-Norway. The subsidy reduces the farmers' economic losses. The challenge for the authorities is, however, to distribute the money in accordance with the goose impact (a subsidy scheme does not imply damage inspection as is the case for compensation schemes). In **Ref.3** we (including Prof. Madsen and Dr. M. Bjerrum from Aarhus University in the team) provided suggestions, and an evaluation of the practice, of how to distribute the money based on goose abundances

(based on a model the research group had provided earlier). It turned out that the regional authorities matched the funding and goose abundances quite well (following the model). In **Ref.4** we also demonstrated how important the establishment of the scheme was for successful goose management. The collaboration with a social scientist, Einar Eythórsson from the Norwegian Institute for Cultural Heritage Research, gave in this paper a further insight of the challenges at the interface farmers–managers–wildlife. Results from these studies (in addition to two other damage-quantification studies not referred to here) adjusted the need for the subsidy, and for the need of goose abundance data. In northern Norway this data collection, carried out by NINA, is today important for the implementation of the subsidy (since 2014 onwards, see later).

Case 3. Optimal goose hunting arrangements: a tool to increase the harvest and regulate populations.
Scaring geese, regardless of method, or subsidize the farmers for crop losses, will not necessarily change the challenges in the future. For species with an open hunting season, recreational hunting can be a regulating tool. In Norway, hunting is in fact an important management measure, and in several scientific projects we have demonstrated the advantage of good hunting arrangements if the purpose is to increase the offtake of geese. In **Ref.5**, ten years of data have been collected (2010–2019), in cooperation with a landowner (O. Jerpstad) who arranged the goose hunting among several landowners, the social scientist E. Eythórsson and a student (F. Fredriksen) and an Associate Professor at Nord University Steinkjer. Fewer hunting days, offering safe areas without hunting and skilled goose hunters collectively gave more geese harvested. In parallel with this study, three other papers were produced based on a PhD project (G.H. Jensen, Aarhus University) as well as several technical reports. Hence, "the key to successful hunting", today commonly referred to as the GOOSEHUNT-model, were well known and several initiatives were established not only in the Trøndelag region where the studies were accomplished but also elsewhere in Norway.

Case 4. Adaptive goose management: the need for basic and applied research
The increasing challenges with geese, along with the significant pool of research knowledge (including the references above), have been the fundament for the establishment of several European goose management plans under the AEWA (*Agreement on the Conservation of the African-Eurasian Migratory Waterfowl*) and the Bonn Convention. **Ref. 6** describes the processes with the management plan for pink-footed goose from its implementation in 2012 and beyond. Here, the core group of initiators are authors. In 2016, the European Goose Management Platform, EGMP, was established (https://egmp.aewa.info/), covering four European goose species with eight different populations (whereas five in Norway). Basically, all monitoring data and much of the research from Norway fuel these plans, which follows an adaptive framework including the participation of governmental representatives, national experts (from Norway, I. Tombre, NINA), managers and relevant stakeholders.

3. References to the research

Case 1, Ref.1. Fox, A. D., Elmberg, J., **Tombre, I. M.** & Hessel, R. 2017. Agriculture and herbivorous waterfowl: a review of the scientific basis for improved management. *Biological Reviews* 92: 854–877. http://onlinelibrary.wiley.com/doi/10.1111/brv.12258/epdf

Case 1, Ref.2. Simonsen, C. E., Madsen, J., **Tombre, I. M.** & Nabe-Nielsen, J. 2016. Is it worthwile scaring geese to alleviate damage to crops? – An experimental study. *Journal of Applied Ecology* 53: 916–924. https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.12604

Case 2, Ref.3. Madsen, J., Bjerrum, M. & **Tombre, I. M.** 2014. Regional Management of Farmland Feeding Geese Using an Ecological Prioritization Tool. AMBIO 43: 801–809. http://link.springer.com/article/10.1007/s13280-014-0515-x

Case 2, Ref.4. **Tombre, I. M.**, Eythórsson, E. & Madsen J. 2013. Towards a solution to the goose-agriculture conflict in North Norway, 1988–2012: the interplay between policy, stakeholder influences and goose population dynamics. *PLOS ONE* August 8 (8), e71912, 1 – 7. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0071912

Case 3, Ref.5. Tombre, I. M., Fredriksen, F., Jerpstad, O., Østnes, J. E., Eythórsson, E. 2022. Population control by means of organised hunting effort: Experiences from a voluntary goose hunting arrangement. Ambio 51, 728–742. https://link.springer.com/article/10.1007/s13280-021-01590-2

Case 4, Ref.6. Madsen, J., Williams, J. H., Johnson, F. A., **Tombre, I. M.**, Dereliev, S. & Kuijken, E. 2017. Implementation of the first adaptive management plan for a European migratory waterbird population: The case of the Svalbard pink-footed goose *Anser brachyrhynchus*. *Ambio* 46: 275–289. http://link.springer.com/article/10.1007%2Fs13280-016-0888-0

All the above peer-reviewed publications were funded by The Research Council of Norway (the projects GOOSEHUNT, MIGRAPOP, Geese Beyond Borders) and the Norwegian Environment Agency. One paper, Tombre et al. 2022, were in fact a follow-up from three previous papers (from 2016 and 2017) and is a ten-year study. Hence, impact based on the results started before the final publication date (see later).

4. Details of the impact

Case 1. Crop damage and conflict reduction

Producing a review and scientific papers do not necessarily bring the message out to those gaining from the knowledge without an active outreach plan. In all goose projects where NINA is involved, a set of collaborators with a variety of competence work together, often at a European scale. This may open for an improved communication with end-users as scientific news may be published at several institutions' webpages. It is, however, not a guarantee for end-users to catch up the news or even implement it. Most of the goose project carried out in NINA have involved stakeholders, often with a reference group linked to the project, lowering the threshold for personal and informal contact (open evening meetings, workshops) with relevant stakeholder groups (bird watchers, conservationists, hunters), managers, and other segments of the society (including journalists). The close collaboration with the Norwegian Farmers Union developed over the years in the research projects has also opened more doors and possibilities to have an impact at an even wider scale. In addition to presentations and communication at local meetings (up to ten meetings on average in Norway, annually over the years 2014–2021), NINA has contributed to three handbooks, where two are related to Case 1 but are still in the printing and quality check phase at the Farmers Union, and one related to Case 3. One of the handbooks, **S1**, are aimed for farmers, informing about the various tools available for goose damage prevention and reduction and their effects under various conditions. All the information is based on scientific results, where NINA is a significant contribution in addition to assisting in the writing and editing of the handbook. The other handbook, **S2**, provides an overview of the content that will be necessary when local (municipality) and regional (county) managers are about to develop local and regional goose management plans following suggested mitigating tools for the agriculture. The identification of useful tools for goose management was also included in a regional management plan, **S3**, where NINA actively participated in the phase of development.

Case 2. Subsidies as a mitigating measure

A tailored subsidy implementation is challenging, but the close cooperation between managers and researchers in the two counties where there is a subsidy scheme for goose damage (for the Svalbard-breeding populations of pink-footed goose *Anser brachyrhynchus* and barnacle

goose *Branta leucopsis*) has facilitated the practice. In Trøndelag, the County Governor use a goose abundance map based on a model developed and tested (**Ref.3**) and has also established a so-called *Collaboration Forum for Geese* (in 2021) where the most affected farmers and managers at the involved municipalities are members. Here NINA is an active partner (in fact the initiator), facilitating the information flow from updated research related to the subsidy scheme etc., **S4**. In the county of Nordland, a corresponding forum has existed since 2016, based on the same premises as in Trøndelag, **S5**. Here, the annual goose abundance assessments, where NINA actively contributes, is a basis for the subsidy at the farm level, **S6**.

Case3. Optimal goose hunting arrangements: a tool to increase the harvest and regulate populations.
Based on the results from several goose hunting projects, and specifically **Ref.5** including the years when it was developed, the GOOSEHUNT-model has been widely announced and incorporated in several hunting and landowner-associations. Several hunting magazines have presented the model, and also the Farmers Union present the practice for their members, as most of the goose hunting in Norway occurs at private land where landowners are the main licence holders. The hunting model is described in the third handbook for goose hunting developed by the Norwegian Farmers Union, **S7**, and in the municipality of Levanger, Trøndelag, the local hunting association has included it in their goose hunting courses they arrange for new hunters, **S8**. Levanger hunting association also use the method at their own hunting fields for members. Over the last ten years, the number of geese harvested in Norway has increased, and an apparent need for a better knowledge of how to handle all the geese harvested has emerged. We have taken this opportunity, as an outreach and a promotion of the complete “goose value chain”, to produce a crossover book. This is a book presenting a number of goose-meat receipts, added with small boxes of information about the geese ranging from their ecology, geese in the culture and history, agriculture damage to conflict aspects as well as hunting methods, all based on our previous research: “Gås og Gourmet” (*Goose and Gourmet*), **S9**. Two master chefs (E. Ramnestedt and L.E. Vesterdal) have created the receipts, a non-fiction author (K. Blom) has led the text quality development, a Farmers Union member (O.M. Gundersen) added the perspectives from farmers and hunters, as well as two researchers representing goose hunting experiences and crop damage (J.J. Aarseth) and cultural and historical aspects (S.B. Holmgaard). The book is published by Orkana Forlag, and the recent sale figures are more than 400 books sold the first month on sale. This outreach project started in 2019, but due to the pandemic it was postponed, and the final book was released in late 2022. Although this is beyond the period referred to in this evaluation, it is still included as the whole implementation process started earlier and final release date could not be earlier.

Case 4. Adaptive goose management: the need for basic and applied research
In the European Goose Management platform, EGMP, **S10**, NINA has a national expert involved(I.Tombre), being a member of the “Modelling Consortium”, “The Communication Group”, all the task forces (five) and coordinate the Agriculture Task Force. EGMP’s main objective is *to provide the mechanisms for a structured, coordinated and inclusive decision-making and implementation process for the sustainable use and management of goose populations in Europe, with the objective of maintaining them at a favourable conservation status, while taking into account concerns of relevant stakeholders and the pertinent legislative frameworks and regulations*. The platform has today more than hundred persons from 16 Range States actively involved, and eight interest organisations (e.g., Wetlands International, Birdlife International, EU Farmers, FACE) and all the model development and plan implementations are based on up-to-date figures from the various populations. Hence, NINA/Norway’s annual goose monitoring and relevant project results (see **Ref.6**) fuel the adaptive processes in this platform. All data, scripts, and sources are open access at the webpage (https://egmp.aewa.info/).

5. Sources to corroborate the impact (indicative maximum of ten references)

Case 1, S1 Handbook for farmers: "*Hvordan forebygge beiteskader av gjes*s» (How to prevent crop damage cause by geese). In print and quality check within the Norwegian Farmers Union, reference: Finn Erlend Ødegård finn.erlend.odegard@bondelaget.no

Case 1, S2 Handbook for wildlife managers: "*Forvaltningsplaner for gås. En veileder*." (Management plans for geese, a handbook.) In print and quality check within the Norwegian Farmers Union, reference: Finn Erlend Ødegård finn.erlend.odegard@bondelaget.no

Case 1, S3 Regional management plan: "Forvaltningsplan for grågås i Nordland, 2019–2025» (Management plan for greylag goose in Nordland, 2019–2025).
https://www.statsforvalteren.no/contentassets/e6872a8fd95046359d3822d5a03338a8/forvaltningsplan-for-gragas-i-nordland.pdf

Case 2, S4
Collaboration Forum for Geese, Trøndelag
https://www.statsforvalteren.no/nb/Trondelag/Landbruk-og-reindrift/Nyheter-landbruk-og-mat/2021/11/har-etablert-samarbeidsforum-for-gas/

Case 2, S5
Collaboration Forum for Geese, Nordland.
https://www.nina.no/apps/evalbiovit/NINA%20Impact_5_Case2_S5_Collaboration%20Nordland.pdf

Case 2, S6
The practice of the goose subsidy scheme in Nordland
https://www.sortland.kommune.no/tjenester/okonomi-naring-og-bevilling/landbruk/miljo-og-okologisk/gas/

Case 3, S7
Handbook for how to arrange optimal goose hunting
https://nettbutikk.bondelaget.no/files/norgesbondelag/Documents/Vedlegg/Veileder%20-%20Jaktomr%C3%A5der%20for%20g%C3%A5s.pdf

Case 3, S8
Local goose hunting course, Levanger municipality
https://www.njff.no/nord-trondelag/levanger/jakt?accordion_section=4-1&#accordion_section=4-1

Case 3, S9
Crossover book; receipts and goose ecology etc.
https://www.orkana.no/produkt/gas-og-gourmet/

Case 4
The European Goose Management Platform https://egmp.aewa.info/

Biovit 1

Institution: Norwegian University of Life Sciences (NMBU)
Administrative unit: Faculty of Biosciences (Biovit)
Title of case study: Foods of Norway- novel protein sources for farmed animals
Period when the underpinning research was undertaken: 2016-2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2015-2022
Period when the impact occurred:2021-2022

1. Summary of the impact

Foods of Norway has successfully developed novel protein sources for farmed animals (Atlantic salmon, pigs, broiler chicken, and dairy cows) based on bio-conversion of forest by-products (cellulose and hemicellulose) into microbial ingredients like yeast. Our research was pioneering in Norway in circular economy based on up-cycling of local side streams to valuable products. The research has reached out to the scientific community, aquaculture, forestry and agricultural industries, and society at large. The work addresses our government strategy that all aquafeeds should come from sustainable sources by 2030. It also has global relevance since the technology can be implemented elsewhere.

2. Underpinning research

The research from Foods of Norway has proven that is possible to produce high-quality protein sources such as yeast from Norwegian forest biomass and chicken hydrolysates. These novel feed resources can be used in diets for Atlantic salmon, piglets, broiler chickens and dairy cows. They can therefore serve as sustainable local alternatives to imported plant ingredients such as soybean meal. This will increase our feed and food security and reduce the environmental impact of food products from farmed animals and fish.

The research was carried out from 2016 to 2022 and is still ongoing. First, it was important to screen different yeast species that could grow well on the feedstocks based on sugar from spruce and chicken hydrolysate. This made it possible to choose the best species for further research. It was also important to optimize fermentation conditions to produce yeast with high yield and nutritional quality, especially regarding the protein content. After the yeast is harvested, the yeast goes throw a down-stream process. That was optimized to increase nutrient digestibility and health-beneficial properties of the yeast. Then, the yeast was chemically characterized and evaluated in a series of experiments with Atlantic salmon, piglets and broiler chickens as well as dairy cows with focus on growth performance, milk yield (dairy cows), health and product quality of the fish, meat and milk products. Further, a techno-economic analysis of 2nd generation sugar for production of microbial ingredients was performed. The environmental impact of the yeast production will be evaluated based on a field trial with Atlantic salmon and pigs.

The work was carried out by Dr Liv Torunn Mydland (researcher, 2016-2022), Dr. Jon Øvrum Hansen (researcher, 2016-2018), Dr Jeleel Agboola (PhD student, 2019-2021), , Dr. Byron Morales Lange (researcher, 2020), Dr. Sergio Rocha (researcher, 2020), Dr. Anna Cruz (PhD student, 2017-2020), Dr Ingrid Marie Håkenåsen (PhD student, 2018-2022), Dr. Leidy Lagos (researcher, 2019-2021), Dr. Brankica Djordjevic, (2017-2019), Dr. Hanne Fjerdingby Olsen (Researcher 2018-2022), and Prof. Margareth Øverland (Professor, 2016-2022).

The research provides important foundation for developing local feed resources and is of high relevance for the aquacultural and agricultural industries, as well as for food security in Norway. The outcome of the project will help develop a circular economy in Norway.

3. References to the research

Agboola, J.O., Mensah, D.D., Hansen, J.Ø., Lapeña, D., Mydland, L.T., Arntzen, M.Ø., Horn, S.J., Øyås, O., Press, C.M., Øverland, M. (2022). Effects of Yeast Species and Processing on Intestinal Health and Transcriptomic Profiles of Atlantic Salmon (*Salmo salar*) Fed Soybean Meal-Based Diets in Seawater. International journal of molecular sciences, *23*(3), 1675. https://doi.org/10.3390/ijms23031675

Agboola, J. O., Lapena˜, D, Øverland M., Øverlie MA, Mydland, LT., Hansen. J. Ø. (2022). Yeast as a novel protein source - Effect of species and autolysis on protein and amino acid digestibility in Atlantic salmon (Salmo salar). Aquaculture 546, 737312, https://doi.org/10.1016/j.aquaculture.2021.737312

Hansen, J. Ø., Lagos, L., Lei, P., Reveco-Urzua, F. E., Morales-Lange, B., Hansen, L. D., Schiavone, M., Mydland, L. T., Arntzen, M. O., Mercado, L., Benicio, R. T., & Øverland, M. (2021). Down-stream processing of baker's yeast (*Saccharomyces cerevisiae*) - Effect on nutrient digestibility and immune response in Atlantic salmon (*Salmo salar*). Aquaculture, 530, 735707 https://doi.org/10.1016/j.aquaculture.2020.735707

Morales-Lange, B., Agboola, J.O., Hansen, J.Ø. Lagos, L., Øyås, O., Mercado, L., Mydland, L.T., & Øverland, M. (2021). The spleen as a target to characterize immunomodulatory effects of down-stream processed *Cyberlindnera jadinii* yeasts in Atlantic salmon exposed to a dietary soybean meal challenge. Frontiers in Immunology, 12, 3345. https://doi.org/10.3389/fimmu.2021.708747

Cruz, A., Tauson, A.-H., Matthiesen, C. F., Mydland, L. T., & Øverland, M. (2020). *Cyberlindnera jadinii* yeast as a protein source for growing pigs: Effects on protein and energy metabolism. Livestock Science, 231. https://doi.org/10.1016/j.livsci.2019.103855

Håkenåsen, I.M., Øverland, M., Ånestad, R., Åkesson, C.P., Sundaram, A.Y.M., Press, C.M., Mydland, L.T. (2020). Gene expression and gastrointestinal function is altered in piglet small intestine by weaning and inclusion of Cyberlindnera jadinii yeast as a protein source. Journal of Functional Foods 73, 104118. https://doi.org/10.1016/j.jff.2020.104118

4. Details of the impact

Results have shown that the novel feed ingredients meet important criteria for feed ingredient development, including nutritional value, palatability, health effects, product quality, technical feed quality, and environmental impact.

The research has established an integrated knowledge platform to get a deeper knowledge on how novel ingredients such as yeast affect growth performance, health and product quality of fish and farmed animals. All this information is essential for a possible up-scaling and commercialization. Because the research is still on-going, the final impact remains to be seen. The described impact has occurred from 2019 until January 2023.

The research outcome provides new technology for producing microbial ingredients in Norway and it provides an important foundation for up-scaling and commercial use of local natural resources. The technology also has global implication since it can be implemented anywhere where suitable natural resources and infrastructure are available for producing industrial scale feed resources.

The work was done in close collaboration with industrial partners in Foods of Norway from blue and green sectors, which taken together have expertise along the value chain from forest biomass to the final fish, meat, and dairy products. The close and integrated work carried out with industrial partners during the centre period allows for rapid transfer of knowledge. The industry partners were included during the planning, discussion, and interpretation of results from the experiments: Final results were presented either in smaller meetings with industry partners or at annual meetings with all partners in the Foods of Norway centre.
The work was also partially carried out with an industrial PhD who was employed by the Feed company Felleskjøpet Fôrutvikling. This facilitated a close collaboration between academia and industry. The PhD candidate is now an employee by the company.

Foods of Norway has also presented the results to a broad audience, including scientific meetings world-wide. Examples are:

- International Symposium Mucosal Health in Aquaculture 2019, 2022
- Fish & Shellfish Immunology, 2019, 2022
- International Symposium on Fish Nutrition and Feeding (ISFNF), 2022
- Aquaculture Europa, 2019 and 2022.

Results have also been presented in many popular science channels. Examples are daily newspapers, Forskning.no, Landbruk 24, Teknisk Ukeblad, and several national and international web-based media channels as well as national TV and radio programs. The results have also been presented at many meetings and seminars with NGOs, politicians and society at large.

The work was carried out in close collaboration with to other faculties at NMBU, Faculty of Chemistry, Biotechnology and Food Science (KBM) and Faculty of Veterinary Medicine (Vet). KBM was responsible for the fermentation of the yeast, while Biovit and KBM collaborated on the down-stream processing of yeast. The health effects, especially histology and immunohistochemistry were carried out by Vet. The work was also done in close collaboration with several industrial partners in the Centre, this included Borregaard, Lallemand, Norilia, Biomar and Felleskjøpet. Based on the result obtained in the centre, the yeast production was upscaled to 1600 kg. e Borregaard produced several 1000 liters of sugars from Norwegian spruce trees, this was shipped to Lallemand's commercial plant in Estonia, where the upscaling took place. The yeast was then used to produce salmon feed by Biomar and NMBU for a large field trial with salmon in Norway where we evaluated effect on growth performance and health responses during grow-out stages in sea cages. The yeast was also used to produce piglet feed for a large field trial carried out by Felleskjøpet Fôrutvikling and NMBU. These experiments provided important information how the novel feeds affect the growth, health and product quality of fish and piglets under practical conditions. LCA model was develop in collaboration with NORSUS.

The feed industry, meat producers, milk producers, farmers can all benefit from the research, especially the partners in Foods of Norway that were involved with the research. Companies like Viken skog, as a provider of forest side streams; the forest biorefinery company, Borregaard as a provider of sugar streams from forest biomass; the fermentation company Lallemand as a provider of yeast; the feed companies from blue and green sector, Felleskjøpet Fôrutvikling and Biomar as users of the novel feed ingredients in their feed manufacture, and the end users, including TINE (milk producer), and Nortura (meat producer).

The outcome of the research has led to new methods for feed development and a knowledge platform on novel feed development and documentation that is essential for up-scaling and commercialization.

5. Sources to corroborate the impact

Scientific review papers:

Øverland, M., A. Skrede. 2017. Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. Journal of the Science of Food and Agriculture. DOI: 10.1002/jsfa.8007

Albrektsen, S., Kortet, R., Skov, P. V., Ytteborg, E., Gitlesen, S., Kleinegris, D., Mydland, L. T., Hansen, J. Ø., Lock, E-J., Mørkøre, T., James, P., Wang, X., Whitaker, R. D., Vang, B., Hatlen, B., Daneshvar, E., Bhatnagar, A., Jensen, L. B., Øverland, M., 2022. Future feed resources in sustainable salmonid production: A review. Reviews in Aquaculture. DOI: 10.1111/raq.12673

Agboola J., Chikwati E. M., Hansen, J.Ø. Kortner T. M. Mydland LT., Krogdahl, Å Djordjevic B., Schrama J W., Øverland, M. 2022. A meta-analysis to determine factors associated with the severity of enteritis in Atlantic salmon (Salmo salar) fed soybean meal-based diets. April 2022. Aquaculture 555:738214. https://doi.org/10.1016/j.aquaculture.2022.738214

Agboola, J. O., Schiavone, M., Øverland M., Morales-Lange, B., Leidy Lagos[1], Arntzen, M. Ø., Lapeña, D., Eijsink, V. G. H., Horn, S. J., François, J. M., Mercado, L., and Hansen, J. Ø.. 2021. Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. Reviews in Aquaculture, 2020, 13, 949–970. DOI: 10.1111/raq.12507

Media articles:

Norway launches expriment on feeding pigs and salmon
(07.10.2021 fishfocus.co.uk)
Growing yeast for feed from Norwegian spruce trees
(30.09.2021 allaboutfeed.net)
Growing yeast from trees – first successful scale-up of microbial feed ingredients from sustainable resources
(13.09.2021 feedplanetmagazine.com)

BIOVIT 2

Institution: Norwegian University of Life Sciences (NMBU)
Administrative unit: Faculty of Biosciences (Biovit)
Title of case study: Towards genomic selection in practical cattle, pig, sheep, goat and salmon breeding schemes.
Period when the underpinning research was undertaken: 2011-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021
Period when the impact occurred: 2014-ongoing

1. Summary of the impact

By 2010, the prerequisites for genomic selection were in place for most livestock species, namely a dense genome-wide panel of genetic markers, cost-effective high-throughput genotyping methodologies, and the theory of genomic selection. Here, we briefly describe the underpinning research during the past 10 years at BIOVIT that enabled the implementation of genomic selection in practical breeding schemes for all the major livestock species, including salmon. Impacts came in the form of an increased genetic improvement of production animals, more sustainable animal production and increased (inter)national sales. These impacts benefitted the breeding industries, farmers and ultimately consumers.

2. Underpinning research

The theoretical principles of genomic selection were laid down in the original paper by Meuwissen et al. (2001; Genetics 157). Genomic selection is the selection of animals based on genome-wide genomics data. The genomics data are here dense marker genotypes, whose large-scale availability is a prerequisite for genomic selection. Large-scale detection of Single-Nucleotide-Polymorphism (SNP) markers came as a by-product of the genome sequencing efforts of livestock species. The SNP-chip genotyping technology proved a cost-effective platform for the genotyping of thousands of animals for ~50k SNPs. For cattle, these prerequisites were first available at around 2007/2008, and other livestock species followed shortly after. This triggered the need for research underpinning the practical implementation of genomic selection in Norwegian breeding schemes for dairy cattle, pigs, sheep, goats and salmon. All these breeding schemes are currently using genomic selection on a daily basis for the genetic improvement of their animals.

The main research questions to underpin the practical application of genomic selection were:

1) How to design a breeding scheme that makes best use of genomic selection? Genomic selection makes the accurate selection of some groups of animals possible, but which animals should be genotyped for it to be cost-effective?
2) What happens with genetic diversity and inbreeding in large scale genomic selection breeding schemes? Should inbreeding be managed by genomic information in genomic selection schemes? If genomic selection schemes run quickly out of genetic diversity and/or move the population towards completely inbred, any possible short-term gains will be lost in the longer term.
3) How to perform the computations for the genomic prediction of breeding values in large national scale breeding schemes? The breeding value estimation approach called GBLUP was used, since it's the computationally the lightest. But still, it needed continuous algorithmic improvements in order keep up with the ever-increasing numbers of genotyped animals. Alternative Bayesian methods are computationally even heavier but yield also somewhat more accurate predictions.

4) Although more and more animals are genotyped, still most animals remain ungenotyped. It is crucial for the accuracy of genomic prediction to also use their information. For methods like GBLUP, which use genomic relationship matrices (GRM), this implies the extension of the GRM with (traditional) pedigree-based relationships. But how to do this and how to deal with unknown pedigrees, and how to avoid any biases when using such completely different estimates of relationships side-by-side? Are there alternatives to the GRM based approach?
5) Genomic selection relies on a reference population that is trait-recorded and genotyped, for the estimation of the marker effects. International large breeds, such as Holsteins, have huge reference populations. Can small breeds benefit from SNP effects estimated in these huge reference populations, i.e., can we predict across breeds? Accurate predictions over large genetic distances are very beneficial, e.g., estimate SNP effects in large US growing-pig herds and use them to select elite Norwegian Landrace breeding pigs.

Names of the key researchers and what positions they held at the administrative unit at the time of the research (where researchers joined or left the administrative unit during this time, these dates must also be stated).

The work was carried out by Prof. Theo Meuwissen (2011-2021); Prof. Bjørg Heringstad (2011-2021); Prof. Peer Berg (2016-2021); Researcher Xijiang Yu (2011-2021); Researcher Tu Luan (2011-2021); PhD-student, Kahsay Nirea (2011-2016); PhD-student, Binyam Dagnachew (2013-2016); Researcher, Tesfaye Belay (2018-2021); PhD-student 2014-2017, PostDoc 2018-2021) Sini Wallen (2014-2021); PhD-student, Rajesh Joshi (2015-2018); PhD-student Maja Iversen Winther (2015-2019); PhD-student Oscar Iheshuilor (2012-2016); PhD-student Maria Kjetså (2016-2021); Associate professor Jørgen Ødegård (2018-2021); PhD-student Vinay Nannuru (2020-2021)

3. References to the research

Belay TK, Eikje LS, Gjuvsland AB, Nordbø Ø, Tribout T, Meuwissen T. (2022) Correcting for base-population differences and unknown parent groups in single-step genomic predictions of Norwegian Red cattle. J Anim Sci. 100: skac227. Correcting for base-population differences and unknown parent groups in single-step genomic predictions of Norwegian Red cattle | Journal of Animal Science | Oxford Academic (doi: 10.1093/jas/skac227)

Lillehammer M, Meuwissen THE, Sonesson AK. (2011) A comparison of dairy cattle breeding designs that use genomic selection. J Dairy Sci. 94:493-500. A comparison of dairy cattle breeding designs that use genomic selection - PubMed (nih.gov).
(doi: 10.3168/jds.2010-3518)

Meuwissen THE, Indahl UG, Ødegård J. (2017) Variable selection models for genomic selection using whole-genome sequence data and singular value decomposition. Variable selection models for genomic selection using whole-genome sequence data and singular value decomposition - PubMed (nih.gov)
(doi: 10.1186/s12711-017-0369-3)

Meuwissen T, van den Berg I, Goddard M. (2021) On the use of whole-genome sequence data for across-breed genomic prediction and fine-scale mapping of QTL. On the use of whole-genome sequence data for across-breed genomic prediction and fine-scale mapping of QTL - PubMed (nih.gov).
(doi: 10.1186/s12711-021-00607-4)

Meuwissen THE, Sonesson AK, Gebregiwergis G, Woolliams JA. (2020) Management of Genetic Diversity in the Era of Genomics. Front Genet. 11:880. Frontiers | Management of Genetic Diversity in the Era of Genomics (frontiersin.org)

(doi: 10.3389/fgene.2020.00880)

Ødegård J, Indahl U, Strandén I, Meuwissen THE.(2018) Large-scale genomic prediction using singular value decomposition of the genotype matrix. Large-scale genomic prediction using singular value decomposition of the genotype matrix | Genetics Selection Evolution | Full Text (biomedcentral.com)
(doi: 10.1186/s12711-018-0373-2)

4. Details of the impact

The underpinning research at BIOVIT (Section 2) enabled the introduction of Genomic selection by Norsvin in pig breeding in 2014, by Aquagen in salmon breeding in 2015, by GENO in cattle breeding in 2016, by NSG in sheep breeding in 2020 and in goats in 2022. The salmon breeders also introduced marker-assisted-selection for a (single) major IPN resistance gene, which made their stocks basically resistant to the IPN virus. The impacts of the introduction of genomic selection on the genetic improvement of production animals are:

- Higher rates of genetic improvement, but these are difficult to document in practical populations where selection is for a broad breeding goal and selection pressures for individual traits vary over time. Improvements in the accuracy of selection of young animals were on average 40% across the traits and species.
- More sustainable breeding goals. The increased accuracy and thus easier improvement of production traits left more room for selection on sustainability traits, such as disease resistance, reproductive and welfare traits.
- Increased sales of semen on (inter)national markets, and increased incomes.
-

Inputs to these impacts by the underpinning research were (following the numbering of section 2):

1) Large-scale computer simulation studies of genomically selected populations were conducted to study genomic selection in dairy cattle, pig, salmon and sheep breeding schemes. In dairy cattle genomic selection increased genetic improvement by 30%, which required replacing the traditional progeny testing scheme (Lillehammer et al., 2011). But this also increased inbreeding, and it was concluded that the number of selected bulls had to increase in a genomic selection scheme in order to keep inbreeding under control. Simulations showed that 100% accuracy could be achieved in salmon sib-testing schemes, which are used to improve disease resistance.
2) Classic inbreeding theory states that identity-by-descent causes both inbreeding and genetic drift, which are thus indistinguishable. Contrary to this classic theory we found that genomic tools which manage homozygosity (inbreeding) cause considerable genetic drift, and *vice-versa* (Meuwissen et al., 2020). We concluded that genomic tools should manage identity-by-descent in order to manage both rates of inbreeding and genetic drift.
3) The most computationally costly step in GBLUP is the inversion of the genomic relationship matrix (GRM). Inverting a GRM with more than 100-200k animals is very challenging. We reduced the dimensionality of the genotypes by a chromosome-wise singular value decomposition,and used the resulting singular components to compute the inverse of the GRM, or directly perform random regression on the singular components to predict breeding values (Ødegård et al., 2018). This

approach was extended to reduce computations in Bayesian variable selection predictions (Meuwissen et al., 2017).

4) The single step method extends the GBLUP method to include non-genotyped animals, which is based on augmenting the genomic relationship matrix with pedigree relationships for non-genotyped animals. One problem is that relationships are always relative to a base population, where relationship recording started. However, genomic and pedigree base populations may be very different. In addition, pedigrees are often incomplete, which is resolved by having several base populations. Belay et al. (2022) solved this by estimating the differences between all these base populations in the genomic prediction model.
5) Genomic prediction over large genetic distances, e.g., across lines/breeds, is expected to require increased marker densities, such as ~500k SNPs or whole genome sequence data. To differentiate between all these SNPs we need a Bayesian variable selection method that can handle huge data sets (in numbers of SNPs and genotyped individuals). Meuwissen et al. (2021) presented a computationally fast implementation of variable selection genomic prediction, that handles whole-genome sequence data on more than 35,000 individuals, but improvements in accuracy of prediction were marginal at 3%. The method proved however powerful in identifying major genes with high mapping precision.

Future research will be needed to reap the benefits from whole-genome sequence data, including large scale identification of causal mutations, developing genomic methods for identity-by-descent based management of genetic diversity, and the inclusion of gene-editing in genetic improvement programs.

The beneficiaries of the impact are firstly breeding organizations in Norway and internationally, secondly the farmers who benefit from the genetically improved stocks, and thirdly the consumers who benefit from more cost-effective and sustainably produced animal products. In addition, increased viability of the animal production sector will support livelihoods in rural parts of Norway. Many national and international collaborations have contributed to this research, including but not limited to the research organizations: Nofima, the Universities of Melbourne, Edinburgh and Wageningen, and the breeding industries: Geno, Tine, Norsvin, NSG, Aquagen, Mowi and Benchmark.

5. Sources to corroborate the impact (indicative maximum of ten references)

2016 - John J. Carty Award by the American National Academy of Sciences to Mike Goddard and Theo Meuwissen (agricultural sciences), For *the development of genomic selection - uniting quantitative genetic theory with genomics technology - revolutionizing the genetic improvement of livestock and crops. Their research also invigorated genomic prediction, which has far ranging implications for fields from human medicine to conservation biology*. (en.wikipedia.org/wiki/John_J._Carty_Award_for_the_Advancement_of_Science#Recipients)

Video from Aquagen : https://www.youtube.com/watch?v=bUVL2yNSKSM

Video/webinar from Geno : https://www.youtube.com/watch?v=omOhguvLeWg

Video from NSG: https://www.youtube.com/watch?v=WFP489JsUSo

Plant breeding: Graminor: https://graminor.no/havreforedling-i-den-genetiske-tidsalder/

Impacts of GS: https://www.kyst.no/aquagen-avl-ik/sa-stor-effekt-har-elleve-generasjoner-med-avl-hatt/635185

https://lactanet.ca/en/impact-genomics/

https://www.geno.no/nyheter/hvilken-verdi-gir-genotyping-av-seminokseemner/

Biovit 3

Institution: Norwegian University of Life Sciences (NMBU)
Administrative unit: Faculty of Biosciences (Biovit)
Title of case study: Optical radiation - plant protection against fungal pest diseases
Period when the underpinning research was undertaken: 2008-2016
Period when staff involved in the underpinning research were employed by the submitting institution: 2008- 2016 (still ongoing with optimization and efficacy improvements)
Period when the impact occurred: Practical impact with field application started in 2011

1. Summary of the impact

Research at BIOVIT during the past 10 years has developed optical radiation as an efficient alternative to fungicides for controlling fungal diseases on crop plants. Environmentally friendly pest management solutions are needed to replace synthetic fungicides, which are increasingly being phased out world-wide. Combining UV light radiation with specific wavelengths have been implemented by the horticultural industry under greenhouse and field conditions in several countries. The integration of the technology on the Thorvald autonomous robotic platform, developed by the robotics group at NMBU (https://sagarobotics.com/crops/), made the technology available for practical usage in several countries, i.e., UK, US and Norway.

2. Underpinning research

Powdery mildews are among the most widespread and destructive fungal pathogens of plants. Their capacity to cause loss and develop resistance against fungicides make them very difficult to control when crops are grown in controlled environments such as greenhouses and table-top tunnel systems.

Powdery mildews lack pigmentation that can protect against UV light; however, they can still repair DNA damages caused by UV by photoreactivation and light-driven photolyases. Work by Dr. Suthaparan and his group provided a novel means to suppress diverse powdery mildews of rose, cucumber, tomato, strawberry, grape, rosemary and aster with supplemental UV light applied during night hours, thereby circumventing photoreactivation. This discovery not only enhanced the efficacy of UV treatments in suppressing diverse powdery mildews; it allowed the use of a lower UV dose that reduced the risk of UV-induced phytotoxicity to the host plant.

The work in Dr. Suthaparan's group subsequently elucidated the photobiochemical and genetic underpinnings of how powdery mildews survive in a daytime environment bathed in UV light. Both blue light and UVA decreased the efficacy of a given dose of UV, presumably because they upregulate the systems that repair UV damage to pathogen DNA. Red light, which itself had been shown to suppress conidiation, was synergistically effective in enhancing the suppression of powdery mildew by UV when applied after UV treatments during nighttime hours. Wavelengths across the range from UVC and UVB to UVA (250 to 400 nm) that were suited to suppress growth of powdery mildews while minimizing damage to the host due to phytotoxicity were identified. This finding provided valuable target parameters for light emitting diode manufacturers seeking to produce LED products that optimize this technology for crop production. The group demonstrated how optimal UV doses were related to prior daily light integrals (Suthaparan *et al.* 2017), and this provided the theoretical as well as practical basis to match UV dose in both field and controlled environments based upon preconditioning of light-mediated DNA repair. The potential lighting strategy, with the possibility of extending the day length by selection and combination of optimal wavelengths within the optical radiation range for the best disease suppressive effect was published in 2018 (Suthaparan *et al.* 2018).

This work was carried out by Aruppillai Suthaparan, researcher (2008-2022); Hans Ragnar Gislerød, professor (2008-2016); Arne Stensvand, professor (2017-2021); Ranjana Pathak, PhD student (2016-2021).

3. References to the research

Suthaparan, A., Pathak, R., Solhaug, K. A., and Gislerød, H. R. Wavelength dependent recovery of UV-mediated damage: Tying up the loose ends of optical based powdery mildew management, 2018, J. Photoch. Photobio. B. 178: 631-640. https://doi.org/10.1016/j.jphotobiol.2017.12.018

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Suthaparan, A., Stensvand, A., Solhaug, K.A., Torre, S., Telfer, K.H., Ruud, A.K., Mortensen, L.M., Gadoury, D.M., Seem, R.C., and Gislerød, H.R. Suppression of cucumber powdery mildew by supplemental UV-B radiation in greenhouses can be augmented or reduced by background radiation quality, 2014, Plant Dis. 98:1349-1357. https://doi.org/10.1094/PDIS-03-13-0222-RE

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3. Details of the impact

The research started when Dr. Suthaparan was a PhD student with Prof. Hans Ragnar Gislerød as supervisor. They worked on a project to use high amounts of light to develop all-year greenhouse production of roses in Norway. During this research it was discovered that powdery mildew was suppressed by high light. Following this discovery, which was published in 2010, further research into testing optical treatment against fungal diseases, especially powdery mildew, was initiated. The research showed that UV light applied during the night can suppress the powdery mildew fungus on several plant species, and that this an alternative non-chemical solution for combatting these diseases. This is especially important since resistance development against fungicides is a big problem for growers.

Several RCN projects with active financial and in-kind support from the horticultural industry have been conducted and several is on-going with Dr. Suthaparan as project leader. Since this research has been supported actively by the industry, the findings were quickly disseminated to the growers. Many articles were published in user-oriented publications, like 'Gartneryrket', the most important horticultural magazine in Norway, Onder Glas, Netherlands, Greenhouse Canada etc. and workshops. The integration of the UV equipment on the autonomous robot Thorvald (https://sagarobotics.com/crops/strawberries/) has been pivotal for practical use of the

technology both under controlled conditions in greenhouses and tunnels, and in fields. This opened great commercial value for these findings and has been widely used in commercial applications in strawberry production systems in Norway (Myhrene Gartneri, see https://gartnerhallen.no/nb/aktuelt/bruker-uv-lys-til-a-forebygge-plantesykdommer/), in California and Florida in the US (the biggest strawberry producers) and in the UK. In addition, cucumber producers in Norway and grape producers in USA adopted the system in commercial practise (see section 4). The research also opened the market value for agricultural robotics for field applications worldwide, with Thorvald robot developed and marketed by the Norwegian company Saga Robotics, which is a spin-off from the robotics group at NMBU, as a good example.

International collaboration has been important for development of this technology. The collaboration has involved NMBU and the Norwegian Institute of Bioeconomy Research (NIBIO) in Norway, and Cornell University, University of Florida, USDA-ARS, and the Lighting Research Center at Rensselaer Polytechnic Institute (https://www.lrc.rpi.edu/) in USA.

Dr. Suthaparan is a leading member of a transdisciplinary team that is now applying the work across a broad range of crops and growing environments, with support of current grants from the Research Council of Norway (RCN), and earlier grants from USDA-SCRI (2014-51181-22381 and 2014-511181-22377), and from the USDA Organic Research and Extension Initiative (2015-51300-24135). This level of support from funding agencies on two continents, spanning the range from highly applied to fundamental, is a good indication of the high regard in which this work is held, and the perceived potential of the findings to lead to entirely new approaches to the suppression of plant diseases through the manipulation of their natural relationships and responses to light.

Even though practically successful, there are still challenges that must be solved for a wide range of applications. Currently, three projects funded by RCN to address these issues with international collaborators at Wageningen University and East China Normal University are ongoing. Outcomes of these projects will expand the potential of optical based management for postharvest diseases like gray mold (*Botrytis cinera*) and some important pests like spider mites.

4. Sources to corroborate the impact

Dr. Suthaparan received the 2018 William Boright Hewitt and Maybelle Ball Hewitt Award for pioneering research on the use of UV and visible light to suppress powdery mildews.

https://www.apsnet.org/members/give-awards/awards/Hewitt/Pages/SuthaparanAruppillai.aspx

https://gartneryrket.no/uncategorized/prestisjefylt-pris-tildelt-planteforsker-aruppillai-suthaparan/

His work was the inspiration for the 2018 ICPP concurrent session "*Why Light Matters: New Concepts, Tools, and Practices and Enhanced Plant Health*" (https://www.apsnet.org/meetings/annual/meetingarchives/ICPP2018/Documents/ICPP2018Program.pdf)

Dr. David Gadoury at Cornell University, US, has been an important collaborator during the practical testing and implementation of the technology in the strawberry and grapevine industry in the US. This also involves integration with autonomous robots.

Some publications by Dr. Gadoury corroborating the importance the discovery made by Dr. Suthaparan:

- The Potential of Light Treatments to Suppress Certain Plant Pathogens and Pests (https://sagarobotics.com/wp-content/uploads/Research-Focus-2019-3-1-Cornell-Light-treatment-David-Gadoury.pdf)
- Use of Ultraviolet Light to Suppress Powdery Mildew in Strawberry Fruit Production Fields (https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-04-20-0781-RE)
- The potential of ultraviolet light to suppress grapevine powdery mildew (https://progressivecrop.com/2021/05/the-potential-of-ultraviolet-light-to-suppress-grapevine-powdery-mildew/)
- Use of Germicidal UV Light to Suppress Grapevine Diseases and Arthropod Pests (https://www.bio-conferences.org/articles/bioconf/pdf/2022/09/bioconf_gdpm2022_01002.pdf

The technology is also being implemented in vineyards in other states of US like in Oregon. (https://americanvineyardmagazine.com/ultraviolet-light-as-an-integrative-pest-management-tool-for-grape-powdery-mildew/)

BIOVIT 4

Institution: Norwegian University of Life Sciences (NMBU)
Administrative unit: Faculty of Biosciences (BIOVIT)
Title of case study: Applying genomics to advance aquaculture and manage wild populations of Atlantic salmon
Period when the underpinning research was undertaken: 2009 to present
Period when staff involved in the underpinning research were employed by the submitting institution: 2009 to present
Period when the impact occurred: 2009 to present

1. Summary of the impact

Atlantic salmon are fish of great social and economic value in Norway and abroad. For 15 years our group has pursued research aimed at building genomic resources allowing us to explore and understand the genome of this iconic species. The enabling genomics tools we have developed have been used by the breeding industry to boost genetic progress, target disease related problems and make step-change improvements in aquaculture. As well as supporting other researchers, our activities have produced essential knowledge regarding evolution in salmonids, improved our understanding of natural biodiversity and created novel tools supporting sustainable management of wild salmon populations.

2. Underpinning research

The Genome Biology research group at BIOVIT possesses internationally recognized expertise within animal genome biology, with key strengths in the application of omics data to understand the genomic architecture of important phenotypes in aquaculture and livestock production. In 2009, we initiated a partnership between leading research institutions in Norway, Chile and Canada, formalized as "*The International Cooperation to Sequence the Atlantic Salmon Genome*". The partnership resulted in the construction and public release of the first salmon reference genome ICSASG_v2 (GenBank Acc. GCA_000233375.4) which is analysed and described in a highly cited Nature article (Lien et al., 2016). Soon after, equipped with this foundational resource, we pioneered larger scale genomic re-sequencing of individuals to detect single nucleotide polymorphisms (SNPs) and used these to produce the first high-density genotyping arrays for Atlantic salmon. Together with the reference, these tools and resources are used by researchers from around the world to investigate genomics underpinning fundamental aspects of salmon biology and fill knowledge gaps related to economically and ecologically important traits. We have formed tight links with Norwegian breeding companies (e.g. AquaGen) and initiated multiple applied research projects addressing key challenges for the aquaculture sector (e.g. infectious diseases, lice infestation, fatty acid composition and filet quality). Furthermore, we have established a professional high-throughput wet-lab genotyping service providing data to national and international breeding companies enabling them to implement genomic selection improve traits through their breeding programs.

The reference published in 2016 represents a single individual genome and did not seek to reflect the wealth of genomic diversity among Atlantic salmon. This shortcoming can be addressed by transitioning to multiple reference genomes and creating pan-genomic resources that capture structural variations (SVs). In the NFR/NMBU-ToppForsk project TRANSPOSE (NFR; 275310), a salmon pan-genome was constructed by using long-read sequencing data to build genome assemblies for multiple salmon individuals spanning the species' range. These resources are currently being used in several projects to explore how genomic variation affect economically and ecologically important traits (e.g. infectious diseases and parasite infestations).

An important addition to any genome sequence is information that describes the function of the genes it contains and how they are regulated. We are currently leading the EU-project AQUA-

FAANG (https://www.aqua-faang.eu/) which is generating functional data to advance our understanding gene regulation and of how genomic variation can lead to phenotypic variation the six most important fish species in European aquaculture, including Atlantic salmon. Infectious diseases, and the genomic basis for disease resistance, is the main focus in the project.

The results and genomic tools were are producing are being made publicly available via a user friendly framework we have developed called Salmobase (https://salmobase.org/), part of the ELIXIR Norway project (https://elixir.no).

The genomic resources developed for Atlantic salmon builds on the infrastructure and competence established in 2003 with the funding of 'Centre for Integrative Genetics – CIGENE' (www.cigene.no) as a national SNP-technology platform responsible for detection, genotyping and interpretation of SNPs. The funding came from the NRC's program for functional genomics 'FUGE'. Since then, CIGENE has grown from a small molecular genetics lab to Norway's foremost SNP-genotyping facility and an internationally important genomics research lab devoted to understanding mechanisms and genetic architecture underlying phenotypes.

Key researchers contributing to the impact case:
Professor Sigbjørn Lien, Associate professor Matthew Kent, Professor Simen Sandve (2015 to present), Professor Stig Omholt (left the unit in 2013)

3. References to the research

1. Lien, S, BF Koop, SR Sandve, et al. (2016). The Atlantic salmon genome provides insights into rediploidization. Nature 533: 200–205. PMID: 27088604. DOI: 10.1038/nature17164. 835 citations.
2. Bourret, V, MP Kent, CR Primmer, et al. (2013). SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). Molecular Ecology 22:532-51. PMID: 22967111 DOI: 10.1111/mec.12003. 277 citations.
3. Barson, N, T Aykanat, K Hindar, et al. (2015). Sex-dependent dominance at a single locus maintains variation in age at maturity in Atlantic salmon. Nature 528:405-408. PMID: 26536110 DOI: 10.1038/nature16062. 490 citations.
4. Moen, T, J Torgersen, N Santi, et al. (2015). Epithelial Cadherin determines resistance to Infectious Pancreatic Necrosis in Atlantic salmon. Genetics 200:1313-26. PMID: 26041276. DOI: 10.1534/genetics.115.175406. 147 citations.
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6. Stenløkk, K, M Saitou, L Rud-Johansen et al. (2022). The emergence of supergenes from inversions in Atlantic salmon. Philosophical Transactions of the Royal Society B: Biological Sciences 377. PMID: 35694753. DOI: 10.1098/rstb.2021.0195. 4 citations.

4. Details of the impact

As the world's largest producer of aquaculture salmon, and host to the largest wild populations anywhere (25% of the global population), Norway has a unique responsibility to ensure sustainable management of both wild and farmed salmon populations. We see a pressing need to develop and deploy genomic strategies to address emerging problems in a fast-expanding aquaculture sector, as well as threats to wild populations resulting from anthropogenic changes. Releasing the Atlantic salmon genome (Lien et al., 2016) represented a major break-through for advancing genomics. Initially, it enabled the efficient detection of millions of mutations (SNPs) which have been used to develop a series of cost-effective genotyping tools (primarily SNP-arrays) that are applied in genome-wide surveys to identify genomic variation effecting economically important traits in aquaculture and studying adaptive evolution in wild salmon populations. More recently, we have used long-read sequencing technology to expand the salmon reference genome into pan-genomic resources that more fully capture the genomic diversity present among individuals. Crucially, these new resources reveal structural variation

(SVs) which have been largely undetected until now despite their tremendous impact on genome architecture.

Over the past decade, advanced genomics has contributed significantly to expedite improvements in salmon aquaculture. While it is difficult to accurately project the positive long-term financial impacts arising from the knowledge we have generated, it is informative to consider the impacts made by genome-based selection for IPN resistance in salmon. Infectious pancreatic necrosis (IPN) is a viral disease that was a primary concern for salmon farming twenty years ago, with frequent outbreaks causing up to 90% mortality in freshwater hatcheries and sea cages. In a series of interrelated projects with AquaGen we contributed to the identification of a major locus that could explain around 80% of genetic variation in IPN resistance. A SNP-based genetic test predicting IPN resistance was developed and implemented in AquaGen's and other companies breeding programs. Consequences of this has been a reduction of mortalities from IPN infection from several million fish per annum to almost none in just a few years, saving the salmon industry many hundreds of millions NOK and improving animal welfare (Moen et al., 2015).

Today, diseases and parasites continue to represent the most important problems for salmon aquaculture causing huge economic losses, poor animal welfare and threatening wild populations. Financial losses caused by pathogens and parasites is estimated at around one-fifth of production, equal to 1,800 M€ per annum in Europe alone. Salmon genomics research has substantially improved our understanding of how genotypes are translated to phenotypes and implementation of this knowledge industrial applications have reduced the negative impact of infectious diseases and parasites in salmon aquaculture. While the IPN example mentioned above may represent an unusually successful case, the industry is so vast that only modest improvements in other diseases and lice infestations can equate to all NRC's investments in salmon genomics research for the last 20 years.

The involvement of Norwegian aquaculture industries (e.g. AquaGen) in collaborative projects provides the means of rapid dissemination of genomic advances and project outcomes to an industry with customer bases world-wide, which also increase impact of the research innovations. Moreover, more sustainable production because of the innovations, along with a more favourable public perception concerning its environmental impacts, is expected to expand the competitiveness of aquaculture bioproduction in Norway.

Unfortunately, the expansion of salmon aquaculture has had unfavourable consequences for wild salmon populations in Norway. Returns of wild salmon to Norwegian rivers have more than halved from the 1 million recorded in the 1980s causing salmon to be red listed as near threatened in 2021. The greatest anthropogenic threats to Norwegian wild salmon are escaped farmed salmon, sea lice and infections related to salmon aquaculture. Advanced genomic provide new tools and step-change improvement in our ability to monitor biodiversity and manage wild salmon populations. Through several projects we have together with other institutions in Norway (mainly NINA, IMR and Nofima) developed targeted SNP-panels that have been used to explore farmed-wild salmon interactions and DNA-based tracking of escapees from fish pens. Moreover, our findings on genetic basis age at maturity (Barson et al., 2015) has been implemented in management programs because of their ability track changes in response to anthropogenic change and establish links to life-history characters.

5. Sources to corroborate the impact

1. Macqueen DJ, CR Primmer, HD Houston, et al. (2017). Functional Annotation of All Salmonid Genomes (FAASG): an international initiative supporting future salmonid research, conservation and aquaculture. BMC Genomics. 18:484. PMID: 28655320. doi: 10.1186/s12864-017-3862-8.
2. Samy JKA, TD Mulugeta, T Nome, et al. (2017). SalmoBase: an integrated molecular data resource for Salmonid species. BMC Genomics.18:482. PMID: 28651544. doi: 10.1186/s12864-017-3877-1.
3. Gillard G, TN Harvey, A Gjuvsland, et al. (2018). Life-stage-associated remodelling of lipid metabolism regulation in Atlantic salmon. Mol Ecol. 27:1200-1213. PMID: 29431879. doi: 10.1111/mec.14533.

4. Pearse DE, NJ Barson, T Nome, et al. (2019). Sex-dependent dominance maintains migration supergene in rainbow trout. Nature Ecology and Evolution 3:1731-1742. PMID: 31768021. DOI: 10.1038/s41559-019-1044-6.
5. Helgeland H, M Sodeland, N Zoric, et al. (2019). Genomic and functional gene studies suggest a key role of beta-carotene oxygenase 1 like (bco1l) gene in salmon flesh color. Sci Rep. 9:20061. PMID: 31882713. doi: 10.1038/s41598-019-56438-3.
6. Bertolotti AC, RM Layer, MK Gundappa, et al. (2020). The structural variation landscape in 492 Atlantic salmon genomes. Nat Commun. 11:5176. PMID: 33056985. DOI: 10.1038/s41467-020-18972-x.
7. Gillard, GB, L Grønvold, LL Røsæg, et al. (2021). Comparative regulomics supports pervasive selection on gene dosage following whole genome duplication. Genome Biology 22, 103. PMID: 33849620. DOI: 10.1186/s13059-021-02323-0.
8. Sinclair-Waters M, T Nome, J Wang, (2022). Dissecting the loci underlying maturation timing in Atlantic salmon using haplotype and multi-SNP based association methods. Heredity 129:356-365. PMID: 36357776. doi: 10.1038/s41437-022-00570-w.
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10. Gundappa MK, TH To, L Grønvold, et al., (2022). Genome-Wide Reconstruction of Rediploidization Following Autopolyploidization across One Hundred Million Years of Salmonid Evolution. Mol Biol Evol. 39:msab310. PMID: 34718723. doi: 10.1093/molbev/msab310.

1 Summary of the impact:

We have developed a game-changing biotechnology for combatting the N_2O-emission from farmland, enabling the agricultural sector to lower its climate footprint, and creating business opportunities for the biogas- and fertilizer-industries. We utilize Non-denitrifying N_2O-respiring Bacteria (**NNRB**), which are nature's own sink for N_2O. By growing our carefully selected NNRB-strains to high cell densities in organic wastes, we produce organic fertilizers which increase the abundance of NNRB in the soil, thereby reducing the N_2O emission by 50-95%. This NNRB-technology has sparked significant interest from agronomic and industrial stakeholders, and we foresee that it will be adopted worldwide.

2. Underpinning research:

The invention and advancement of NNRB-technology drew heavily on the fundamental research conducted in a creative and innovative intellectual environment at our faculty. NMBU Nitrogen Group (NMBUNG), established in 2005, has become an international frontrunner in basic research on microbial nitrogen transformations, with a specific focus on the biology of N_2O transformations. The NNRB research has extensively utilized the specialized infrastructure for studying respiratory biology under low oxygen conditions provided by NMBUNG, as well as the proteomics platform created by the Protein Engineering Group group (PEP), and the expertise in bioinformatics of the Bioinformatics and statistics group (BIAS), both at KBM.

Approximately ~1/3 of the global warming effect of food production is due to N_2O-emission from farmland. The ultimate driver of the emission is nitrogen fertilization, which boosts a plethora of microbial nitrogen transformation which produce and consume N_2O in the soil.

The invention, conceived in 2016, is to use organic wastes, destined to be used as fertilizers, as substrates for NNRB, thus vectoring massive amounts of NNRB into agricultural soils. NNRB are bacteria which cannot produce N_2O themselves, but their anaerobic respiration consumes N_2O produced by other organisms, reducing it to harmless N_2.

While the NNRB-technology is simple, the development was not. The challenge was to identify and isolate NNRB which can grow fast in organic wastes, and remain active in soil throughout a growth season. At first we used conventional enrichment culturing in unsterilized waste, monitored by meta-genomics and -proteomics to identify growing strains, thus guiding the isolation. This provided strains whose catabolic profile was streamlined for growth in the waste, not in soil (Jonassen et al 2022a). Strains with broader catabolic profiles were obtained by adopting a novel dual substrate enrichment culturing, alternating between sterilized soil and waste (Jonassen et al 2022b).

We now have a set of NNRB-strains, and have conducted agronomic field experiments, where the emissions of N_2O was monitored by our unique field robot, providing high-resolution emission data. **The field experiments show that fertilization with an NNRB-enriched organic waste reduce N_2O emission by 50-95%, depending on the pH of the soil, and the effect was long-lasting. This is no less than sensational, and will be published soon.**

Key persons and their roles. The concept emerged in 2005 through discussions within NMBUNG, led by professor Åsa Frostegård and Lars Bakken. The work was launched as a collaboration with other

research groups at the faculty: The bioprocessing group (Svein Jarle Horn) and the Protein engineering Group, PEP (Vincent Eijsink). The core team is:

Kjell Rune Jonassen, from start (PhD student): pioneering work, and main responsible, now leading the follow-up project NOX2N, employed by VEAS. Silas Vick joined in 2019 (postdoc), microbiology and genomics. Elisabeth G Hiis PhD-student joined 2021: field experiments and phenotyping. Lars R Bakken: from start (professor): inventor and leader. Lars Molstad from start (engineer): modelling and robotics.

Others: Åsa Frostegård (professor), Live Heldal Hagen and Magnus Arntzen (Researchers, PEP group), Torgeir Hvidsten (Professor, BIAS).

3. References to the research:

Fundamental research:

Lycus P, Bøthun KL, Bergaust L, Shapleigh JP, Bakken LR, Frostegård Å (2017) Phenotypic and genotypic richness of denitrifiers revealed by a novel isolation strategy. The ISME Journal 11:2219-2232. https://www.pnas.org/doi/abs/10.1073/pnas.1805000115

Lycus P, Soriana-Laguna, Kjos M, Richardson DJ, Gates AJ, Milligan DA, Frostegård Å, Bergaust L, Bakken LR (2018) A bet-hedging strategy for denitrifying bacteria curtails their release of N2O. PNAS 115: 11820–11825. https://www.nature.com/articles/ismej201782

Bakken LR, Frostegård Å (2020) Emerging options for mitigating N2O emission from food production by manipulating the soil microbiota. Current Opinion in Environmental Sustainability 47:89-94. https://www.sciencedirect.com/science/article/pii/S1877343520300646

Gao Y, Mania D, Mousavi SA, Lycus P, Arntzen M, Woliy K, Lindström K, Shapleigh JP, Bakken LR, Frostegård Å (2021) Competition for electrons favors N2O reduction in denitrifying *Bradyrhizobium isolates*. Environmental Microbiology 23:2244-2259. https://ami-journals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.15404

NNRB-technology development:

Jonassen KR, Hagen LH, Vick SHW, Arntzen MØ, Eijsink VGH, Frostegård Å, Lycus P, Molstad L, Bakken LR (2022a) N2O-respiring bacteria in biogas digestates for reduced agricultural emissions. The ISME Journal 16:580-590. https://doi.org/10.1038/s41396-021-01101-x

Jonassen KR, Ormåsen I, Duffner C, Hvidsten TR, Bakken LR, Vick SHW (2022b) A dual enrichment strategy provides soil- and digestate-competent nitrous oxide-respiring bacteria for mitigating climate forcing in agriculture. mBio online https://journals.asm.org/doi/full/10.1128/mbio.00788-22

4 Details of impact

The promising results have convinced VEAS to proceed with upscaling the NNRB technology to pilot level, and to elaborate it to fit with their new line of fertilizer production from digestates. This work received funding from The Research Council of Norway for a 3-year project: "Transformation of biogas digestates to a fertilizer which reduce N_2O emissions", with a short-name **NOX2N**. Several government organizations, industries and relevant stakeholders joined in as advisory board

members:, Norwegian Farmers organization (Norges Bondelag), IVAR IKS, Tine, Scanship AS, Air Liquide (France), Yara International, N2Applied AS, Biokraft AS, VOW AS, Rogaland Biogassnettverk,The Norwegian Environmental Agency (Miljødirektoratet), Bellona and NGI .

Link to the project description:
https://prosjektbanken.forskningsradet.no/project/FORISS/331811?Kilde=FORISS&distribution=Ar&chart=bar&calcType=funding&Sprak=no&sortBy=score&sortOrder=desc&resultCount=30&offset=0&Fritekst=N2O

The NOX2N project commits the partners (VEAS and the core team at NMBU) to establish an upstart company to promote the technology. The company will assist biogas industries to implement the NNRB technology in their fertilizer products, be it as liquid fertilizers, sludge or dry pellets.

Fertilizer producers are beginning to develop dried/pelleted organic fertilizers, with or without mineral fertilizers as supplements. We are in the process of seeking funding from industries and the Research Council for a R&D-project to prepare the NNRB for such dry commodities, which are expected to take an increasing share of the fertilizer market.

5. Sources to corroborate the impact

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[Administrative unit short name] [case number]

Institution:
Administrative unit:
Title of case study: New redox enzymes and processes for more efficient processing of polysaccharide-rich biomass
Period when the underpinning research was undertaken: 2010 - 2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2010 - 2022
Period when the impact occurred: 2010 - now

1. Summary of the impact (indicative maximum 100 words)

We have discovered a completely new class of redox enzymes (LPMOs) that oxidatively cleave recalcitrant polysaccharides such as chitin and cellulose. This discovery has changed our understanding of the enzymatic conversion of polysaccharide biomass, both in nature and in industrial biorefineries. Today these enzymes are studied and used industrially all over the world. Upon the initial discovery in 2010 (with a prelude in 2005), we have combined fundamental research with applied research through multiple collaborations with industries of varying intensity. In 2016 we made another breakthrough discovery shedding light on how these powerful but "complicated" enzymes best can be used. Industrial application of the 2016 discovery has reached the level of 4.5 cubic meters / 4000 kg working weight in the Borregaard industrial biorefinery pilot as reported in a 2020 publication (Costa et al; see below) and Borregaard now considers the technology ready for full scale industrial implementation. The technology has brought closer the prospect of converting Norwegian biomass to single cell protein for salmon feed (see 2020 publication by Lapena et al., below). Completely new applications of these unique enzymes, in chemical catalysis and conversion of synthetic polymers, are being explored in a 2020-2026 ERC Synergy project.

2. Underpinning research (indicative maximum 500 words)

Efficient enzymatic processing of polysaccharide-rich non-edible biomass, such as cellulose-containing straw and woody materials ("lignocellulose"), and (chitin-containing) shrimp shells, is considered a major component of a future greener economy.

In 2005, then PhD student Gustav Vaaje-Kolstad in the group studied a small, 170 residue protein called "chitin-binding protein" (out of pure curiosity) and showed that this protein was capable of boosting the activity of classical hydrolytic enzymes (chitinases) in the degradation of the recalcitrant, crystalline polysaccharide chitin. The mechanism behind this phenomenon remained an enigma. After Gustav's post-doc abroad, on another topic, and a period of little research funding in the lab, Gustav and the group managed to return to the topic and we discovered, in 2010, that this protein catalyzes oxidative cleavage of glycosidic bonds (Science paper, cited 1198 times, Google scholar; see below; 7 authors, all from the group). It was immediately clear that such proteins also existed for cellulose, a biopolymer of much wider (industrial) interest, and these were indeed described in 2011, by us and others. These enzymes are called lytic polysaccharide monooxygenases (LPMOs) and are, today, studied and used all over the world, including in commercial cellulolytic enzyme cocktails for (industrial) processing of lignocellulosic biomass.

Because of the huge industrial importance, intriguing catalytic properties, and natural abundance of LPMOs, we then managed acquisition of more research funds, which were used along multiple connected paths:

1. Mapping the natural diversity of LPMOs
2. Structure function-studies of LPMOs
3. Understanding the catalytic properties, including unravelling of the many factors that determine LPMO performance (activity and stability), in the lab and in the bioreactor.

4. Application of LPMOs in biomass processing, in collaboration with companies including Novozymes, DSM, Borregaard (Norway), and Cambi (Norway), and in applied EU projects with company involvement on bioprocessing of chitin-rich biomass.

Since 2010 work on LPMOs has been funded by the Research Council of Norway, through company collaborations, and by the EU through collaborative projects, a Marie Curie ITN and, since 2020, an ERC-Synergy grant.

Next to permanent staff Profs Vincent Eijsink and Svein Jarle Horn and Gustav Vaaje-Kolstad, key contributors and some of their major achievements related to the impact include:

- PhD student/post-doc/researcher Zarah Forsberg (2010 – today); has done fundamental work focusing of characterizing novel LPMOs and structure-function studies focusing on the structural determinants of substrate specificity and oxidative regioselectivity, as well as the role of appended carbohydrate-binding domains. Her work provides a fundament for much of the more applied research in the group.
- Post-doc Bastien Bissaro (2015 – 2018); brilliant post-doc, who discovered that LPMOs in fact are peroxygenases (published dec 2016 on BioRxiv; patent application), thus shining totally new light on how these enzymes could/should be used in bioprocessing; landmark 2017 paper (see below).
- Post-doc/researcher Aniko Varnai (2013 - today); supervising the applied studies, leading the enzyme work package of a national center for biofuel research, and collaborative work with industry, together with Prof. Svein Horn.
- PhD student Piotr Chylenski (2013-2017); LPMOs in applied settings, focusing on the Borregaard substrate and on upscaling to 1 liter in bioreactors wit controlled pumping if hydrogen peroxide, process development based on the 2016-2017 discoveries; industry collaboration; papers published in 2017-2018.
- PhD student Gerdt Müller (2013-2017); as Chylenski, but with addition of studies on the use of LPMOs in simultaneous saccharification and fermentation (= an industrially important biorefining approach); industry collaboration; papers published in 2015-2018.
- Post-doc Thales De Freitas Costa (2018-2020); hired to drive the transition from the 2018 results of Muller and Chylenski (1 liter bioreactors) to the Borregaard demo plant (4000 L scale); landmark 2020 paper (see below).
- PhD student Anton Stepnov (2018–2022); fundamental discoveries, of great practical importance, on reaction parameters that affect LPMO performance, papers published in 2021-2022.
- PhD student Eirik G Kommedal (2018–2022); studying the impact of visible light on LPMO reactions; papers published in 2020-2022.
- PhD student Lene D Hansen (2018–2022); studying how pretreatment of lignocellulosic biomass affects LPMO activity and, thus, overall saccharification efficiency; the use of LPMOs in simultaneous saccharification and fermentation, adapted to the 2016-2017 discoveries; industry collaboration; papers published in 2022.
- PhD student Heidi Østby (2018–2022); applied studies with real substrates, looking at the effects of reaction temperatures, H2O2-delivery systems, hemicellulolytic activities of LPMOs and the potential of using naturally thermostable enzyme cocktails; papers published in 2020-2022.

3. References to the research (indicative maximum of six references)

The fundamental discovery (2010):
An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides.
Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sørlie M, Eijsink VGH.
Science. 2010 Oct 8;330(6001):219-22. doi: 10.1126/science.1192231.

Example of early fundamental work (2014):

Structural and functional characterization of a conserved pair of bacterial cellulose-oxidizing lytic polysaccharide monooxygenases.
Forsberg Z, Mackenzie AK, Sørlie M, Røhr ÅK, Helland R, Arvai AS, Vaaje-Kolstad G, Eijsink VGH.
Proc Natl Acad Sci U S A. 2014 Jun 10;111(23):8446-51. doi: 10.1073/pnas.1402771111.

Towards applications (work with company Novozymes, Denmark; 2015):
Harnessing the potential of LPMO-containing cellulase cocktails poses new demands on processing conditions.
Müller G, Várnai A, Johansen KS, Eijsink VG, Horn SJ.
Biotechnol Biofuels. 2015 Nov 25;8:187. doi: 10.1186/s13068-015-0376-y.

Towards applications (work with company Borregaard, Norway; 2017):
Enzymatic degradation of sulfite-pulped softwoods and the role of LPMOs.
Chylenski P, Petrović DM, Müller G, Dahlström M, Bengtsson O, Lersch M, Siika-Aho M, Horn SJ, Eijsink VGH.
Biotechnol Biofuels. 2017 Jul 11;10:177. doi: 10.1186/s13068-017-0862-5. eCollection 2017.

A second breakthrough (2016-2018):
Oxidative cleavage of polysaccharides by monocopper enzymes depends on H2O2.
Bissaro B, Røhr ÅK, Müller G, Chylenski P, Skaugen M, Forsberg Z, Horn SJ, Vaaje-Kolstad G, Eijsink VGH.
Nature Chem Biol. 2017 Oct;13(10):1123-1128. doi: 10.1038/nchembio.2470.

Process for degrading a polysaccharide employing a lytic polysaccharide monooxygenase
Bissaro B, Eijsink VGH, Vaaje-Kolstad G
US2019233861/EP3519580/WO2018060498 (priority date Sep 30, 2016)

The impact of hydrogen peroxide supply on LPMO activity and overall saccharification efficiency of a commercial cellulase cocktail.
Müller G, Chylenski P, Bissaro B, Eijsink VGH, Horn SJ.
Biotechnol Biofuels. 2018 Jul 24;11:209. doi: 10.1186/s13068-018-1199-4.

*Towards improved applications, **pilot scale** (work with company Borregaard, Norway; 2020):*
Demonstration-scale enzymatic saccharification of sulfite-pulped spruce with addition of hydrogen peroxide for LPMO activation. Since then, Borregaard has used this process regularly for pilot scale production of spruce-derived sugars.
Costa THF, Kadic A, Chylenski P, Várnai A, Bengtsson O, Lidén G, Eijsink VGH, Horn SJ.
Biofuels, Bioprod. Bioref. 2020, 14:734–745. Doi: 10.1002/bbb.2103.

Towards applications (work with company DSM, The Netherlands; 2020):
Characterization of an AA9 LPMO from *Thielavia australiensis*, TausLPMO9B, under industrially relevant lignocellulose saccharification conditions.
Calderaro F, Keser M, Akeroyd M, Bevers LE, Eijsink VGH, Várnai A, van den Berg MA.
Biotechnol Biofuels. 2020 Nov 30;13(1):195. doi: 10.1186/s13068-020-01836-3

Towards novel applications (2020):
Spruce sugars and poultry hydrolysate as growth medium in repeated fed-batch fermentation processes for production of yeast biomass
Lapena D, Olsen PM, Arntzen MO, Kosa G, Passoth V, Eijsink VGH, Horn SJ
Bioprocess Biosystems Engineering, 2020; 43:723-736. Doi: 10.1007/s00449-019-02271-x.

4. Details of the impact (indicative maximum 750 words)

The discovery of LPMOs, i.e. oxidative cleavage of glycosidic bonds by small easy-to-produce secreted proteins that employ only a single copper ion as co-factor, has generally had large impact because LPMOs:

- have unprecedented catalytic centers doing unprecedented chemistry (-> impact on the scientific community).
- are abundant in nature (suggesting that they are important) (-> impact because the topic is broad and important).
- provide a novel and powerful tool for the enzymatic conversion of lignocellulosic or chitin-rich biomass, providing clear and experimentally proven benefits (-> direct impact on the biorefining industry)

LPMOs are today central components of commercial enzyme preparations for processing of lignocellulosic biomass. There is a huge patent landscape, including a patent from our group covering the original 2010 discovery, which we sold to Novozymes (only cellulose applications, chitin applications still owned by NMBU) in 2011. Multiple companies are using and selling LPMOs or LPMO-containing enzyme blends.

Recent developments point at other potential impact:

- LPMOs clearly play a role in microbial pathogenicity, suggesting that hitherto not-identified additional substrates exist.
- LPMOs are explored as a scaffold to engineer enzymes acting on other recalcitrant polymers, such as plastics, or to carry out other specific oxidations, for example of aromatic compounds or even alkanes.

By maintaining a well-balanced portfolio of fundamental and applied research projects, our group is a leader in the field, serving both academia and industry. Through the years we have published our LPMO findings in process-oriented journals (see above), as well as in journals more interested in publishing fundamental breakthroughs, such as PNAS and Nature Communications. Key patents were written in 2010 (sold in 2011) and 2016 (still in NMBU's possession).

Through the years, we have actively communicated with companies, especially with the Norwegian company Borregaard, under appropriate non-disclosure agreements to protect our intellectual property, to ensure rapid implementation of or findings. Our 2016-2017 discovery that LPMOs in fact are peroxygenases accelerated these communications and boosted the collaboration, as exemplified by the 2020 4000 liter-scale study described by Costa et al., 2020.

Other companies with whom we have been and still are collaborating are Novozymes (Denmark), DSM (The Netherlands) and St1 (Finland). It has turned out that, while the positive impact of LPMOs is clear and significant, optimizing the use of LPMOs in industrial biorefining is not straightforward. Therefore, much research is still ongoing, and our leading expertise is sought after.

LPMOs and our LPMO competence have been, and still are, central in several national and international projects involving collaboration with a multitude of research partners and companies. Some examples:

- Foods of Norway, a national Centre for Research-based innovation ("SFI"), 2015-2023; the Centre focuses on developing new feed sources for animals and fish, and its members are multiple groups at NMBU and 20 non-academic external partners, mostly companies.
- Centre for environmentally friendly energy ("FME") - Bio4Fuels; 2017-2025; this national Centre comprises 3 Universities, 4 Research Institutions and 40 companies and includes activities on pretreatment and enzymatic saccharification of lignocellulosic biomass.
- Centre for Research-based Innovation ("SFI") within Industrial Biotechnology; 2020 – 2028. This Centre comprises 2 Universities, 2 Research Institutions and 15 companies and its activities vary from the production of vaccines and antibiotics to the development of green solutions based on enzyme and fermentation technologies.

- Four Horizon/Era-Net projects on enzymatic biorefining of cellulose or chitin (since 2012; last one just started).

Of note, one popular topic in Norway when discussing the power of biotechnology, is the fact that today, salmon “eat Norwegian spruce” (see Lapena et al., 2020, above; spruce -> sugar -> single cell protein). Work om commercializing this technology is in progress (in the Foods of Norway Center). This would not have been possible without the discovery and industrial implementation of LPMOs.

Completely new applications of these unique enzymes, in chemical catalysis and conversion of synthetic polymers, are being explored in a 2020-2026 ERC Synergy project (four groups: Ås, Norway; Oslo, Norway (Unni Olsby); Turin, Italy (Silvia Bordiga); Mullheim, Germany (Serena DeBeer).

5. Sources to corroborate the impact

Gudbrand Rødsrud – Technology Director Business Development, Borregaard AS; phone: (+47) 48 14 06 03; E-mail: gudbrand.rodsrud@borregaard.com

Oskar Bengtsson – Section Manager, Business Development R&D, Borregaard AS; phone: (+47) 47 24 29 83; E-mail: oskar.bengtsson@borregaard.com

Marco van den Berg – Senior Science Fellow Food Technology, DSM Food & Beverage; phone: +31 6 12045117; E-mail: marco.berg-van-den@dsm.com

Impact case guidelines

Each case study should include sufficiently clear and detailed information to enable the evaluation committee to make judgements based on the information it contains, without making inferences, gathering additional material, following up references or relying on members' prior knowledge. References to other sources of information will be used for verification purposes only, not as a means for the evaluation committee to gather further information to inform judgements.

Timeframes

- The impact must have occurred between 2011 and 2021
- Some of the underpinning research should have been published in 2010 or later
- The administrative units are encouraged to prioritise recent cases

Page limit

Each completed case study template will be limited to **five pages** in length. Within the annotated template below, indicative guidance is provided about the expected maximum length limit of each section, but institutions will have flexibility to exceed these so long as the case study as a whole remains no longer than **five pages** (font Arial size 10,5 or similar). Please write the text into the framed template under the sections 1–5 below. The guiding text that stands there now, can be deleted.

Maximum number of cases permitted per administrative unit

For up to 10 researchers: one case; for 10 to 30 researchers: two cases; for 30-50 researchers: three cases; for 50-100 researchers: four cases, and up to five cases for units exceeding 100 researchers.

Naming and numbering of cases

Please use the standardised short name for the administrative unit, and the case number for the unit (1,2,3, etc) in the headline of the case. Each case should be stored as a separate PDF-document with the file name: [Administrative unit short name] [case number]

Publication of cases

RCN plans to publish all impact cases in a separate evaluation report. By submitting the case the head of the administrative units consents to the publication of the case. Please indicate below if a case may not be made public for reasons of confidentiality.

If relevant, describe any reason to keep this case confidential:

KBM Case 3

Institution: Norwegian University of Life Sciences,
Administrative unit: Faculty of Chemistry, Biochemistry and Food Science
Title of case study: The benefit of microbiota analyses in the dairy industry
Period when the underpinning research was undertaken: 2000-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2000-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact (indicative maximum 100 words)

Impact case: Microbiota in raw milk and its influence on udder health, and quality and shelf life of dairy products.
Our work on dairy microbiota has given the Norwegian dairy industry new possibilities to optimize their production processes and adjust shelf life of dairy products. This has led to changes in farming practices, raw milk treatment, and several innovation projects to better understand the fermentation process during cheese production. The cheese microbiota projects has resulted in changes in the production of starter at the dairy plants, changes during fermentation and revised cleaning procedures. This has resulted in a more stable product quality and less quality problems which reduce waste.

2. Underpinning research

The research group has worked with cheese research and the development of cheese flavour for decades. The development of a completely new cheese variety "**Jarlsberg"** in the 50' has been a flagship for the research group ever since. One of the focus areas the last 20 years has been the development of flavour compounds in cheese. From 1997-2004 the RCN funded research project "**Cheese ripening**" generated new knowledge about the interaction between starter bacteria and non-starter lactobacilli for the flavour development in Scandinavian cheese (Kieronzyk et al 2003). Both Arla and TINE joined the project after the project had started, as they considered the project to have a high innovation potential. The project resulted in an RCN innovation project for the industrial sector lead by TINE (2009-2014) "**Identification and use of NSLAB in cheese**" which then led to a EUROSTAR project (2013-2017) "**E! 8454 Towards the next generation cheeses: Engineering of excellent Flavours for healthy cheeses**" and full-scale production experiments were performed. As the industry does not communicate their use, we have limited knowledge of the fate of the results from this research.

The RCN funded project "**Healthy cheese**" (2008-2013) studied the influence of fat on microbial growth in cheese. In parallel with this project, the use of molecular methods for the studies of microbial communities developed, and this methodology was adapted for cheese (Porcellato & Skeie, 2016) and later taken further to milk (Porcellato et al. 2018, Skeie et al. 2019, Porcellato et al. 2021) in the RCN funded projects "**Bacterial flora and dynamics in Norwegian milk and dairy products: Potential for spoilage and disease**" (2015-2019), "**CLOBIO: Clostridia and other microbiota in raw milk and milk products: importance for product quality and food safety**" (2019-2024), *"***The udder microbiota in dairy cows: importance for animal health and welfare, mastitis and milk quality**" (2017-2022), "**Increasing sustainability of Norwegian food production by tackling streptococcal infections in modern livestock systems**" (2018-2022) and the ongoing project "**HoliCow: A holistic approach to an old problem: deciphering complex host-microbiome-pathogen relationships in bovine mastitis**" (2021-2024). This load of work has led to a very specific knowledge on milk and cheese microbiota, of which the industry has been using to improve their way of advising farmers, reducing udder infections (Porcellato et al. 2020, Winther et al. 2022), plan their production and processing of milk and how to adjust the cheese-making processing to improve quality and reduce loss. The RCN funded innovation project for the industrial sector lead by TINE (2019-2023) "**DNA analyses for better control of cheese production**" utilises the knowledge produced by this research and has led to substantial

changes in how TINEs cheese production plants handle their starter and production technology.

 Names of the key researchers and what positions they held at the administrative unit at the time of the research

Professor Siv Skeie – PI of the raw milk/liquid milk and cheese microbiota projects (2011-2022)
Professor Judith Narvhus – PI of the Udder microbiota project and has been responsible for activities related to studies growth of psychotropic bacteria in milk until her retirement in June 2021 (2011 – June 2021)
Associate Professor Davide Porcellato – PI of the HolyCow project, took over as PI for the Udder microbiota project after Professor Judith Narvhus retirement. Responsible for microbiota research activities (2011-2013, PhD student; 2014 – 2016, PostDoc; 2017-2021 (March) Research scientist; 2021 (April) – 2022 Associate Professor (tenure)).
Associate Professor Hilde Østlie – Responsible for research activities in the NSLAB project from 2011-2013. (2011-2022)
PostDoc Vinicius da Silva Duarte – Responsible for research activities in the HolyCow project (2020-2022)
Research Scientist Misty Dawn Finton – Responsible for research activities in CLOBIO and DNA analyses projects from 2020 to 2022. (Has previously been connected to another project as a PhD student (2017-2020)

3. References to the research (indicative maximum of six references)

Cheese:
Kieronczyk, A., Skeie, S., Langsrud, T., Yvon, M. 2003. Cooperation between Lactococcus lactis and non-starter lactobacilli in the formation of cheese aroma from amino acids. Applied and Environmental Microbiology. 69: 734-739. https://doi.org/10.1128/AEM.69.2.734-739.2003

Porcellato, D., Skeie, S. (2016). Bacterial dynamics and functional analysis of microbial metagenomes during ripening of Dutch-type cheese. International Dairy Journal, 61, 182–188. http://dx.doi.org/10.1016/j.idairyj.2016.05.005

Raw and liquid milk quality:
Porcellato D., Aspholm M., Skeie S., Monshaugen, M., Brendehaug, J., Mellegård, H. 2018. Microbial diversity of consumption milk during processing and storage. International Journal of Food Microbiology 266:21:30
https://doi.org/10.1016/j.ijfoodmicro.2017.11.004

Skeie, S. B., M. Håland, I. M. Thorsen, J. Narvhus, and D. Porcellato. (2019). Bulk tank raw milk microbiota differs within and between farms: A moving goalpost challenging quality control. Journal of Dairy Science, 102(3):1959-1971. https://doi.org/10.3168/jds.2017-14083

Porcellato, D., M. Smistad, A. Bombelli, A. Abdelghani, H. J. Jørgensen, and S. B. Skeie. 2021. Longitudinal Study of the Bulk Tank Milk Microbiota Reveals Major Temporal Shifts in Composition. Frontiers in Microbiology 12:348. https://doi.org/10.3389/fmicb.2021.616429

Udder microbiota:
Porcellato D., Meisal R., Bombelli A., Narvhus, J.A. 2020. A core microbiota dominates a rich microbial diversity in the bovine udder and may indicate presence of dysbiosis. Scientific Reports, 21608. https://www.nature.com/articles/s41598-020-77054-6

Winther A.R., Narvhus, Smistad M., da Silva Duarte, V., Bombelli, A., Porcellato, D., 2022. Longitudinal dynamics of the bovine udder microbiota. Animal Microbiome, 4 (1):26
https://doi.org/10.1186/s42523-022-00177-w

4. Details of the impact

It is very stimulating for a research group which aims at being in the forefront of its research field (food microbiology), to experience that the industry utilises and is interested in the research made. At least that is the interpretation we make when the industry initiates and start innovation projects based on our research, where 50 % of the costs must be covered by themselves. Also, the fact that the same industry comes to us with completely confidential projects, confirms that our research is relevant and of use for the industry. We interpret that the industry adopts our research and implement results in their production.

The relevance of our research was clear already with the project “Cheese ripening”, which also made a foundation for long ripened cheese in Norway, where two of the largest dairy companies in Scandinavia (TINE and Arla) joined the project after it had started. TINE has been involved as an active partner in all the projects mentioned in section 2. At the same time scientific results has been published in high impact journals of the field. TINE has been actively participating in project meetings giving them access to project results way ahead of publication. This has benefitted both farmers, dairy processing facilities and consumers by leading to better farming practices, better processing practices, less waste and higher quality products. A very concrete result is longer shelf life on liquid milk. We experience that the fact that the industry partners get access to information by active participation in research projects, actually results in an increased research activity in total from the industry partner. They also more actively exploit the knowledge in their production.

In all the microbiota research made by the research group we have benefitted from the high microbiota competence at KBM, we have also cooperated with other research institutions were beneficial:

- In the cheese projects we have been participating in different international networks, and Professor Skeie was heading the NorFond funded “NordOst” network project encompassing research groups from all Nordic and the Baltic countries.
- In the NSLAB project, the High Throughput analytical technology at SINTEF was used.
- Several of the project dealing with milk and udder microbiota has been made in cooperation with researchers at the Veterinary faculty or the Veterinary institute (3 of the projects had their PI from these institutions), where we have had the responsibility for the analysis of microbiota and dairy technology part, while the veterinarians has focused on udder health and pathogenic bacteria.

As this research is used by a large industry partner (TINE is the largest agricultural industry in Norway) they do not (and should not, of competitive reasons) communicate how they benefit and when they benefit from the research made. However, the fact that they continue to actively participate in the research projects is a very strong indication that the research has an impact.

5. Sources to corroborate the impact (indicative maximum of ten references)

Jorun Øyaas, Research Scientist, TINE, jorun.oyaas@tine.no
Anne Cathrine Whist, Research Director, TINE Anne.Cathrine.Whist@tine.no
Eirik Selmer Olsen, Head of TINE Advice and Member service, TINE eirik.selmer-olsen@tine.no

[MINA] – [impact case 1]

Institution: NMBU
Administrative unit: Faculty of Environmental Sciences and Natural Resource Management (MINA)
Title of case study: Using biochar to fortify low-input farming in sub-Saharan Africa
Period when the underpinning research was undertaken: 2011- ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 2011- ongoing
Period when the impact occurred: still on-going

1. Summary of the impact (indicative maximum 100 words)

The team has shown that biochar, an alkaline and carbon-rich pyrolysis product made of different types of organic waste, is a low-cost, locally available soil enhancer increasing crop production in Zambia. Due to its recalcitrance, biochar was shown to increase soil carbon content, while enhancing fertility and water holding capacity. Consequently, biochar addition to soil combines climate change mitigation and adaptation. Type and availability of feedstock, charring method, soil quality as well as farm management practices are important factors determining the long-term potential of biochar and thus adoption of the technology for increased food security and on farm income generation in sub-Saharan Africa.

The established impact is the adoption of biochar as a soil enhancer by numerous small-scale farmers affiliated to the partner NGOs in Zambia.

2. Underpinning research (indicative maximum 500 words)

The impact has two sides, an 'underpinning', technical, agronomical one (i.e. does it work?), and a societal one (i.e. is it adopted?). The former requires well-designed experimental work at the local field scale, whereas the latter requires a network of extension services reaching out to farmers, and ideally controlling for potential bias due to overoptimism as well as co-benefits or selectivity linked to participation.

The long-term cooperation on this subject builds on a series of successive projects supported by a variety of sponsors. It has also been an avenue for capacity building, a.o. with the Soil Sciences Department of the University of Zambia (UNZA), later also with Gulu University in Uganda. Currently, Mirriam Phiri (UNZA) is finalising her PhD on biochar from pigeon pea biomass at NMBU. Prior to this, two other PhD projects on biochar were completed in Zambia. The latest projects ("Climate smart agriculture in Sub-Saharan Africa", Norad-funded, QZA-21/0182-NORHEDII and RCN-funded "Climate-smart innovations in agriculture in Uganda; Improved food security, iivelihoods and soil carbon", Norglobal 302713) involve a new cohort of 6 PhDs and 2 post-docs that has started up in autumn 2022. Research topics include mechanistic studies and implementation research in Uganda, South Sudan and Ethiopia in addition to Zambia.

Agronomic research has focused on feed stock availability for the local production of high quality biochar. Novel, low-tech, small scale and clean pyrolysis units were successfully developed for on-farm use. Biochar produced from maize cobs or pigeon pea biomass had a positive effect on yield and farmer income in the dry and sandy regions of Zambia. Also, and importantly, an LCA has been carried out (Sparrevik et al. 2013) comparing different types of biochar production technologies. The key conclusion was that the use of traditional kilns would lead to adverse health effects for the biochar producer – this can be overcome with improved kilns. Crop yields did improve overall, but less so in relatively fertile soils. Addition of biochar into sandy soils increased water retention, enhanced soil aggregation and adds nutrients (Obia et al., 2016, 2020).

The societal part is strongly supported by the Conservation Farming Unit (CFU) and its successor Climate Smart Farming Solutions (CSFS) and their network of farming extension workers (cf. https://conservationagriculture.org/). The potentially promising, new practice of locally produced biochar from crop residues that is utilized on-site has been rolled out among the farmer network of the CFU (> 200,000 small holders). One recent project (CLIMCHAR, Nordic Climate Facility-funded, 2019-2021; Lindhjem et al, 2021) assessed the adoption of biochar from pigeon pea biomass. Out of the targeted 500 farms in the CFU network, a third (168) in the end also adopted the use of biochar. Among these adopters, the on-farm effects on soil organic carbon as well as maize yield were robustly positive. However, it is simply realistic to understand and accept that only a part of a population will be early adopters within the life span of a single project. Drop-outs will occur also after having received training for a range of reasons when a novel technology may be looked upon as complicated, laborious, and uncertain in its effects. At the same time, adoption by these 168 in different regions may well be the sufficient seeding number that will start a snowball effect.

So, all in all, the impact on the livelihoods of local small-scale farmers has been demonstrated to be positive. This first generation of early adoption farmers is the demonstrable impact. A gradual, step-by-step dissemination of the technique and a broader adoption into agricultural practice will require time and is still also subject to favourable reception among the farmers involved and their communities. Here the CFU and the new NGO Climate Smart Farming Solutions (CSFS) again have been pivotal in monitoring longer-term robustness. The long-term cooperation of a varied and evolving partnership likely is an important key to a sustained impact of this novel technology. The latest NORHED II research cooperation project lasting well into the current decade will also ensure a sustained research effort providing support to the on-going snow-ball adoption of biochar 'on the ground'.

The team:

Prof Dr Jan Mulder (MINA, NMBU)
Prof Dr Gerard Cornelissen (Norwegian Geotechnical Institute and MINA NMBU)
Dr Vegard Martinsen (MINA, NMBU)
Dr Sarah Hale (Norwegian Geotechnical Institute)
Dr Alfred Obia (PhD NMBU 2013-2015, currently lecturer Gulu University Uganda).
Dr Jose Munera-Echeverri (PhD NMBU 2016-2019, currently postdoc at INRAE, France)

3. References to the research (indicative maximum of six references)

Cornelissen, G., Martinsen V., Shitumbanuma V., Alling V.,.Breedveld, G Rutherford D., Sparrevik M., Hale S., Obia A., Mulder J.. 2013. Biochar effect on maize yield and soil characteristics in five conservation farming sites in Zambia. Agronomy 3:256-274.

Hale S., Alling V., Martinsen V., Mulder J., Breedveld G.D., Cornelissen, G. 2013. The adsorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. Chemosphere 91, 1612–1619.

Martinsen V, Mulder J, Shitumbanuma V, Sparrevik M, Børresen T, Cornelissen G.. 2014. Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. J Plant Nutr Soil Sci 177, 681-695.

Munera-Echeverri, J. L., Martinsen V., Strand L.T., Cornelissen G., Mulder, J. 2020. Effect of conservation farming and biochar addition on soil organic carbon quality, nitrogen mineralization, and crop productivity in a light textured Acrisol in the sub-humid tropics. Plos One 15:e0228717

Obia, A., Mulder, J., Martinsen, V., Cornelissen, G., Børresen, T., 2016. In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils. *Soil & Tillage Research* 135, 35-44.

Obia, A., Cornelissen G., Martinsen V., Smebye A.B., Mulder, J. 2020. Conservation tillage and biochar improve soil water content and moderate soil temperature in a tropical Acrisol. Soil and Tillage Research 197:104521.

4. Details of the impact (indicative maximum 750 words)

As outlined in section 2, this impact has two sides, an agronomical and a societal side. Without the sound experimental design of the soil sciences experiments carried out in real-world farming situations across Zambia, carried out under the agronomy flag, the other side, that is the extension and adoption of biochar for soil improvement would never have taken off. The agronomical side is well published in the international scientific literature and thus amply documented (cf section 3). This documentation is less the case for the adoption of the technique among small-holder farmers in Zambia's sandy districts. The LCA carried out by Sparrevik et al (2013) also points at potential pitfalls along the roll-out of a new technology, for example in the type of biochar kiln that would be most profitable but not necessarily is feasible for low-income smallholders. However, CFU and now also CSFS are dedicated NGOs to do exactly this outreach to and adoption by rural communities. We refer to Lindhjem (2021) for such documentation.

The biochar team (cf section 2, key researchers) has demonstrated to be able to cooperate over a longer period of at least a decade and find funding for their work from a range of different sources. This is more because of the common interest in the subject shared by the group than because of formal agreements. It is such a long-term cooperation that is a prerequisite for a sound testing of the sustainability of any innovation in a sub-Saharan farming system under strong pressure. The larger soil sciences research group at MINA provides a well-equipped background for experimental design, analytical instrumentation and sample processing.

The ultimate impact will gradually show from the wider-scale adoption of locally produced biochar beyond those 168 early adopters in Zambia, which then will go hand-in-hand with an increased productivity over the full board of the farmers associated with the Conservation Farming Unit in Zambia. Also, the work is expanded into other sub-Saharan countries where small-scale farmers meet comparable climatic and soil quality challenges, e.g. in Uganda (NORHEDII and RCN NORGLOBAL projects), Kenya and South Africa (ERA-LEARN project BICEPS coordinated by SLU). In addition, new initiatives are on their way. For example in Tanzania, where the NORAD-funded project ELCAP is currently undertaking farmer training. Also the coffee industry is showing interest, as there is a potential for carbon credits in biochar.

5**. Sources to corroborate the impact (indicative maximum of ten references)**

Lindhjem H, 2021. Testing biochar-pigeon pea agroforestry businesses in Zambia (ClimChar Zambia), Zambia, NCF7, Nordic Climate Facility -C7-091, Final report Menon. Oslo, Norway; see https://www.ndf.int/newsroom/agroforestry-shows-potential-for-climate-adaptation-in-zambia.html

Sparrevik M, JL Field, V Martinsen, GD Breedveld, G Cornelissen. 2013. Life cycle assessment to evaluate the environmental impact of biochar implementation in conservation agriculture in Zambia. Env Sci Tech 47,1206-1215

Website of the CFU: https://conservationagriculture.org/
(CSFS is developing its website)

News items on biochar in sub-saharan Africa:

- https://www.nmbu.no/en/faculty/mina/news/node/37537
- https://www.aftenposten.no/viten/i/o2nR/det-groenne-kullet
- https://forskning.no/partner-landbruk-nmbu-norges-miljo-og-biovitenskapelige-universitet/forhatt-plante-kan-brukes-som-biokull/1205086

- http://www.nationen.no/debatt/enrevolusjon-er-i-gang/ This is a long-read from Hans Peter Melby in the Norwegian daily newspaper Nationen of May 6, 2014, a well-read newspaper with the agricultural sector as its audience. Melby was agricultural officer at the Norwegian embassy in Lusaka at that time. He makes a point for biochar. The text is no longer available through the link, but we can provide a pdf upon request.

|[MINA] – [impact case 2]

Institution: NMBU
Administrative unit: Faculty of Environmental Sciences and Natural Resource Management (MINA)
Title of case study: Knowledge for better monitoring and management of Scandinavia's large carnivore species (ROVQUANT - Integrated analysis for management of large carnivores in Scandinavia)
Period when the underpinning research was undertaken: 2011- ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-ongoing
Period when the impact occurred: still expanding

1. Summary of the impact (indicative maximum 100 words)

This case study describes the impact of the work by the Applied Quantitative Ecology Group (AQEG) led by Richard Bischof at MINA, NMBU. The AQEG is changing how we quantify, understand, and communicate information about the status and future of wildlife populations. The conceptual and methodological advances that emerged from the group's work are being adopted in Scandinavia and beyond for answering both applied and fundamental questions, with a strong focus on mapping spatial and temporal dynamics of wildlife populations. Importantly, the detailed maps have provided much needed facts in one of the most polarized topics in natural resource management: large carnivores.

2. Underpinning research (indicative maximum 500 words)

Concept development
Bischof initiated the concept by developing one of the first open-population spatial capture recapture models (OPSCR) during the NFR project "Non-invasive monitoring of carnivores" (NFR 204202; 2011-2014). OPSCR uses multi-year wildlife monitoring data (mostly camera trap and eDNA) to produce timeseries of maps and estimates of density.OPSCR accounts for the ubiquitous challenge in wildlife monitoring, that not all individuals are detected. This resulted in a key paper showing the importance of the transboundary nature of wildlife populations [1].

Development of the framework
Motivated by the need of national wildlife management agencies, the project RovQuant (funded by the Norwegian Environment Agency and the Swedish Environmental Protection Agency) extended the OPSCR approach to map and estimate populations of bears, wolves, and wolverines. RovQuant (2017-ongoing) dovetailed with the NFR-funded project WildMap (NFR 286886; 2019-ongoing): the former has an applied scope and the latter also includes fundamental questions. We developed a suite of methods for analyzing monitoring data, estimating population density, size and vital rates. We have now been able to scale-up the estimates to the level of landscapes and entire populations. Close collaboration between ecologists and computer scientists led to an R package (nimbleSCR, [2]), which is now being used by a growing number of researchers in France, Italy, Spain, Germany, Canada, and the USA. Methods development is ongoing, as we strive to make the tools applicable to a wider range of species and conditions.

Monitoring and estimation of large carnivore populations
The AQEG has been producing maps and estimates of large carnivore populations annually, since 2019. The first range-wide estimates of density and population dynamics of wolves, bears and wolverines in Scandinavia resulted from RovQuant and WildMap and were published in PNAS [3]. It quickly became apparent that this framework had the potential to answer other key but elusive concepts in wildlife ecology. OPSCR allowed us to quantify the impact of GPS-collaring [4], estimate the role of spatial drivers on survival [5], and will

eventually help to identify more efficient designs for monitoring of wildlife at very large spatial scales [6].

Broader application and assimilation Researchers from Italy, Germany, France, Spain, Canada, and the US have begun using nimbleSCR developed during WildMap. AQEG has established collaborations with researchers in Italy, France, Germany, the USA, and Canada. For example, within the EU LIFE project WolfAlps (https://www.lifewolfalps.eu/), AQEC has helped generate the first comprehensive wolf population size estimate across Italy. The framework has also been tested and extended for ungulates such as red deer moose, roe deer, and wild boar.

The team
Dr Richard Bischof (professor, MINA, NMBU)
Dr Cyril Pierre Milleret (researcher, MINA, NMBU)
Dr Pierre Dupont (researcher, MINA, NMBU)
Ehsan Moqanaki (current PhD student, MINA)
Dr. Mahdieh Tourani (former PhD student, MINA)
Dr. Joseph Chipperfield (former postdoc, MINA)
Dr Soumen Die (former postdoc, MINA, NMBU)

3. References to the research (indicative maximum of six references)

[1] Bischof, R., Brøseth, H., & Gimenez, O. (2016). Wildlife in a Politically Divided World: Insularism Inflates Estimates of Brown Bear Abundance. *Conservation Letters*, *9*(2), 122–130. https://doi.org/10.1111/conl.12183
[2] Bischof, R., Turek, D., Milleret, C., Ergon, T., Dupont, P., & de Valpine, P. (2020). *nimbleSCR: Spatial Capture-Recapture (SCR) Methods Using "nimble". R package version 0.1.0*. https://cran.r-project.org/web/packages/nimbleSCR/index.html
[3] Bischof, R., Milleret, C., Dupont, P., Chipperfield, J., Tourani, M., Ordiz, A., de Valpine, P., Turek, D., Royle, J. A., Gimenez, O., Flagstad, Ø., Åkesson, M., Svensson, L., Brøseth, H., & Kindberg, J. (2020). Estimating and forecasting spatial population dynamics of apex predators using transnational genetic monitoring. *Proceedings of the National Academy of Sciences*, *117*(48), 30531 LP – 30538. https://doi.org/10.1073/pnas.2011383117
[4] Milleret, C., Bischof, R., Dupont, P., Brøseth, H., Odden, J., & Mattisson, J. (2021). GPS collars have an apparent positive effect on the survival of a large carnivore. Biology Letters, 17(6).
[5] Milleret, C., Dey, S., Dupont, P., Brøseth, H., Turek, D., de Valpine, P. and Bischof, R. (2023), Estimating spatially variable and density-dependent survival using open-population spatial capture–recapture models. Ecology. Accepted Author Manuscript e3934. https://doi.org/10.1002/ecy.3934
[6] Milleret, C., Dupont, P., Åkesson, M., Brøseth, H., Svensson, L., Kindberg, J., and Bischof, R., 2022.Estimates of wolf density, abundance, and population dynamics in Scandinavia, 2013–2022- MINA fagrapport 77. 35 pp. Available from: https://www.researchgate.net/publication/362092835_Estimates_of_wolf_density_abundance_and_population_dynamics_in_Scandinavia_2013-2022

4. Details of the impact (indicative maximum 750 words)

Better use of existing data and involving the public
The analytical framework allows integration of multiple sources of data for drawing inferences, including eDNA samples collected by management authorities and opportunistically by the public, recoveries of dead animals, and observation reports by the public. These data have been collected for 1-2 decades across Scandinavia and compiled primarily in 2 international databases: rovbase,no and skandobs.no. The research team's efforts have enabled comprehensive exploitation of existing long-term data collected at landscape scale. This not only facilitates value-creation but also increases the visibility of the international effort and public participation that large carnivore monitoring in Scandinavia represents. As large

carnivore monitoring relies heavily on citizen scientist participation, demonstrated use of these data and communication of results are imperative. Results were communicated to data collectors via various channels, including workshops, seminars, reports, and integration of outputs into the rovbase webapp. This intensive involvement of the public has contributed importantly to a broader understanding in the polarised debate on large carnivore management. of the contentious issue of Close collaboration and coordination between the team and the Norwegian Institute of Nature Research (NINA), which administers the Scandinavian large carnivore database, was instrumental.

Contribution to international coordination in natural resource monitoring and management

The Norwegian Environment Agency and the Swedish Environmental Protection Agency are co-signees of an international agreement ("Memorandum of Understanding Regarding the establishment and continuance of a monitoring system for large carnivores in Sweden and Norway"). The MoU 1) recognizes the trans-boundary nature of large carnivores in Scandinavia, 2) outlines the need to use standardized monitoring methods, and 3) highlights the intent to implement actions that benefit both parties with respect to monitoring population status and addressing research needs. The work performed by the AQEG (coordinated and targeted analysis of data collected as part of national large carnivore monitoring schemes in Scandinavia) falls within the scope of this MoU and the various outputs (methods, maps, estimates) facilitate the developing coordination in management through the recognition of shared cross-border populations.

Large carnivore population estimates

One of the main impacts of the team's work has been the provision of robust estimates of large carnivore populations and their dynamics to the management agencies in Norway and Sweden. The project provides density maps, population-level abundance estimates, as well as jurisdiction-specific numbers with associated uncertainty. The abundance numbers and other parameters for wolf and wolverine have been used annually by agencies to assess population status and inform management strategy – down to the numbers that are allowed to be culled. Density maps generated by the team are integrated into the rovbase webapp and are thus accessible to management agencies. In addition, products, such as raster data of annual carnivore density, are freely available via a dedicated repository on GITHUB (see section 5 below). These layers are already being used for investigations into carnivore ecology and impacts in Scandinavia. One concrete application was to inform the compensation scheme for carnivore impacts on semi-domestic reindeer husbandry in reindeer herding areas administered by Sámi parliament in Sweden. Sensitivity of the issue so far has prevented any publication on this case. The team had close interactions with management agencies and their staff throughout its existence. Meetings and workshops have facilitated an effective 2-way exchange and ensured that method development targeted applied needs of those charged with large carnivore monitoring and management.

Wildlife monitoring design

Monitoring carnivores is a challenging and expensive endeavour, especially at the large spatial scales at which it is conducted in Scandinavia – but that are appropriate for the populations of these animals. Empirical and simulation studies conducted by the AQEG have contributed to updated and more efficient monitoring protocols for wolf (started in 2017) and wolverine (started in 2022) and are currently being considered for a change of brown bear monitoring in Sweden.

Methods and tools

AQEG developed an open-access R package for large-scale spatial capture-recapture analysis, nimbleSCR, which is being continuously improved and extended. Close collaboration with researchers at two Universities in the US (UC Berkeley and Williams College) have been particularly instrumental in overcoming massive computation challenges associated with analysis of individual-based data collected at the landscape-scale. Researchers from Italy, Germany, France, Spain, Canada, and the US have begun using nimbleSCR and other tools

developed during WildMap for mapping and estimating wildlife populations. Researchers from Germany, Canada, and Italy have visited NMBU to learn from and work with team members. The methods have been adopted for the first large scale estimation of wolf populations in Italy, estimation in wolverine in Canada, and population estimation of several ungulate species (Chamois, red deer, roe deer) in different regions in Germany.

Scientific papers and technical reports (and associated citations)
The AQEG's activity related to non-invasive wildlife monitoring and population estimation has resulted in 18 peer-reviewed scientific publications since 2018, with a combined number of 286 citations. In addition, the work has resulted in 6 technical reports, primarily targeted at wildlife management agencies and policy makers in Scandinavia. Results are frequently picked up by media outlets in Scandinavia and beyond and the team has also published several popular articles/editorials describing its work (see section 5 below).

5. Sources to corroborate the impact (indicative maximum of ten references)

Data products at GITHUB
https://richbi.github.io/ ; https://github.com/richbi/RovQuantPublic/tree/main/technicalReports

Website AQEG
https://www.nmbu.no/en/faculty/mina/about-us/organization/sections/ecology-and-natural-resource-management/aqeg/node/46428

Selected from popular media
https://www.nmbu.no/en/faculty/mina/news/node/46501
https://kommunikasjon.ntb.no/pressemelding/oppdaterte-bestandsestimater-for-ulv-bjorn-og-jerv-i-norge-og-sverige?publisherId=17847876&releaseId=17950817

Management agencies and managers involved
Jens Andersson (Swedish Environmental Protection Agency, jens.andersson@naturvardsverket.se)
Robert Ekblom (Swedish Environmental Protection Agency, robert.ekblom@naturvardsverket.se)
Terje Bø (Norwegian Environment Agency, terje.bo@miljodir.no)
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At the partner research institute
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Jenny Mattisson (Norwegian Institute for Nature Research, jenny.mattisson@nina.no)

[MINA] – [impact case 3]

Institution: NMBU
Administrative unit: Faculty of Environmental Sciences and Natural Resource Management (MINA)
Title of case study: Advancing forest inventory and monitoring by integrating ground-based sample surveys and emerging remote sensing technologies – from local management to global applications
Period when the underpinning research was undertaken: 1995 - ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 1989 - ongoing
Period when the impact occurred: still expanding

1. Summary of the impact (indicative maximum 100 words)

The Forest Inventory and Monitoring Group (SKOGROVER, www.forestinventory.no) has developed the airborne laser scanning technology for operational mapping and estimation of forest resources in Fennoscandia and North America to a standard tool. In recent years more cross-disciplinary research has been focused in collaboration with e.g. archaeologist and wildlife biologists. The potential of combining field observations and novel sensor and sensing technologies operated on ground and from drones, airplanes and satellites, and use of emerging data analysis techniques such as machine learning, artificial intelligence and data assimilation is explored. Furthermore, SKOGROVER has contributed substantially to technical guidance for tropical forest monitoring.

2. Underpinning research (indicative maximum 500 words)

Forest inventory for operational forest management – from research to industrialization
The Forest Inventory and Monitoring Group (SKOGROVER, www.forestinventory.no) at MINA has been developing methods and technologies that increase our understanding of the status of forests, from very fine spatial scales (sub-hectare scales) to continental scales. This has been carried out in close cooperation with the forestry sector in Norway, including major industrial partners, sectoral organisations and government institutions. International cooperation across Fennoscandia and Northern America is equally important. It started back in 1995 when professor Næsset as the first researcher globally started to explore the potential of use of emerging airborne laser scanning technology for operational mapping and estimation of forest resources. Already in 1997, the potential was documented, and a potential operational application was outlined, followed up by a pilot study which has become the most seminal study in this field of science [1], and led to a full-scale proof of concept as well as the documentation of the first operational and commercial application of the methods for forest inventory for management purposes {2]. This series of studies was part of an overall strategy, incorporating operationalization and commercialization of research findings in Europe and North America, plus continuous improvement of the methodology, which is still ongoing. The overall innovation and research strategy is detailed in retrospect in [3]. Since around 2001 Gobakken took active part in this endeavour, and the research group has since gradually grown into a productive and method-innovation-oriented team (approx. 100 international peer-reviewed articles on remote-sensing based forest inventory).

New interdisciplinary areas of research
In recent years, the research focus of the group has expanded as the competence of the team has been broadened by recruitment of young scientists with educational background in biology, ecology, computer science, mathematical statistics, geomatics and geophysics, which in turn has stimulated more cross-disciplinary research (e.g. [4],[5]). Important contributions have also been made in collaboration with archaeologist and wildlife biologists. Important directions of the research since 2008 have been related to alpine and tundra treeline dynamics, albedo

seasonality and belowground carbon stock assessments. A common denominator of this research has been to explore the potential of combining field observations and novel sensor and sensing technologies operated on ground and from drones, airplanes and satellites, and use of emerging data analysis techniques such as machine learning, artificial intelligence and data assimilation as the growing volumes of data have permit effective use of such techniques. Research in this area has been curiosity- and technology-driven and has thus far had less of a focus on utility for private and public sectors in the short term. However, the work related to operational inventory applications (detailed above) and large-scale monitoring for climate mitigation (see below) has benefited from lessons learned here.

Global forest monitoring applications supporting international climate mitigation ambition

A contrasting line of research since 2009 has been quantification of forest biomass and carbon loss and uptake in tropical forests – from local to continental scales; in dry regions in Africa and in the Amazon basin, through a series of international research projects. This has built on advanced statistical approaches to estimation over vast tracks of land - first explored in Norway in collaboration with partners such as NASA and Yale University, and subsequently demonstrated and applied in tropical regions and in close collaboration with international bodies, such as UN/FAO and (e.g., [6]).

Research team:

Prof Dr Erik Næsset (all MINA, NMBU)
Prof Dr Terje Gobakken
Dr Ole Martin Bollandsås
Dr Hans Ole Ørka
Dr Victor Strimbu
Dr Svetlana Saarela
Dr Lennart Noordermeer
Dr Eirik Næsset Ramtvedt
Bjørn-Eirik Roald (technician)
Julius Wold (Researcher)

Current (PhDs: Ana Claudia Ferreira Aza, Benjamin Allen, Marie-Claude Jutras-Perreault, Claire Devos, Maria Åsnes Moan, Jaime Candelas Bielza.

3. References to the research (indicative maximum of six references)

[1] Næsset E, 2002. Predicting forest stand characteristics with airborne scanning laser using a practical two-stage procedure and field data. Remote Sens Env 80, 88-99.
[2] Næsset E, 2004. Accuracy of forest inventory using airborne laser-scanning: evaluating the first Nordic full-scale operational project. Scand J For Res 19, 554-557.
[3] Næsset, E. 2014. Area-based inventory in Norway – from innovation to an operational reality. In: Forestry Applications of Airborne Laser Scanning. Concepts and Case Studies (Maltamo, M., Næsset, E. & Vauhkonen, J. (eds)), Springer, pp. 215-240.
[4] Ramtvedt EN, Bollandås OM, Næsset E, Gobakken T.,2021. Relationships between single-tree mountain birch summertime albedo and vegetation properties. Agr Forest Meteor 307, 108470.
[5] Devos CC, Ohlson M, Næsset E, Bollandås OM, 2022. Soil carbon stocks in forest-tundra ecotones along a 500 km latitudinal gradient in northern Norway. Sci Rep 12(1), 1-10.
[6] Næsset E, McRoberts RE, Pekkarinen A, Saatchi S, Santoro M, Trier ØD, Zahabu E, Gobakken T, 2020. Use of local and global maps of forest canopy height and aboveground biomass to enhance local estimates of biomass in miombo woodlands in Tanzania. Int J Appl Earth Obs Geoinf 82, 102109.

4. Details of the impact (indicative maximum 750 words)

Forest inventory for operational forest management
Under the leadership of Næsset the team has over more than 25 years grown into a highly productive and competitive research group, as witnessed from the continuous success in acquiring externally funded projects (>50 representing more than 100M NOK since 2014) and the flow of publications (> 200 peer reviewed papers since 2011), and PhDs (15 since 2011) delivered by the group.

The impact of the research in the forest sector is obvious from the stepwise adoption of the new, continuously improved methodologies for forest resource assessment for operational forest management. A breakthrough came in 2002 when the methods pioneered by SKOGROVER were the basis for the first operational and commercial remotely sensed mapping of forest resources for practical forest management in Norway (Næsset 2004, 2014). It has since been modified and adopted to local conditions as a preferred methodology for commercial applications across Fennoscandia (e.g., Maltamo et al.2014), and beyond, including many countries in Europe, America, Australia and even Africa (e.g., Vauhkonen et al. 2014). Næsset received the Swedish Marcus Wallenberg prize for this breakthrough in 2011, considered the second most prestigious scientific prize awarded in Sweden (next to the Nobel prizes). As stated by the Prize Selection Commitee, the prize was awarded to Næsset "... *for his path breaking research that incorporates the airborne laser scanning method as an integral part of forest inventory. Prof. Næsset has significantly contributed to the development of methods for operational use of laser scanning in forestry which provides high quality estimates of forest variables at reduced costs. Professor Næsset's scientific work demonstrates prudently the progression of a research idea to implementation and how to make a scientifically developed method fully operational in practical forestry globally.*" (MWP, 2011).

Global forest monitoring applications supporting international climate mitigation ambition
The contribution to the international community at the interface between science and politics is evident from the intervention that was made in 2011 when Næsset initiated a dialogue between the scientific community and FAO at the beginning of an era that arose with establishment of the UN-REDD program (REDD: "Reducing emissions from deforestation and forest degradation in developing counties") where the Government of Norway played a leading role as demonstrated by prime minster Jens Stoltenberg's declaration at the Conference of the Parties to the UNFCCC in Bali, 2007. FAO reported: "*In February 2011, twelve prominent experts in forest inventory wrote to FAO and suggested holding a scientific meeting on forest inventory in relation to the requirements of REDD+. The concern expressed is that current approaches to REDD+ monitoring of forest carbon do not appear to take statistical analysis aspects sufficiently into consideration. Given the long experience of the developments in the science and application of national forest inventories, it may be valuable to the UNFCCC process to review this knowledge and its application for REDD+ monitoring. … On 31 May and 1 June 2011, the Expert meeting on assessment of forest inventory approaches for REDD+ was held at FAO headquarters in Rome, Italy. The meeting objectives were to develop preliminary criteria for evaluating the design of national forest inventory systems with respect to C and delta-C estimates; assess the needs and opportunities to contribute to the methodological guidance to be prepared by UNFCCC and IPCC; define how a science forum, facilitated by FAO, can be organized to advise countries on national forest inventories/monitoring for REDD+ and prepare key preliminary messages for the above."* (UN-FAO, 2011).

The impact of the subsequent scientific experience gained through the tropical research is evident from the new IPCC 2019 guidelines for greenhouse gas inventory under UNFCCC, in which methodologies that SKOGROVER has contributed to have been approved and adopted. Næsset served as lead author on the IPCC 2019 Refinement on greenhouse gas inventory on multiple sections, including new guidance on use of global biomass data from remotely sensed information for such inventories (IPCC, 2019), where concluding recommendations were based on research by SKOGROVER and collaborators (see McRoberts et al., 2022).

Likewise, SKOGROVER contributed substantially to technical guidance for tropical forest monitoring in compliance with the IPCC ("Methods and Guidance from the Global Forest Observation Initiative", GFOI 2020), which is being used by governmental agencies in many tropical countries and by the World Bank and other bodies managing funds in performance-based partnerships for reducing tropical forest loss and increased carbon uptake (GFOI, 2020). Næsset served as author on multiple sections of this guidance over the period 2011-2020. The work is to be resumed in 2023. The importance of the research underpinning the guidance in GFOI (2020) is evident from the 14 cited peer-review articles by SKOGROVER and international collaborators.

5. Sources to corroborate the impact (indicative maximum of ten references)

GFOI, 2020. Integration of remote-sensing and ground-based observations for estimation of emissions and removals of greenhouse gases in forests. Methods and Guidance from the Global Forest Observation Initiative, 3rd edition, 300 pp, https://www.reddcompass.org/mgd/resources/GFOI-MGD-3.1-en.pdf

IPCC, 2019. Volume 4, Chapter 2, Generic Methodologies Applicable to Multiple Land-Use Categories. In: 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Inventories, The Intergovernmental Panel on Climate Change, https://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_02_Ch2_Generic.pdf

McRoberts, et al. 2022. Statistically rigorous, model-based inferences from maps. Remote Sensing of Environment, 279, 113028, https://doi.org/10.1016/j.rse.2022.113028

Maltamo, M. & Packalen, P. 2014. Species-specific management inventory in Finland. In: Forestry Applications of Airborne Laser Scanning. Concepts and Case Studies (Maltamo, M., Næsset, E. & Vauhkonen, J. (eds)), Springer, pp. 241-252.

MWP, 2011. The Marcus Wallenberg Prize, 2011 Erik Næsset. Marcus Wallenberg Foundation, Sweden. https://www.mwp.org/%20Previous%20Winners/2011-erik-naesset-norway/?_gl=1*1nurc96*_up*MQ..*_ga*MTUzNzkzODIzOS4xNjczMzU0NzAy*_ga_9JZ4EGMVKB*MTY3MzM1NDcwMi4xLjEuMTY3MzM1NDcxMS4wLjAuMA.

Næsset, E. 2004. Accuracy of forest inventory using airborne laser-scanning: evaluating the first Nordic full-scale operational project. Scand. J. For. Res. 19, 554-557.

Næsset, E. 2014. Area-based inventory in Norway – from innovation to an operational reality. In: Forestry Applications of Airborne Laser Scanning. Concepts and Case Studies (Maltamo, M., Næsset, E. & Vauhkonen, J. (eds)), Springer, pp. 215-240.

UN-FAO, 2011. Expert meeting on assessment of forest inventory approaches for REDD+. Meeting Report. MRV Report 8, 2010. UN-REDD Programme, 18 pp. https://www.un-redd.org/sites/default/files/2021-10/Report8.pdf

Vauhkonen, J., et al. 2014. Inventory of forest plantations. In: Forestry Applications of Airborne Laser Scanning. Concepts and Case Studies (Maltamo, M., Næsset, E. & Vauhkonen, J. (eds)), Springer, pp. 253-268.

[MINA] – [impact case 4]

Institution: NMBU
Administrative unit: Faculty of Environmental Sciences and Natural Resource Management (MINA)
Title of case study: Stakeholder involvement in radiation risk management
Period when the underpinning research was undertaken: 2011 - ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 - ongoing
Period when the impact occurred: still expanding

1. **Summary of the impact** (indicative maximum 100 words)

The Environmental Chemistry Section at MINA has been addressing the broader societal impacts of radiation risks for over two decades. This has resulted in two key areas where management of radiation risks has changed in order to reflect the new knowledge. First, there is now a wide recognition in international nuclear emergency preparedness that assessment of radiation risks needs to go beyond assessment of the direct health impacts of radiation exposure, and include societal and psychological consequences. This has been particularly evident in the follow-up to the Fukushima accident in 2011. Second, addressing these broader dimensions requires stakeholder involvement in all areas of radiation risk management.

2. Underpinning research (indicative maximum 500 words)

Prior to 2011, Researchers at NMBU (Oughton) had worked extensively on the societal and ethical aspects of radiation risk management, in particular in connection with the Chernobyl accident. Following the Fukushima accident in 2011, there was an increased international interest in the broader societal aspects of risk management and especially the role of stakeholder involvement. The key hypothesis is that management of radiation risks needs to go beyond the direct physiological impact of radiation exposure (i.e., cancer risk) and address broader aspects such a psychosocial effects, socioeconomic consequences such as demographic changes and access to infrastructure, as well as ethical issues arising from the distribution of risks and benefits and consequences for vulnerable populations [1-3]. Likewise addressing the societal impact of radiation risks requires the participation of a range of stakeholders, and in particular the publics impacted by the decision. At the time of Fukushima, existing emergency preparedness recommendations had a strong technical focus, with less attention paid to social, ethical, psychological issues and the information tended to be directed towards the decisions made by experts rather than for support of affected populations.

Post 2011, this research was developed in a range of EU projects (PREPARE, NERIS, CONFIDENCE, SHAMISEN), as well as international initiatives such as the International Atomic Energy Agency (IAEA) Fukushima report (2017), International Commission on Radiological Protection (ICRP) on the Ethical Foundation of Radiological Protection (2018), as well as field studies and research in Fukushima in collaboration with Japanese researchers and institutions (2012-present). In 2016, Oughton and Tomkiv also participated in a project led by UK researchers focusing on opening up restricted areas in the Chernobyl Exclusion zone, where they had the responsibility for stakeholder engagement activities in the project.

Research carried out in Fukushima and Chernobyl has documented these broader aspects of radiation risks in affected populations, resulted in the implementation of stakeholder engagement in affected populations, and led to revisions in international guidelines for emergency preparedness (see concrete details in the following section). Broadly the impact can be defined as a systematic and on-going effort to enhance societal impact through two established bottom-up pathways, institutional stakeholder involvement and public grass roots citizen science engagement [4].

Lately, the research has been also been taken up in the EU RADONORM project (2020-2025), which also includes research on the social dimension of radon risk management [5], including an ongoing citizen science pilot in Gjøvik (Tomkiv) See: https://www.nmbu.no/en/services/centers/cerad/research/ra4/umb4c/radon-og-folkeforskning

The team:
Prof Dr Deborah Oughton
Dr Yevgeniya Tomkiv (postdoc MINA)
Prof Dr Lindis Skipperud
Prof Dr Ole Christian Lind
Dr Rani Lill Anjum

The research resulted in a PhD in Risk Communication (Yevgeniya Tomkiv, defended 2019), continuing in a post-doc project.

3. References to the research (indicative maximum of six references)

[1] Oughton DH, Howard BJ, 2012. The social and ethical challenges of radiation risk management. ethics, Policy Environ 15, 71-76 https://doi.org/10.1080/21550085.2012.672690
[2] Oughton DH, Liutsko L, Midorikawae S, Pirard P, Schneider T, Tomkiv Y. 2021. An ethical dimension to accident management and health surveillance. Env Int 153, 106537 https://doi.org/10.1016/j.envint.2021.106537
[3] Oughton DH, Hansson SO (eds), 2013. Societal and ethical aspects of radiation risk management (Elsevier: Amsterdam) ISBN 978-0-08-045015-5
[4] Tomkiv Y, Liland A, Oughton D, Wynne B, 2017. Assessing quality of stakeholder engagement: from bureaucracy to democracy. Bull Sci Tech Soc 37, 167-178. https://doi.org/10.1177/0270467618824027
[5] Muric M, Thijssen P, Turcanu C, Perko T, Tomkiv Y, 2022. Foxes caught in the same snare: a methodological review of social radon studies. J Risk Res. 10.1080/13669877.2022.2127850

4. Details of the impact (indicative maximum 750 words)

Our research on the societal impacts of radiation risks has been taken up by a number of national and international organizations working on strategies for nuclear emergency preparedness. Oughton led work on the societal impacts of the Fukushima accident and stakeholder engagement for the IAEA Review of the Fukushima accident published in 2017 [1]. The ICRP initiated a publication on the Ethical Foundations of Radiation Protection, led by Oughton 2014-2016 [2]. The research has also been brought forward in a number of EU projects, of which the SHAMISEN project has had the most direct impact on the way radiation risks are managed after a nuclear accident, and gives a good example of both the types of societal impacts and practical consequences of stakeholder engagement.

The EU-OPERRA SHAMISEN project started in December 2015, with the goal of producing a set of recommendations that would contribute to health surveillance and related communication with affected populations after nuclear accidents. SHAMISEN was co-ordinated by Elisabeth Cardis, ISGlobal, Spain, an internationally recognized epidemiologist. Within the SHAMISEN project, Deborah Oughton (NMBU/CERAD) was WP leader and responsible for the final SHAMISEN Recommendation document [3], as well as leading work on ethical aspects (e.g., changes in legal and ethical requirements for health surveillance and epidemiological studies related to data protection).

The SHAMISEN recommendations are based on reviews of guidelines in existence at the time of the Chernobyl and Fukushima accidents and of the actions which were taken, highlighting successes and limitations. The SHAMISEN Recommendations have had an impact at the international level, both by being recognized and endorsed by leading international organizations, including a direct impact on the management of radiation risks in Fukushima. The SHAMISEN recommendations were presented and discussed at an International Stakeholder Workshop in Paris on 24th March 2017 [4]. The final recommendations were widely disseminated in a variety of different formats (including infographics) and translated into Japanese, Russian, Spanish and French [5], and resulted in changes in which health impacts on affected populations are monitored. Recognition that monitoring needs to go beyond assessment of the direct radiation effects and include well-being and mental health, has been endorsed by the OECD Nuclear Energy Agency (NEA/OECD) work on Integration of Non-Radiological Aspects of Emergency Planning [6] and WHO through its Radiation Emergency Medical Preparedness and Assistance Network (REMPAN) programme [7]. Although the psychological impacts of nuclear accidents have been noted since the Three Mile Island and Chernobyl accidents, this is the first time that international organisations have proposed practicable recommendations to tackle the issue. Previously these were sidelined as the consequences of public irrationality without a deeper understanding of the factors that lead (and can potentially alleviate) public anxiety.

The work has been followed up by dedicated projects on the use of Health and Dosimetry Apps by affected populations, as well as examples of ethical challenges with personal dosimetry in Fukushima being addressed in other international projects on big data and machine learning [8]. All these projects have a strong, and practical stakeholder engagement dimension. Again, building on previous MINA research, SHAMISEN stressed the importance of remediation that not only addressed dose reduction but also supports community resilience and ensure sustainable infrastructure (jobs, hospitals schools). After an accident populations are not only concerned about health effects, but also about whether they will be able to lead a normal life, and with particular concern for children and future generations. These recommendations are supported by international developments including IAEA, NEA and ICRP [9, 10].

5. Sources to corroborate the impact (indicative maximum of ten references)

[1] IAEA, 2017. The Fukushima Daiichi Accident; Technical Volume 4/5. Radiological Consequences. IAEA: Vienna. Available from: https://www.iaea.org/publications/10962/the-fukushima-daiichi-accident

[2] ICRP, 2018. Ethical Foundations of the System of Radiological Protection. (Authors: K-W. Cho, M-C. Cantone, C. Kurihara-Saio, B. Le Guen, N. Martinez, D. Oughton, T. Schneider, R. Toohey, F. ZöLzer), ICRP Publication 138, Sage Journals https://doi.org/10.1177%2F0146645317746010

[3] Oughton, D., Albani, V., Barquinero, F., Chumak, V., Clero, E., Crouail, P., Fattibene, P., Kesminiene, A., Laurier, D., Liutsko, L., Ohba, T., Ostroumova, E., Pirard, P., Rogel, A., Sarukhan, A., Schneider, T., Tanigawa, K., Tomkiv, Y., Vale, L., Cardis E. 2017. Recommendations and procedures for preparedness and health surveillance of populations affected by a radiation accident. Shamisen report, pdf available at: https://issuu.com/isglobal/docs/recommendations_booklet

[4] SHAMISEN Stakeholder Workshop Paris, 2017. https://www.isglobal.org/en/new/-/asset_publisher/JZ9fGljXnWpI/content/el-proyecto-europeo-shamisen-concluye-conuna-reunion-de-actores-relevantes-en-paris

[5] SHAMISEN Infographics and media pages. https://www.isglobal.org/en/-/recomendaciones-para-mejorar-la-salud-de-poblaciones-en-caso-de-accidentenuclear

[6] Carr, Z., Maeda, M., Oughton, D., Weiss, W. 2018. Non-radiological impact of a nuclear emergency: Preparedness and response with the focus on health. Radiation Protection Dosimetry, 182: 112-119. https://doi.org/10.1093/rpd/ncy163

[7] OECD/NEA Workshop “Towards a better integration of Non-Radiological Public Health Aspects of protection strategies during Radiation Emergency Planning and Response“, March 2020. Specifically references SHAMISEN work. https://www.oecdnea.org/hans/
[8] WHO REMPAN The 15th meeting of the WHO Radiation Emergency Medical Preparedness and Assistance Network (REMPAN), Geneva 3-5 th July, 2017. See July Newsletter and Dropbox for lectures and presentations: https://www.who.int/ionizing_radiation/a_e/rempan/en/
[9] UNESCO Committee for Ethics of Scientific Knowledge and Technology, COMEST. Concept notes on Artificial Intelligence (AI) and Internett of Things (IoT). http://www.unesco.org/new/en/social-and-human-sciences/themes/comest/
[10] ICRP Update of Publication 109 and 111 http://www.icrp.org/icrp_group.asp?id=85

[MINA] – impact case 5

Institution: NMBU
Administrative unit: Faculty of Environmental Sciences and Natural Resource Management
Title of case study: Developing the world's first vaccine for honeybees
Period when the underpinning research was undertaken: 2011- ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-ongoing
Period when the impact occurred: still expanding

2. Underpinning research (indicative maximum 500 words)

The team of NMBU researcher Gro Amdam built the launch-pad for the first vaccination programs for honeybees. Honeybees are the most important commercial pollinators in agriculture, but diseases are causing problems — making fewer colonies available for producing honey and pollinating berries, fruits and vegetables. The most fatal disease of young bees is American foulbrood. American foulbrood is caused by a bacterium that attacks the bees while they are in the early larval stages. Amdam's team discovered how honeybee queens can vaccinate their young by consuming the pathogen orally, digesting the bacterium, and transferring bacterial pieces into their eggs (Salmela et al 2015). These pieces trigger the innate immune system so larvae are protected when they hatch. The vaccine developed from this principle was patented by Amdam's postdoc and team member Dalial Freitak, and Amdam's former PhD student and postdoc Heli Havukainen Salmela: PCT/FI2016/050541 – Patent "Edible vaccination against microbial diseases". The vaccine was approved for distribution in the USA in January 2023. Amdam continues to study the vaccine mechanism i.e. the physiology that allows the vaccines to be effective which includes the multifunctional protein Vitellogenin. Amdam's team is currently leading the world in research on this protein.

The core team:
Prof Gro Amdam, PhD (MINA NMBU and Arizona State University)
Prof Dalial Freitak, PhD (formerly postdoc. at MINA NMBU, currently Institute of Biology, University of Graz, Graz, Austria)
Heli Salmela, PhD (formerly at KBM NMBU during her graduate studies, currently Faculty of Biological and Environmental Sciences, University of Helsinki)
Vilde Leipart, PhD, (MINA NMBU during her graduate studies, starting her postdoc at MINA NMBU with mobility placement at London University College UK in July 2023)
Gyan Harwood, PhD (graduate student at Arizona State University)
Claus Kreibich, MS (technician MINA NMBU)

3. References to the research (indicative maximum of six references)

Leipart V, Ludvigsen J, Kent M, Sandve Simen, To T-H; Árnyasi, Kreibich CD, Dahle B, Amdam GV, 2022. Identification of 121 variants of honey bee Vitellogenin protein sequences with structural differences at functional sites. Protein Science 31 (7) e4369

Leipart V, Montserrat-Canals M, Cunha ES, Luecke H, Herrero-Galán E, Halskau Ø, Amdam GV, 2022. Structure prediction of honey bee vitellogenin: a multi-domain protein important for insect immunity. FEBS Open Bio 12: 51–70. doi: 10.1002/2211-5463.13316. Jounal cover: Volume 12, Issue 1 January 2022

Harwood G, Salmela H, Freitak D, Amdam G, 2021. Social immunity in honey bees: royal jelly as a vehicle in transferring bacterial pathogen fragments between nestmates. J Exp Biol. 224(Pt 7):jeb231076. doi:10.1242/jeb.231076.

Leponiemi M, Amdam GV, Freitak D, 2021. Exposure to inactivated deformed wing virus leads to trans-generational costs but not immune priming in honeybees (Apis mellifera), Frontiers in Ecology and Evolution 9, 626670

Harwood G, Amdam G, Freitak D, 2019. The role of Vitellogenin in the transfer of immune elicitors from gut to hypopharyngeal glands in honey bees (Apis mellifera). Journal of Insect Physiology 112:90-100.

Salmela H, Amdam GV, Freitak D., 2015. Transfer of Immunity from Mother to Offspring Is Mediated via Egg-Yolk Protein Vitellogenin. PLoS Pathogens 11(7):e1005015. doi: 10.1371/journal.ppat.1005015.

4. Details of the impact (indicative maximum 750 words)

Citing the USDA approval press release of Dalan Animal Health, which is the company of core team members Dalial Freitak and Heli Salmela:

“Honeybees are a critical component of agriculture. One-third of the global food supply relies on pollination, and healthy commercial hives are essential to secure high crop yields. However, honeybees are plagued by American Foulbrood, with previously no safe and sustainable solution for disease prevention. Overt clinical cases of American Foulbrood are notifiable in the USA and Canada, and former treatment methods relied on the incineration of bees and infected hives and equipment. We are committed to providing innovative solutions to protect our pollinators and promote sustainable agriculture. Global population growth and changing climates will increase the importance of honeybee pollination to secure our food supply.

Our vaccine is a breakthrough in protecting honeybees. We are ready to change how we care for insects, impacting food production on a global scale,”

Read more:
https://www.businesswire.com/news/home/20230104005262/en/First-in-Class-Honeybee-Vaccine-Receives-Conditional-License-from-the-USDA-Center-for-Veterinary-Biologics

5. Sources to corroborate the impact (indicative maximum of ten references)

Relevant project:
Launching the first vaccination programs for a beneficial, pollinating insect. FRIMEDBIO 2017-2022 Project Number: 262137

From the press:
https://www.nysgjerrigper.no/bladet/2016-2/bidronningen-lager-egen-vaksine/

https://forskning.no/immunforsvaret/har-lagd-vaksine-for-a-minske-biedod/1273930

https://www.nrk.no/viten/norsk-forsker-utvikler-vaksiner-mot-sykdom-som-dreper-biene-1.14318985

https://www.nettavisen.no/bier/usa/vaksine/godkjenner-verdens-forste-vaksine-for-honningbier/s/5-95-837587

https://www.dagbladet.no/nyheter/godkjente-vaksine-for-bier/78231478

https://www.cnn.com/2023/01/07/us/honeybee-vaccine-usda-approval-scn-trnd/index.html

https://www.nytimes.com/2023/01/07/science/honeybee-vaccine.html

https://www.bbc.com/news/world-us-canada-64180181.amp

VET Case number 1
Department of Paraclinical Sciences

Institution: NMBU
Administrative unit: Faculty of Veterinary Medicine (VET)
Title of case study: Piscine orthoreovirus (PRV) pathogenesis
Period when the underpinning research was undertaken: 2012 – 2015
Period when staff involved in the underpinning research were employed by the submitting institution: 2009 – 2022
Period when the impact occurred: 2012 - 2022

1. Summary of the impact (indicative maximum 100 words)

Piscine orthoreovirus (PRV) is the causative agent of heart and skeletal muscle inflammation (HSMI), a widespread and important disease in Atlantic salmon aquaculture. There have been significant knowledge gaps in the pathogenesis of the disease and no specific preventive measures to prevent disease. Research undertaken at the Department of Paraclinical Sciences identified red blood cells as a main target cell for PRV essential to the pathogenies. This finding enabled several new research projects, generating novel information about the virus and disease as well knowledge that can be used to mitigate the disease impact in aquaculture.

2. Underpinning research (indicative maximum 500 words)

The initial key research finding was the identification of red blood cells as a major target cell for not PRV. The red blood cell of mammals lacks nucleus and therefore do not support any virus replication, and due to this it was an intellectual and mental hurdle to cross before being able to realize that the fish' red blood cells, that are nucleated, may support virus replication. The infection of red blood cells was demonstrated through challenge experiments in fish (Ref 1) as well as under controlled laboratory settings in isolated red blood cells (Ref 2).

The underpinning research was headed by two researchers at Department of Clinical Sciences (Professor Espen Rimstad, Dr Øystein Wessel) from 2012 to 2015, and performed in collaboration with Dr Maria Dahle at the Norwegian Veterinary Institute and Dr Marie Løvoll at VESO Vikan. The initial research was funded internally by the VET faculty as part of a strategic effort to fill knowledge gaps for PRV and HSMI after the genetic mapping of PRV in 2010.

The initial findings were followed up by the same researchers at Department of Paraclinical Sciences which has led to a number of fundamental findings:

- Demonstration of cause relationship between the virus and disease (Ref 3)
- Development of experimental vaccine against HSMI (Ref 4)
- Inactivation studies for PRV (Ref 5)
- Demonstration of virulence differences for PRV variants (Ref 6)

In general, the initial key discoveries performed at Department of Paraclinical Sciences strategically supported by the VET faculty, had a significant impact which laid the foundation for a broad field of research within the salmon virus PRV. This includes several successful grant applications, establishment of international collaborations, a number of doctoral degrees (both at NMBU and at collaborating institutions such as DTU-Copenhagen) post-doctoral and

research positions as well a significant number of scientific publications including knowledge that could be applied by the Atlantic salmon aquaculture industry. (Details in section 4)

Key researchers at the administrative unit

- Professor Espen Rimstad
- Dr Øystein Wessel (First PhD student, Subsequent Post-doc position)

3. References to the research (indicative maximum of six references)

1) Finstad OW, Dahle MK, Lindholm TH, Nyman IB, Lovoll M, Wallace C, et al. Piscine orthoreovirus (PRV) infects Atlantic salmon erythrocytes. Vet Res. 2014;45(1):35.
2) Wessel Ø, Olsen CM, Rimstad E, Dahle MK. Piscine orthoreovirus (PRV) replicates in Atlantic salmon (Salmo salar L.) erythrocytes ex vivo. Vet Res. 2015;46(26).
3) Wessel Ø, Braaen S, Alarcon M, Haatveit H, Roos N, Markussen T, et al. Infection with purified piscine orthoreovirus demonstrates a causal relationship with heart and skeletal muscle inflammation in Atlantic salmon. PLoS One. 2017;12(8):e0183781.
4) Wessel O, Haugland O, Rode M, Fredriksen BN, Dahle MK, Rimstad E. Inactivated piscine orthoreovirus vaccine protects against heart and skeletal muscle inflammation in Atlantic salmon. J Fish Dis. 2018;41(9):1411-9.
5) Wessel Ø, Hansen EF, Løvoll M, Inami M, Husby A, Kruse G, et al. Inactivation of Piscine orthoreovirus. J Fish Dis. 2020.
6) Wessel Ø, Hansen EF, Dahle MK, Alarcon M, Vatne NA, Nyman IB, et al. Piscine Orthoreovirus-1 Isolates Differ in Their Ability to Induce Heart and Skeletal Muscle Inflammation in Atlantic Salmon (Salmo salar). Pathogens. 2020;9(12):1050.

4. Details of the impact (indicative maximum 750 words)

The initial identification of Øystein Wessel that red blood cells are a major target cell for PRV had a significant impact generating a number larger projects, doctoral candidates, international collaboration, new research tools and knowledge that could be applied by the aquaculture industry.

Externally funded projects

Many projects were initiated based on PRV infection of red blood cells in which Department of Paraclinical Sciences had a central role. These included studies of the consequence on fish health (Project 1). The infection of red blood cells was also utilized to develop a method to purify PRV, which is a key tool in virus research. Purified virus was used as a fundamental tool in several projects including vaccine experiments (Project 2), inactivation studies and study virulence differences (Project 3) as well as identification of the receptor used by PRV to enter red blood cells (Project 4).

1. HSMI-more (FHF #901001, 2014-2016)
2. Development of vaccines against HSMI and CMS (NFR#245286, 2015-2018)
3. PRV characterization: Inactivation and virulence (FHF #901305, 2017-2020)
4. VERIFIcation: Virus entry and receptors in fish (NFR #301477, 2020-2025)

Doctoral candidates

The projects included seven PhD candidates at NMBU which all studied PRV, where most have continued into different positions in the aquaculture industry.

- Øystein Wessel (2009-2014)
- Hanne Haatveit (2013-2017)

- Morten Lund (2014-2017)
- Magnus Vikan Røsæg (2014 – 2019, industrial PhD)
- Dhamotharan Kannimuthu (2016 - 2019)
- Salman Malik (2018 - 2021)
- Nina Askim Vatne (2019 -)

And at the collaborating institution: Technical University of Denmark:
- Niccolò Vendramin (2014-2018)
- Juliane Sørensen (2020 –)

International collaboration

The projects have included collaboration with a number national and of international partners, which has generated a solid network from both academia and the industry.

- PatoGen AS (Norway)
  - Magnus Devold, Morten Lund
- Pharmaq (Norway)
  - Marit Rode, Øyvind Haugland
- Norwegian Veterinary Institute (Norway)
  - Maria K Dahle
- VESO Vikan (Norway)
  - Marie Løvoll
- Nofima (Norway)
  - Gerrit Timmerhaus
- Fisheries and Oceans (Canada)
  - Kyle Graver, Mark Polinski
- Technical University of Denmark (Denmark)
  - Niccolo Vendramin
- University of Pittsburgh (USA)
  - Terrence Dermody, Danica Sutherland

Patents

One patent has been filed regarding viral genetic markers of PRV virulence (#346211).

Applied knowledge

The outcome of the projects has provided a significant contribution in the basic understanding og the virus and disease. Most of the projects has worked closely with the industry to provide information that has applied value. We have provided key data on inactivation of the virus which can be implemented by the aquaculture industry. The identification of variants with different virulence has provided an opportunity to target high virulent variants which is an ongoing approach to reduce the risk of disease.

5. Sources to corroborate the impact

- Maria Dahle (Norwegian Veterinary Institute)
- Marie Løvoll (VESO Vikan)
- Trine Marie L'Abée-Lund Normann (Norwegian Institute of Public Health)
- Sven Martin Jørgensen (Norwegian Seafood Research Fund)

VET Case number 2
Department of Preclinical Sciences and Pathology

Institution: NMBU
Administrative unit: Faculty of Veterinary Medicine (VET)
Title of case study: Melanin in salmon fillet
Period when the underpinning research was undertaken: 2008 – 2015
Period when staff involved in the underpinning research were employed by the submitting institution: 2000 – 2022
Period when the impact occurred: 2012 - 2022

1. Summary of the impact (indicative maximum 100 words)

Pigmented cells in the muscle of the Atlantic salmon form inflammatory clusters in the muscle or fillet that causes losses of an estimated NOK 1 billion a year in the Norwegian aquaculture industry. The changes must be manually removed before the fillet is sent out on the market, and on average, about 20% of all produced salmon is affected. The problem is also substantial in other salmon-producing countries, but data is not available due to lack of industrial transparency. At Section of Anatomy, we have shown that the lesions start with muscle bleedings that may develop into chronic inflammatory changes that attract immune cells capable of melanin synthesis, explaining the nature of these costly lesions.

2. Underpinning research (indicative maximum 500 words)

The initial key research finding was the identification of immune cells in salmon capable of melanin synthesis (Refs 1,2). Further, we showed that such cells were present in the melanised lesions and that they produced melanin on site (Ref. 3).

The underpinning research was headed by one at Department of Preclinical Sciences and Pathology (then Associated Professor Erling Olaf Koppang) from 2008 to 2015 with PhD students. At this point, melanin changes in the muscle were not a problem, and the aim of this work was to study the pigmentary component in the immune system of fishes. Later, the problem with pigmented lesions emerged in the industry, and then this research attracted attention from a broad audience.

The initial findings were followed up by the same researchers at Department of Preclinical Sciences and Pathology but now also in collaboration with the Virology group at Department of Paraclinical Sciences which has led to a number of fundamental findings:

- Demonstration of cause relationship between Piscine orthoreovirus (PRV) and severe pigmented lesions (Ref 4)
- The development and progression of bleedings into pigmented lesions (Ref 5)
- The inability of PRV to cause the changes in itself (Ref 5)
- Costal fractures may be involved in the initial muscle bleedings (Ref 6)

In general, the initial key discoveries performed at Department of Preclinical Sciences and Pathology strategically supported by the VET faculty, had a significant impact which laid the foundation for a broad field of research within the pigmentary system of fishes. This includes

several successful grant applications, establishment of international collaborations, a number of doctoral degrees as well a significant number of scientific publications including knowledge that could be applied by the Atlantic salmon aquaculture industry. (Details in section 4)

Key researchers at the administrative unit

- Professor Erling Olaf Koppang
- Associate Professor Håvard Bjørgen (First PhD student, Subsequent Associate Professor)

3. References to the research (indicative maximum of six references)

1) Haugarvoll E, Thorsen J, Laane M, Huang O, Koppang EO (2006) Melanogenesis and evidence for melanosome transport to the plasma membrane in a CD83+ teleost leukocyte cell line. *Pigment Cell Research* **19**, 214-225.
2) Thorsen J, Høyheim B, Koppang EO (2006) Isolation of the Atlantic salmon tyrosinase gene family reveals heterogenous transcripts in a leukocyte cell line. *Pigment Cell Research*, **19,** 327-336.
3) Larsen HAS, Austbø L, Mørkøre T, Thorsen J, Hordvik I, Fischer U, Jirillo E, Rimstad E, Koppang EO (2012) Pigment-producing granulomatous myopathie in salmon: a novel inflammatory response. *Fish and Shellfish Immunology,* **33,** 277-285.
4) Bjørgen H, Wessel Ø, Fjelldal PG, Hansen T, Sveier H, Sæbø HR, Enger KB, Monsen E, Kvellestad A, Rimstad E, Koppang EO (2015) *Piscine orthoreovirus* (PRV) in red and melanised foci in white muscle of Atlantic salmon (*Salmo salar*). *Veterinary Research*, Sep 8;46:89. doi: 10.1186/s13567-015-0244-6.
5) Bjørgen H, Haldorsen R, Oaland Ø, Kvellestad A, Kannimuthu D, Rimstad E, Koppang EO (2019) Melanized focal changes in skeletal muscle in farmed Atlantic salmon after natural infection with *Piscine orthoreovirus* (PRV). *Journal of Fish Diseases* **42**, 935-945.
6) Brimsholm M, Fjelldal PG, Hansen T, Trangerud C, Knutsen GM, Asserson CF, Koppang EO, Bjørgen H. Anatomical and pathological characteristics of ribs in the Atlantic salmon (*Salmo salar* L.) and its relevance to soft tissue changes. *Anatomica, Histologia, Embryologia*, in press.

4. Details of the impact (indicative maximum 750 words)

The initial identification that melanin production may occur in salmon leukocytes had a significant impact generating a number larger projects, doctoral candidates, international collaboration, new research tools and knowledge that could be applied by the aquaculture industry.

Externally funded projects

Many PhD projects were initiated based on the knowledge that melanin synthesis may occur in the immune system. And then, the problems of melanin in the fillet occurred, opening up the possibilities for external funding. Until then, the research on melanin in the immune system was a despised branch within the research community, as it did not follow the traditional research trends where all jump simultaneously in the same directions and good luck to get funding if you don't. These projects included a study addressing the impact of vaccination on the association of pigmented lesions (Project 1). Further projects have addressed the nature of the lesions (Project 2), the impact of virus infection of the pathogenesis (Project 3) and the development from red to black spots (as we found that to be the case under Project 3) (Project 4).

Projects:

1) Side-effects as a consequence of vaccination in salmonids; The Fishery and Aquaculture Research Fund, Project number: 900631, 2011 (headed by Erling Olaf Koppang)
2) Black spots in salmon; The Fishery and Aquaculture Research Fund, Project number: 900824, 2012-2015 (headed by Dr. Turid Mørkøre, NOFIMA)
3) The Piscine Orthomyxoreovirus and its impact on red and melanised Atlantic salmon muscle changes; The Fishery and Aquaculture Research Fund, Project number: 901221, 2026-2018 (headed by Erling Olaf Koppang)
4) Red spots and transition to black spots in salmon fillet; The Fishery and Aquaculture Research Fund, Project number: 901501 2018 – 2024 (headed by Erling Olaf Koppang)

Doctoral candidates

The projects included four PhD candidates at NMBU who all studied melanin in fish and now work as public servants, industry owners or within the academy.

- Erlend Haugarvoll (2005-2008)
- Jim Thorsen (2005-2008)
- Hilde Fagerland Larsen (2009-2013)
- Håvard Bjørgen (2017 – 2021)

International collaboration

The projects have been conducted without international collaboration. The substantial problem is that the foreign aquaculture industry is very secretive with respect to their problems with melanin. They are eager to talk to us, but when we ask to get something in return, they run away as fast as they can. Thus, it is also difficult for foreign researchers to get funding for this. The research abroad on this theme is probably conducted in dark rooms by the industry itself. My speculation, but it has to be so. So we have just given this up – waste of time.

Patents

No patents.

Applied knowledge

The outcome of the projects has provided a significant contribution in the basic understanding of a costly production disease in Norway. Further, we have put the melanin system together with the immune system in our research, and this is a fact, even though it is not main-stream research. Most of the projects have worked closely with the industry to provide information that has applied value. We have provided key data on the development of black spots and factors that may contribute to this, even though we cannot say what causes the initial bleedings. We have investigated the impact of virus infection in the pathogenesis and shown a connection towards severe cases.

5. Sources to corroborate the impact

- Sven Martin Jørgensen (Norwegian Seafood Research Fund)

VET case number 3
Department of Production Animal Sciences

Institution: NMBU
Administrative unit: Faculty of Veterinary Medicine (VET)
Title of case study: Fish mortality – classification of causes
Period when the underpinning research was undertaken: 2018 - 2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2012 to current
Period when the impact occurred: 2020 and ongoing

1. Summary of the impact

The case study comprises research undertaken to describe patterns of mortality throughout the life of farmed Atlantic salmon in Norway. Losses of fish during the sea-phase of production varies between units, and high mortality is not sustainable financially or for animal health and welfare reasons. Targeted management factors to prevent loss of fish can be employed if the underlying causes of death are known. A new classification system for fish mortality was developed by researchers at NMBU and is now implemented by the industry.

2. Underpinning research (indicative maximum 500 words)

Losses in salmonid aquaculture in Norway were found to be stable at around 13% in the period from 1998 to 2008, while the volume of production increased substantially. Questionnaire data related to fish groups transferred to sea in 2010/2011 investigated risk factors for mortality grouped into fish-specific factors (e.g. species, genetics, and generation), input factors (e.g. vaccines and smolt quality), environmental factors (e.g. geographical location), and managerial factors (e.g. ownership). Mortality varied by factors related to management, but also between smolt-groups suggesting that variable quality if incoming smolt will impact production at sea. The most important result was that production losses to a large extent are explainable, which implies that it should be possible to reduce these losses (Pincinato et al., 2021).

Both infectious diseases and management factors such as delousing can lead to mortality in farmed fish (Østevik et al., 2022 a, b, Sviland Walde et al., 2021). Mortalities vary substantially between fish-groups (Persson et al., 2021). Knowledge regarding the underlying causes of mortality in a certain group will enable targeted preventive measures to minimize losses. A project was therefore initiated with the objective to develop a standardized system for classification of mortality and losses in aquaculture. The WHO's International Classification of Disease (ICD) served as a model tool. The system is based on causality with the underlying cause of death as the principal variable to monitor and the classification system is organized with an alphanumeric code in a hierarchical structure with three levels. The hierarchy allows both registration and retraction of data at each of the three different levels. Fish farmers can use the coding system to monitor the total effect of underlying causes, both in biological and monetary terms. This will allow targeted interventions against mortality, to maximize effect of preventive measures. Through the establishment of a uniform classification system, it is possible to standardize registrations between area of losses within a company, between companies and regions, and potentially also between countries (Aunsmo et al., 2022).

The research related to classification of mortality causes was performed by researchers from the department of production animal clinical sciences in collaboration with collaborators from NMBU and externally. The research related to production losses and mortality was carried out in the period from 2018 onwards and published scientifically from 2020. Production databases were accessed retrospectively such that the described mortalities happened in historical production cycles, prior to 2018.

 Key researchers and their positions at the department of production animal clinical sciences:

Marit Stormoen, Associate professor, 2018 to current
Ane Nødtvedt, Professor, 2012 to current

Arnfinn Aunsmo, Associate professor II until 2022, Professor II 2022 to current
David Persson, PhD student, 2018 to current

3. References to the research

Pincinato RBM, Asche F, Bleie H, Skrudland A, Stormoen M. 2020. Factors influencing production loss in salmonid farming. Aquaculture
doi:https://doi.org/10.1016/j.aquaculture.2020.736034:736034.

Østevik L, Stormoen M, Hellberg H, Kraugerud M, Manji F, Lie K-I, Nødtvedt A, Rodger H, Alarcon M. 2022. A cohort study of gill infections, gill pathology and gill-related mortality in sea farmed Atlantic salmon (Salmo salar L.): Descriptive analysis. Journal of Fish Diseases
doi: https://doi.org/10.1111/jfd.13662

Østevik L, Stormoen M, Evensen Ø, Xu C, Lie K-I, Nødtvedt A, Rodger H, Skagøy A, Manji F, Alarcón M. 2022. Effects of thermal and mechanical delousing on gill health of farmed Atlantic salmon (Salmo salar L.). Aquaculture 552:738019.
doi: https://doi.org/10.1016/j.aquaculture.2022.738019

Sviland Walde C, Bang Jensen B, Pettersen JM, Stormoen M. 2021. Estimating cage-level mortality distributions following different delousing treatments of Atlantic salmon (salmo salar) in Norway. Journal of Fish Diseases 44:899-912.
doi: https://doi.org/10.1111/jfd.13348

Persson D, Nødtvedt A, Aunsmo A, Stormoen M. 2021. Analysing mortality patterns in salmon farming using daily cage registrations. Journal of Fish Diseases
https://doi.org/10.1111/jfd.13560

Aunsmo, A., D. Persson, M. Stormoen, S. Romstad, O. Jamtøy and P. J. Midtlyng (2022). "Real-time monitoring of cause-specific mortality- and losses in industrial salmon farming." Aquaculture
doi: https://doi.org/10.1016/j.aquaculture.2022.738969

4. Details of the impact

Our research into causes of mortality in farmed Atlantic salmon indicated that losses vary by smolt group, and also that there are large differences between fish-groups even within a site. If managers could understand the risk factors for mortality related to individual groups, targeted preventive measures could be employed where they yield the largest effect. Classification of losses into cause-specific mortality will aid in achieving the goal of effective health management and decreased losses. The proposed cause-specific mortality classification system developed by Aunsmo et al. (2022) has recently been incorporated in two of the major production system databases in salmon farming (Fishtalk and Mercatus). The system (levels 1 and 2) is further included in the revised national standard for disease classification NS9417 "Salmon and rainbow trout - Unambiguous terminology and methods for documentation of production" as part of a chapter on fish health (personal communication, Olav Jamtøy). A unified method of cause specific mortality classification has a potential to aid monitoring and strategic diseases control at a regional and national level. Beneficiaries are fish farmers, site managers in salmon aquaculture and companies producing salmon, because by decreasing mortality the profitability of fish farming will increase as output increases. The monetary benefit is particularly high during times when the price of salmon is high. The benefits in terms of improved health and welfare for millions of farmed fish are also substantial. High mortality at sea is not compatible with good animal welfare, and as a veterinary institution we are committed to improving health and welfare for animals in all production systems.

5. Sources to corroborate the impact

1. AKVA fishtalk - AKVA group
2. Mercatus Farmer | ScaleAQ
3. NS 9417:2012 (standard.no) (under revision)

[Nofima] [1]

Institution: Nofima
Administrative unit: Nofima
Title of case study: Documenting fish welfare in commercial aquaculture
Period when the underpinning research was undertaken: 2011 – 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 – 2021
Period when the impact occurred: 2018 - 2021

1. Summary of the impact

Fish welfare in aquaculture has been key thematic research area for Nofima since the field began gaining prominence in the late 1990's. This research area is complex and multifaceted and has a wide-ranging impact upon the aquaculture industry both within Norway and beyond. Nofima aims to better document and ultimately improve the welfare of farmed fish via our research, outreach and dissemination. We have adopted an integrated and multi-disciplinary approach to ensure the operational and societal utility of our research, both at the fundamental and applied level. In this case study we will outline the approaches we have used to developing tools for helping external stakeholders document fish welfare (and health) in aquacultural settings, and how we have applied these to differing species, life stages, rearing systems, routines and operations. Specifically, we will summarise how these tools have been compiled into operational toolboxes for commercial stakeholders in order to ensure that the tools are scientifically validated and fit for purpose for their farms. This case study will outline where our approach, in partnership with others, has had a large impact upon key Norwegian farmed fish species and also on how they have shaped how farmers and other interested stakeholders audit the welfare of the fish that are produced.

2. Underpinning research

For many years Nofima has undertaken fundamental and applied research addressing aquatic animal welfare, especially fish welfare in aquaculture. Whilst the theme is diverse, Nofima have adopted a comprehensive research portfolio, integrating differing fields to answer complex questions, such as the fundamental relationships between fish health and welfare or the bio-economic costs and benefits of implementing different types of welfare interventions in commercial aquaculture. A further aspect of our research in recent years has been to study the potential risk drivers and mitigation strategies for fish welfare challenges in aquaculture, primarily salmonid aquaculture, but also in other aquatic animals. The approach has generally been species-specific and is both wide-ranging and multi-disciplinary.

In the early 2000's it was noted by the research community that the there was a lack of knowledge on what welfare means from a fish's perspective, specifically on species- and life-stage specific welfare needs of different fish species. There was also a knowledge gap on how to document the fulfilment of these needs in farm settings. Welfare indicators are the tools that are used to audit the fulfilment of welfare needs and it was soon acknowledged that data on the type, selection and application of these indicators was also lacking, especially in relation to other key terrestrial farmed species. This became a key thematic area for Nofima, and our researchers have therefore developed a knowledge-building programme on differing aquaculturally applicable environmental and animal-based health and welfare indicators, identifying potential welfare related thresholds (where applicable) and also developing novel indicators that allow stakeholders to identify and document specific welfare threats.

A range of articles have been published from both fundamental and applied welfare research. Key insights on the development, application and utility of differing operational welfare indicators (OWIs) have been published and these have helped build a platform for the

development of species and life stage specific OWI toolboxes for key Norwegian farmed species. For example, going back to 2012, a series of review articles were published as an output of an EU COST Action (867 Welfish) on fish welfare, where Nofima researchers contributed to knowledge on the potential use and application of animal based OWIs in aquaculture including:

- A review on the use of fish behaviour as an OWI: A wide-ranging article on how behaviour can be used as an operational welfare indicator (OWI) for various farmed fish species, involving two Nofima researchers (Børge Damsgård and Chris Noble). It outlined the range of behaviours that have utility as OWIs, ways to document these behaviours and also the number and types of drivers that can influence how behaviour is expressed.
- A review on fish health as an OWI: A far-reaching paper on the utility of fish health as a fish welfare indicator, involving Hilde Toften and looking at the relationships between farming conditions and fish health and welfare as well as the application of health-related parameters as OWIs,
- Review on injuries and their welfare impacts: An extensive article on the influence of injury based OWIs upon aquacultural production and fish welfare, led by Chris Noble of Nofima and involving Børge Damsgård, Kjell Ø. Midling and Bjørn-Steinar Sæther. The article catalogued the different types of injuries farmed fish can be subjected to and their potential impacts upon fish welfare and aquaculture production, in additional to outlining some scientifically based mitigation strategies for reducing the occurrence of these injuries.

Further work has been carried out by Nofima in relation to the impacts of environmental based indicators upon fish welfare in aquaculture, in relation to existing and emerging welfare threats. An example of this is some of the work that has been carried out under the CtrlAQUA SFI project (RCN: 237856): during 2016-2017 an intensive experiment was carried out on the thresholds and limit values for levels of water-borne carbon dioxide in land-based recirculating aquaculture systems (RAS). This work was led by Vasco Mota of Nofima and Atlantic salmon post-smolts were exposed varying levels of CO_2 in RAS systems before being exposed to a standardised non-CO_2 exposure period for 6-weeks (sea water phase). Skin dermal thickness was significantly thinner at 40 mg/L CO_2 and growth was significantly reduced at CO_2 concentrations ≥ 12 mg/L. Additionally, blood parameters of stress and osmoregulatory properties were linearly correlated with water CO_2 concentration. The study concluded that post-smolt in RAS should be raised in CO_2 levels below the established recommendation of 15 mg/l CO_2, generating science-based knowledge that can be used to revise fish welfare guidelines if needed.

This type of knowledge can then be used to assemble OWI toolboxes for relevant farmed fish species, which is a challenging endeavour, even in cases where there is a comprehensive catalogue of science-based literature to build upon. From 2015 – 2020, the Norwegian Seafood Research Fund (FHF) funded a 9.2 MNOK project entitled “FISHWELL: Kunnskapssammenstilling om fiskevelferd for laks og regnbueørret i oppdrett” led by Nofima in partnership with scientists from the Institute of Marine Research (IMR), the Norwegian Veterinary Institute (NVI), Nord University (NU) in Norway and the University of Stirling in the UK. The aim was to collate and synthesise knowledge on the welfare of Atlantic salmon and rainbow trout in aquaculture and use this knowledge to develop fit-for-purpose OWI toolboxes for differing rearing systems and farming operations. Two handbooks outlining the latest knowledge on the welfare of Atlantic salmon and rainbow trout, in additional to appropriate and fit for purpose OWI toolboxes for different rearing systems and operations were release in 2018 and 2020 in both English and Norwegian language versions. Each comprehensive handbook was split into three parts: Part A addressed state of the art knowledge regarding the welfare of each species in relation to their welfare needs, Part B outlined fit-for-purpose OWI toolboxes for differing rearing systems and Part C adapted the same approach for differing a husbandry procedures and operations.

Names of key researchers involved:

- Fish behaviour review article: Chris Noble (Senior Scientist Nofima, 2007 – present); Børge Damsgård (Senior Scientist Nofima, 1995 - 2015)
- Fish health review article: Hilde Toften (Research Director Nofima, 1991- present)
- Injuries review article: Chris Noble (Senior Scientist Nofima, 2007 – present); Børge Damsgård (Senior Scientist Nofima, 1995 - 2015); Bjørn-Steinar Sæther (Senior Scientist Nofima, 2000 – 2018, part time 2018 - present); Kjell Ø. Midling (Senior Scientist Nofima, 1989 - 2017)
- Carbon dioxide article: Vasco Mota (Scientist Nofima, 2016 - 2023); Jascha Gerwins (Research technician Nofima, 2016 - 2021); Elisabeth Ytteborg (Scientist Nofima, 2006 - present); Grete Bæverfjord (Senior Scientist Nofima, 1987 - present); Jelena Kolarevic (Senior Scientist Nofima, 2008 – 2020; part time 2021- present); Bendik Terjesen (Senior Scientist Nofima, 2001 - 2017)
- Welfare indicator handbooks (both Atlantic salmon and rainbow trout): Chris Noble (Senior Scientist Nofima, 2007 – present); Jelena Kolarevic (Senior Scientist Nofima, 2008 – 2020, part time 2021- present); Bjørn-Steinar Sæther (Senior Scientist Nofima, 2000 – 2018, part time 2018 – present); Åsa M Espmark (Senior Scientist Nofima, 2001 - present); David Izquierdo-Gomez (Scientist Nofima, 2015 - present); Bjørn Roth (Senior Scientist Nofima, 2007 - present); Kjell Ø. Midling (Senior Scientist Nofima, 1989 - 2017)

3. References to the research

References are provided below with Nofima researchers highlighted. Google scholar citations also listed.

- Martins, C. I., Galhardo, L., **Noble, C.**, **Damsgård, B.**, Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J.C., Carter, T. and Planellas, S.R., (2012). Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38(1), pp.17-41. https://doi.org/10.1007/s10695-011-9518-8 Citations: 342.
- Segner, H., Sundh, H., Buchmann, K., Douxfils, J., Sundell, K.S., Mathieu, C., Ruane, N., Jutfelt, F., **Toften, H.** and Vaughan, L., (2012). Health of farmed fish: its relation to fish welfare and its utility as welfare indicator. Fish physiology and biochemistry, 38(1), pp.85-105. https://doi.org/10.1007/s10695-011-9517-9 Citations: 218.
- **Noble, C.,** Cañon Jones, H.A., **Damsgård, B.**, Flood, M.J., **Midling, K.Ø.**, Roque, A., **Sæther, B.S.** and Cottee, S.Y., (2012). Injuries and deformities in fish: their potential impacts upon aquacultural production and welfare. Fish physiology and biochemistry, 38(1), pp.61-83. https://doi.org/10.1007/s10695-011-9557-1 Citations: 110.
- **Mota, V.C.,** Nilsen, T.O., **Gerwins, J.,** Gallo, M., **Ytteborg, E., Bæverfjord, G., Kolarevic, J.,** Summerfelt, S.T. and **Terjesen, B.F.**, (2019). The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. Aquaculture, 498, pp.578-586. https://doi.org/10.1016/j.aquaculture.2018.08.075 Citations: 48.
- **Noble, C.,** Gismervik, K., Iversen, M. H., **Kolarevic, J.,** Nilsson, J., Stien, L. H., & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare. 351pp. ISBN 978-82-8296-556-9 https://nofima.com/press-release/download-the-fishwell-handbooks/ Citations: 153
- **Noble, C.,** Gismervik, K., Iversen, M. H., **Kolarevic, J.,** Nilsson, J., Stien, L. H., & Turnbull, J. F. (Eds.) (2020). Welfare Indicators for farmed rainbow trout: tools for assessing fish welfare. 310 pp. ISBN 978-82-8296-620-7 https://nofima.com/press-release/download-the-fishwell-handbooks/ Citations: 23

4. Details of the impact

Fish welfare is key to the overall sustainability of an aquaculture venture. Better tools and indicators for documenting fish welfare out on the farm mean farmers and other interested stakeholders can implement better welfare monitoring programmes. This in turn means welfare challenges can be detected at an earlier stage, and potential solutions or mitigation strategies can then be developed and implemented whilst a problem is still in its infancy. The impacts of better threat detection and early intervention will ultimately contribute to improved animal welfare in salmon farming, with reduced mortalities throughout the production cycle. Better fish welfare during production can also lead to a better-quality fish product on the slaughter and filleting line. This will lead to a more environmental, economical and societal sustainable salmon farming industry, both in Norway and beyond.

The FISHWELL project and its resulting handbooks on fish welfare have been well received by the salmon and trout industry and other interested stakeholders. All associated partners, led by Nofima, including the Institute of Marine Research (IMR), the Norwegian Veterinary Institute (NVI), Nord University (NU) (all Norway) and the University of Stirling (UK) utilised their communication departments to coordinate press releases and social media communications at the time each handbook was released. Project partners also hosted stakeholder workshops. This work was also coordinated with the communications team of the Norwegian Seafood Research Fund (FHF) that funded the project. Over 3000 PDFs of the handbooks have been downloaded from Nofima's internet servers (and this does not include those copies digitally shared by others) and over 1750 hard copies of the handbook have been freely distributed to Norwegian stakeholders. The rainbow trout handbook has also been translated into Finnish by the Finnish Fish Farmers' Association in 2021, see https://www.kalankasvatus.fi/wp-content/uploads/2021/01/Ty%C3%B6kalut-kalojen-hyvinvoinnin-arviointia-varten-.pdf

- Within months of release of the handbooks in 2018, Mowi, Cermaq Global and Grieg Seafood, some of the worlds biggest salmon farming companies, based their companywide fish welfare monitoring policies and frameworks upon FISHWELL.
- From 2019 onwards, the FISHWELL welfare monitoring toolboxes have also been used as the basis for fish welfare documentation programmes with regard to developing new technologies for the production and rearing of Atlantic salmon on land and in the sea.
- From 2019 onwards, newly developed welfare monitoring technologies that use computer vision and AI algorithms to document fish welfare in real time also use the FISHWELL OWIs as a basis for their algorithm development and utilisation.
- Since 2020, the Norwegian Food Safety Authority (Mattilsynet) have used FISHWELL as foundation material for some of their welfare guidance to Atlantic salmon farmers
- Since 2019 FISHWELL has also been used by various contracted Fish Health Services in their monthly health and welfare controls of Atlantic salmon farms in Norway.
- Some of the findings and outputs of FISHWELL are being utilised in an updated NS 9417 Standard "Salmon and rainbow trout — Unambiguous terminology and methods for documentation of production" by Standards Norway, with regard to fish health and welfare documentation terminology. This Norwegian standard is currently under final review and will be released in 2023.
- Since 2018 FISHWELL has been utilised in various University BSc and MSc courses on fish welfare in aquaculture e.g., at the University of Tromsø
- Since 2019, knowledge gaps identified in FISHWELL have also generated further research projects, including: the ongoing FHF project CrowdMonitor (901595) "Monitoring and optimising the crowding of Atlantic salmon using emerging health and welfare indicators", a partnership between Nofima and IMR; the Research Council of Norway project (295200) "Optimising Feed Withdrawal for Safeguarding Fish Welfare" a partnership between IMR and Nofima; the FHF project LAKSVEL "Utvikling og evaluering av metode for rutinemessig velferdsovervåkning av laks i norske matfiskanlegg" a partnership between IMR, NVI, Nord University and Nofima; the RCN project Welfare Severity (326980) "Frameworks for classifying the welfare of farmed Atlantic salmon based upon the principles of severity assessment", a partnership

between IMR, NVI and Nofima; and Digital Aqua - Digitalisation for better measurement of fish welfare (2019-2023, Nofima beacon funded project).

For supporting references, see below.

5. Sources to corroborate the impact

- Cermaq Global: a top three global salmon producer has implemented and based their fish welfare policy upon FISHWELL, see https://www.cermaq.com/news/cermaq-introduces-fish-welfare-policy
- MOWI, the world's largest salmon farmer, base their fish welfare documentation and monitoring programme upon FISHWELL (amongst others), see e.g., page 4 of https://mowi.com/wp-content/uploads/2021/07/210729-Mowi-Salmon-Welfare-Policy.pdf
- Grieg Seafood As is also utlising FISHWELL in the development of their welfare monitoring framework, see e.g., page 270 https://www.annualreports.com/HostedData/AnnualReportArchive/g/OTC_GRGSF_2020.pdf
- It is used a s a basis for welfare monitoring and documentation in new technologies and development licenses e.g., Cermaq/BioSort iFarm, see https://www.fiskeridir.no/Akvakultur/Tildeling-og-tillatelser/Saertillatelser/Utviklingstillatelser/Brev-og-vedtak/_/attachment/download/fa040e24-0b61-497d-b2cc-049f1ed83029:a8de8cc4ed2ebe3c0b0721085283cf5780b59653/tilsagn-Cermaq.pdf and SalMar Oceanfarm 1 https://www.salmar.no/wp-content/uploads/2016/06/OF_SR_16122019.pdf
- Numerous welfare monitoring technology companies use FISHWELL and its follow up LAKSVEL to standardise the documentation of fish injuries e.g., https://www.aquabyte.no/ny-teknologi-kan-bidra-til-a-lose-naeringens-storste-utfordring-fiskevelferd/
- The Norwegian Food safety Authority (Mattilsynet) base some of their fish welfare guidance on FISHWELL see e.g., https://www.mattilsynet.no/om_mattilsynet/gjeldende_regelverk/veiledere/veileder_om_fiskevelferd_ved_utvikling_og_bruk_av_metoder_utstyr_teknologi_mv_i_akvakultur_oppdatert_juni_2020.20481/binary/Veileder%20om%20fiskevelferd%20ved%20utvikling%20og%20bruk%20av%20metoder,%20utstyr,%20teknologi%20mv%20i%20akvakultur%20(oppdatert%20juni%202020)
- FISHWELL has been used as a basis for fish health and welfare documentation terminology for NS 9417 "Salmon and rainbow trout — Unambiguous terminology and methods for documentation of production" which is currently under final review https://www.standard.no/nyheter/nyhetsarkiv/fiskeri-akvakultur-og-mat/2021-nyheter/terminologi-for-laks-og-regnbueorret--standard-pa-horing/
- FISHWELL is used in guidance to document operational fish welfare by contracted Fish Health Services e.g., https://www.akerbla.no/vare-tjenester/fiskehelse/fiskehelse-og-fiskevelferd
- FISHWELL is used in various university courses on fish welfare in aquaculture e.g., at the University of Tromsø, BIO-3613 FISKEVELFERD I HAVBRUK - 5 STP https://uit.no/utdanning/emner/emne?p_document_id=786587

If specific referees are required:

- Karl Fredrik Ottem (Leader, Fish health, Cermaq Norway AS); Olai Einen (Head of Feed and Fish Health, Cermaq Global); Christine Thomassen (Fish Health Manager, Andfjord Salmon AS); Berit Seljestokken (Production Director, Grieg Seafood); Kjell Maroni (Director - Aquaculture, FHF); NS 9417 – Standards Norge

[Nofima] [2]

Institution: Nofima
Administrative unit: Nofima
Title of case study: Aquaculture breeding and genomics
Period when the underpinning research was undertaken: 2011 – 2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 – 2022
Period when the impact occurred: 2015 – 2022

1. Summary of the impact

Advancing traditional aquaculture selective breeding programmes to include genomics information, which results in increased genetic improvements of 20-50% per generation compared to traditional selection methods. These advancements increase sustainability of the aquaculture breeding programmes and of the production they serve, because they enable improved selection accuracy for e.g. disease resistance traits that have low accuracy when using traditional selection methods, and therefore increase survival rates. Both national and international beneficiaries have been targeted.

2. Underpinning research

It is well-known that selective breeding is a very powerful tool to improve traits of interest in closed populations of livestock. In aquaculture, we typically see genetic improvements of 5-10% per generation, and these improvements are cumulative over generations. We have contributed to the advancement of traditional selective breeding to also include genomic data for species and industry specific cases of aquaculture, which result in increased genetic improvements of 20-50% per generation compared to traditional selection methods.

Underpinning research activities include:

- Development of genomic tools, including linkage maps, SNP arrays and RAD (2b-RAD and ddRAD) marker sets;
- Gene mapping, gene expression and epigenetics studies;
- Optimisation of designs of genomic selective breeding programs by computer simulations, accounting for biological limitations and possibilities of aquaculture species and maturity of sectors;
- Selection methods that utilise genomics tools, including selection methods to reduce genotyping costs and single step methods for accounting for genotyped and non-genotyped individuals. Selection methods to constrain rates of inbreeding in ongoing genomic breeding programmes have also been developed.

All these genomics activities aim for increasing genetic improvement of aquaculture production, either directly by increasing accuracy of selection and/or by increasing genetic knowledge of the traits under selection. The activities include production of large datasets and quantitative genetics, bioinformatics and statistics skills required and fit for the specific purposes here. The genetic work has been accompanied by social science through ELSA (Ethical, legal and social aspects) and RRI (Responsible Research and Innovation) activities.

Research activities were on economically important traits related to productivity, feed efficiency, survival to specific diseases and product quality.

Research activities were on finfish, e.g. A. salmon, rainbow trout, A. cod, European seabass, gilthead seabream, barramundi and roho carp. We have also worked with lumpfish, tiger shrimp, whiteleg shrimp, abalone and lately also on low trophic species, such as Pacific oyster, blue mussel, Manila clam and seaweed species. Some features are similar across all aquaculture species, e.g. that large numbers of offspring are produced, but some reproductive and genomic features require specific adaptations. The maturity of the different sectors also affects the impact.

The above list shows activity for Norwegian aquaculture sectors and sectors from other countries in Europe and globally. The work has been done in collaboration with industry. For this work, the main national R&D partner is NMBU-IHA and the main international R&D partner is University of Edinburgh/Roslin Institute.

The research activities have taken place in the whole period 2011-2021.

These researchers have all contributed to the work described above, but in different degree:
Aslam, Muhammad Luqman, researcher 2015-2021, senior researcher 2021-
Bangera, Rama, -2015
Baranski, Matthew, researcher -2016
Boison Antwi, Solomon, researcher 2015-2018
Dagnachew, Binyam Sime, researcher 2018-2021, senior researcher 2021-
Storteig Horn, Siri, PhD student 2015-2018, researcher 2018-2021
Jacq, Celeste, researcher -2021
Kettunen, Anne Helena, researcher 2017-
Khaw, Hooi Ling, researcher 2017-2019
Leder, Erica, 2018-2020
Lillehammer, Marie, researcher
Moghadam, Hooman Khaleghi, researcher 2012-2018
Nielsen, Hanne-Marie, researcher 2007-2015
Olesen, Ingrid, research director -2019, senior researcher 2019-
Robinson, Nicholas, senior researcher
Sae-Lim, Panya, PostDoc 2015-2017, researcher 2017
Sonesson, Anna Kristina, senior researcher -2019, research director 2019-
Ødegård, Jørgen, -2012

3. References to the research

Robinson N.A., Robledo D., Sveen L. et al. Applying genetic technologies to combat infectious diseases in aquaculture, *Reviews in Aquaculture* 2022,1-45. doi:10.1111/ raq.12733..

Aslam, M.L., Robledo, D., Krasnov, A., Moghadam, H. K., Hillestad, B., Houston. R.D., Baranski, M., Boison, S., and Robinson, N. A. Quantitative trait loci and genes associated with salmonid alphavirus load in Atlantic salmon: implications for pancreas disease resistance and tolerance. *Scientific Reports* 2020, 10, 10393, doi.org/10.1038/s41598-020-67405-8

Lillehammer, M., Bangera, R., Salazar, M. et al. Genomic selection for white spot syndrome virus resistance in whiteleg shrimp boosts survival under an experimental challenge test. Scientific reports 2020, 10, 2057, doi: 10.1038/s41598-020-77580-3

Boison, S. A.; Ding, J.; Leder, E.; Gjerde, B.; Bergtun, P.H.; Norris, A.; Baranski, M.; Robinson, N.A. QTLs Associated with Resistance to Cardiomyopathy Syndrome in Atlantic Salmon. *Journal of Heredity* 2019, 110, 727-737, doi:10.1093/jhered/esz042

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4. Details of the impact

Atlantic salmon. The development of the NOFSAL SNP arrays, which contained ~55000 SNP markers across the genome in 2014 for the SalmoBreed and Mowi strains was an important starting point for the implementation of genomic selection in these two strains and a game changer for these two selective breeding programmes. Earlier work in collaboration with NMBU had presented optimal genomic selective breeding programme designs that built on existing salmonid breeding programmes. We had shown that genomic selection was robust to different sire/dam mating ratios (2016), which is important for the accuracy and bias of the breeding values. We had also shown the effects of genomic selection on inbreeding and developed methods to select parents with high genetic improvement rates while controlling rates of inbreeding in collaboration with NMBU (2011 and 2020). The SNP arrays also increased the activities on gene mapping studies for a multitude of traits in these two strains. There was a large focus on specific disease traits, e.g. resistance to pancreas disease and cardiomyopathic syndome/piscine myocarditis viruses and amoebic gill disease but also quality related traits, such as omega-3 content in the fillets. Similarly, gene expression studies gave information on the functional genomics of the same traits.

Other species. Introduction of genomics data in selective breeding programmes has also been done in several other species. Many of these aquaculture sectors are smaller than the A. salmon sector, so focus has been on developing methods to reduce genotyping costs, e.g. through within-family genomic selection (2013), pooling of groups with extreme phenotypes (2021). Also, we have optimised genomic selective breeding programme designs that keep families mixed (2010), which is the case for many global breeding programmes, because the investment needed to keep separate families is very high. Actually, the genomic data is essential when mixing families in breeding programs; it can be used to determine genetic relationships and to correct for structure in the data when performing the analysis.

Examples of species-specific genomic research activity in other species are:

- SNP arrays, including linkage maps: rohu carp and tiger shrimp (2014), lumpfish (2019), whiteleg shrimp (2020), European seabass, gilthead seabream and Pacific oyster (2021) and rainbow trout and A. cod (2022).
- QTL: resistance to aeromonas disease in rohu carp (2014), lice eating ability in lumpfish (2019), resistance to photobacteriosis in gilthead seabream (2018), resistance to infectious pancreatic necrosis in rainbow trout (2018), resistance to viral nervous necrosis in A.cod (2018) and European seabass (2021), resistance to *Sparicotyle chrysophrii* in gilthead seabream (2020) and resistance to ostreid herpes virus in Pacific oyster (2021).

Our main industry collaborators and primary beneficiaries of the work represent selective breeding companies or companies that have integrated selective breeding activities. They represent industries from the Atlantic salmon, rainbow trout, European seabass, gilthead seabream, barramundi, abalone, tiger shrimp and whileleg shrimp sectors. Some breeding programs are owned by universities or institutes, e.g. in rohu carp and Atlantic cod. A large number of aquaculture producers also benefit from the genetically improved stocks.

Nature and extent of impact. The implementation of genomic selection leads to increased sustainability of the breeding program and of the production it serves, because it enables improved selection accuracy for e.g. disease resistance traits that have low accuracy when using traditional selection methods. A key example is the measured impact of genomic selection on the resistance to white spot syndrome virus, a worldwide disease causing major loss, in the BMK Genetics whiteleg shrimp breeding population (2020). One generation of genomic selection resulted in 14% average improved survival.

For breeding companies that sell genetic material on the market, genomic selection and mapped genes are used to develop specialised products, thereby increasing their sales.

5. Sources to corroborate the impact

- How do we innovate? - Mowi Company Website and Mowi helps make an impact in breeding and nutrition - Mowi Company Website
- Three is the magic number for PD survival (fishfarmingexpert.com)
- Benchmark Genetics uses DNA tools to produce resistant shrimp - Benchmark Genetics (bmkgenetics.com)
- European aquaculture: FISHBOOST Newsletter 8 - January 2019 (campaign-archive.com)
- Global Aquaculture Genomics, Genetics, and Breeding Market | Size ,Share , Forecast 2019-2025 (briefingwire.com)

[Nofima] [3]

Institution: Nofima
Administrative unit: Nofima
Title of case study: Omega-3 fatty acids in feed for robust salmon
Period when the underpinning research was undertaken: 2013 – 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2013 – 2022
Period when the impact occurred: 2015 - 2021

1. Summary of the impact

Our projects showed that Atlantic salmon has a higher requirement for the omega-3 fatty acids EPA and DHA under challenging environmental conditions in sea than previously believed. This resulted in a paradigm shift in the Norwegian aquaculture industry, promoting them to increase the dietary levels of omega-3 fatty acids to secure fish robustness and fillet quality. Our projects also resulted in new knowledge on two novel omega-3 sources; oil from genetically modified canola crop and microalgae *Schizochytrium*. Results showed that both ingredients are safe, resulting in good fish performance, health and fillet quality. Both sources are commercially available and contribute to securing the total global availability of omega-3 ingredients and thereby securing further growth of aquaculture industry.

2. Underpinning research

Research projects related to omega-3 requirement

The **FHF project nr. 900770** (2013-2015)" *Optimized utilization of omega-3 throughout the life cycle*", with Dr. Gerd Berge as project leader, showed that the salmon body levels of EPA and DHA prior to sea transfer were of importance to salmon survival later in the sea. This knowledge was followed up in the project; "*Minimum requirements for omega 3 fatty acids in Atlantic Salmon"*, **NRC nr. 224913** (2013-2016). However, in this project the fish was followed only to 1.2 Kg, but new funds from **FHF, project nr. 900957** (2013-2015) "*Long-term effects of low omega-3 levels in feed on salmon growth, health and quality"*, enabled us to follow the fish further in sea cages under challenging environmental conditions until they reached 3,5 kg. These projects were led by Prof. Bente Ruyter, and with Dr. Marta Bou Mira as a PhD student. The main results showed higher mortalities after repeated delousing procedures and paler color of fillets in the groups fed low dietary levels of EPA and DHA. The barrier tissues, intestine and skin, were largely affected in their fatty acid compositions, a finding which was followed up in the **FHF project nr. 901265** (2016-2019); "*The importance of nutrition for skin, intestine and gill health in salmon*", led by Dr. Gerd Berge. Results showed that dietary omega-3 levels were of importance for the wound healing process in skin, Zn-uptake, and the intestinal barrier function. Findings from the aforementioned projects were followed up in co-financed **NRC nr. 273202** and **FHF nr. 901882** (2017-2021) projects; "*Optimizing dietary fatty acids and lipids of Atlantic salmon to secure their health and welfare through varying environmental conditions"*. The projects were led by Prof. Bente Ruyter and with Dr. Esmail Lutfi as a Postdoc. The main results showed that increasing dietary levels of EPA and DHA from 1.3% to 3.5% in feed improved fish health, red colour of fillet, and significantly reduced the occurrence of black melanin spots. In the ongoing project **FHF nr. 901656** «*Interaction between nutrients and significance for salmon health and quality*" (2021-2024), led by Prof. Bente Ruyter, the interactions between nutrients, in particular omega-3 fatty acids and Zn, and significance for salmon health and quality is further studied.

Research projects related to novel omega-3 sources:

In the **NRC project nr. 234057**; "*Heterotrophic microalgae for future marine omega-3 rich salmon feeds*" (2014-2017)**,** led by Dr. Katerina Kousoulaki**,** the importance of dietary microalgae as an omega-3 source at early life stages was studied. In the **FHF project nr. 901037** "*New sources of omega-3 in salmon feed"*, (2015-2019) led by Prof. Bente Ruyter, the

fish were followed further in sea cages to 3.5 Kg. The results showed that *Schizochytrium sp.* biomass is a good source of DHA in the salmon diet, leading to improved fillet quality without any negative effects on growth. The EPA requirement in salmon fed microalgae as DHA source is followed up further in the **NRC project IPN 319987** (2021-2024); "*Millennial salmon*", led by Dr. Katerina Kousoulaki. In the **FHF project 901037** "*New omega-3 sources*", another novel omega-3 source, DHA canola oil from genetically modified plant crops (DHA-CA), was also tested at different life stages. Analysis of number of health markers showed that DHA-CA is a safe omega-3 rich oil source to use in salmon feed in both freshwater and seawater providing similar growth performance as fish oil and improving color of skin and muscle.

Names of the key researchers:

Prof. Bente Ruyter, Senior scientist in Nofima, project leader.
Dr. Gerd Marit Berge, Senior scientist in Nofima, project leader.
Dr. Katerina Kousoulaki, Senior scientist in Nofima, project leader.
Dr. Marta Bou Mira, PhD student in 2013-2017, Research scientist in Nofima from 2017 to date
Dr. Esmail Lutfi, Postdoc 2018-2020, Research scientist in Nofima from 2020 to date.
Other researchers in Nofima that has made a major contribution to the research:
Dr. Tone Kari Østbye, Dr. Bjarne Hatlen, Dr. Trine Ytrestøyl, Dr. Elisabeth Ytteborg, Prof. Turid Mørkøre all senior scientists in Nofima.

Key contextual information about this area of research.
The "*UnderCurrent News"* report 2018 (*Editor: Tom Seaman),* pointed to the lack of omega-3 fatty acids EPA and DHA on the world market as the major bottleneck preventing further growth of the Aquaculture industry. The report highlighted the need for research on requirement for the essential omega-3 fatty acids in Atlantic salmon and for creating knowledge on novel omega-3 ingredients for use in Aquafeed.

3. **References to the research**

Selected published articles and reports related to omega-3 fatty acid requirement

Bou, M., Berge, G. M., Baeverfjord, G., Sigholt, T., Østbye, T.-K., Romarheim, O. H., Hatlen, B., Leeuwis, R., Venegas, C., & Ruyter, B. (2017a). Requirements of n-3 very long-chain PUFA in Atlantic salmon (Salmo salar L): effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. *British Journal of nutrition, 117*(1), 30-47. . DOI: https://doi.org/10.1017/S0007114516004396

Bou, M., Berge, G. M., Baeverfjord, G., Sigholt, T., Østbye, T.-K., & Ruyter, B. (2017b). Low levels of very-long-chain n-3 PUFA in Atlantic salmon (Salmo salar) diet reduce fish robustness under challenging conditions in sea cages. *Journal of nutritional science, 6*. DOI: 10.1017/jns.2017.28

Berge, G. M., Ytteborg, E., Østbye, T.-K. K., Sundh, H., Rud, I., Sveen, L., Bæverfjord, G., Karlsen, C., Krasnov, A., & Ødgaard, J. (2019). The importance of nutrition for skin, intestines and gill health in salmon. FHF report project 901265, *Nofima report 17/2019*. SBN: 978-82-8296-596-5 (pdf), ISSN 1890-579X.

Lutfi, E., Berge, G. M., Bæverfjord, G., Sigholt, T., Bou, M., Larsson, T., Mørkøre, T., Evensen, Ø., Sissener, N. H., & Rosenlund, G. (2022). Increasing dietary levels of the n-3 long-chain PUFA, EPA and DHA, improves the growth, welfare, robustness and fillet quality of Atlantic salmon in sea cages. *British Journal of nutrition*, 1-19. DOI: 10.1017/S0007114522000642

Selected published articles and reports related to novel omega 3 sources

Ruyter, B., Sissener, N. H., Østbye, T.-K., Simon, C. J., Krasnov, A., Bou, M., Sanden, M., Nichols, P. D., Lutfi, E., & Berge, G. M. (2019). n-3 Canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon. *British Journal of nutrition, 122*(12), 1329-1345. DOI: 10.1017/S0007114519002356

Kousoulaki, K., Berge, G. M., Mørkøre, T., Krasnov, A., Bæverfjord, G., Ytrestøyl, T., Carlehogs, M. & Ruyter, B. (2020). Microalgal Schizochytrium limacinum biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. *Frontiers in Marine Science, 7*, 57. DOI: 10.3389/fmars.2020.00057

Ruyter, B., Bou, M., Berge, G. M., Mørkøre, T., Sissener, N. H., Sanden, M., Lutfi, E., Romarheim, O.-H., Krasnov, A., & Østbye, T.-K. K. (2022). A dose-response study with omega-3 rich canola oil as a novel source of docosahexaenoic acid (DHA) in feed for Atlantic salmon (Salmo salar) in seawater; effects on performance, tissue fatty acid composition, and fillet quality. *Aquaculture, 561*, 738733. DOI: 10.1016/j.aquaculture.2022.738733

4. Details of the impact

It is reported 10-20% mortalities of Atlantic salmon in commercial aquaculture after sea transfer. Further it is estimated that approximately 20% of salmon farmed in Norway suffer from black melanin spots and there are many observations on pale/uneven fillet colour, all factors leading to production losses. Our research showed that increasing dietary levels of EPA and DHA, improved salmon survival, barrier tissue health, red fillet colour and reduced occurrence of dark melanin spots. These results promoted the aquaculture industry to increase the dietary levels of omega-3 in fish diets and thereby leading to improved fish performance, robustness and fillet quality. Our research showed that two novel omega-3 sources, DHA-CA and microalgae *Schizochytrium* are safe sustainable ingredients in fish diet and leads to good fish performance and fillet quality. Both sources are commercially available and contribute to the total global availability of omega-3 ingredients for aquaculture. The following industry internet

articles refer to the importance of Nofima's research; (*https://nuseed.com/study-finds-increasing-dietary-levels-of-epa-dha-in-fish-increases-weight-dietary-value/*); (*https://www.corbion.com/-/media/Corbion/Files/Misc-PDFs-1-of-6/1420011-cor-onepager-algaprime_v4_890878.pdf*); (*https://www.corbion.com/-/media/Corbion/Files/Misc-PDFs-1-of-6/1420011-cor-onepager-algaprime_v4_890878.pdf*). The reference states; Microalgae omega-3s are now included in more than 25% of Norwegian salmon feed. And the following article points to our omega-3 research of importance for rainbow trout omega-3 requirement; (*https://www.ewos.com/uk/news/unique-omega-3-research-on-trout*).

 Evidence of the nature/extent of the impact, **and dates when the impact occurred.** Figure 1. shows a major reduction in the fillet levels of omega-3 fatty acids from 2011 to 2016. The levels increased again in 2017, coinciding with our research on omega-3 requirement.

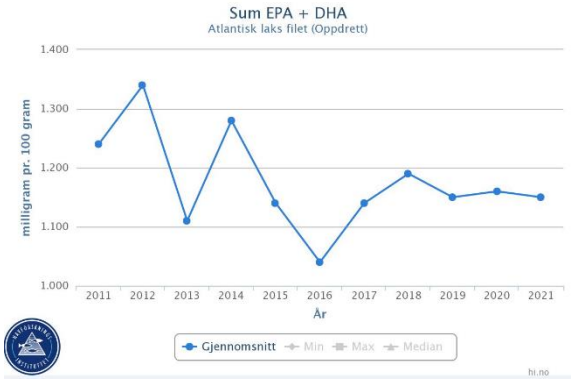

Figure 1. Historical overview of EPA and DHA content in salmon fillet over year. The figure is collected from institute of Marine Research (IMR) Sjømat data. https://sjomatdata.hi.no/#/substance/5550/-1

 Other key research collaborators that contributed to the impact
Institute of Marine Research (Dr. Nini Sissener), University of Stirling, UK (Prof. Brett Glencross), University in Oslo (Prof. Tor Gjøen), University of Gothenburg (Prof. Kristina Sundell), NMBU (Prof. Øystein Evensen).

 Details of the beneficiaries — how they have benefited, been affected, or impacted on. **Aquaculture industry**: More robust salmon with improved survival, pigmentation and increased EPA+DHA, and potentially improved sustainability, perceived sustainability by the consumers or future sustainability of salmon feeds and salmon products. **Fish feed producers:** Knowledge of omega-3 fatty acid requirement and novel sources for use in feed securing further growth of the industry. **Ingredients industry**: Producers of novel omega-3 sources. **fish consumers:** More healthy fatty acid profile and improved colour and reduced black melanin spots of salmon fillet.

5. Sources to corroborate the impact
 Dissemination: Results from the project were presented in dialogue meetings with industry, at national and international conferences and published in FHF reports in addition to scientific peer reviewed journals. There were also a series of News articles (a small selection shown below) which helped the research knowledge to reach the industry in order to be implemented.

Popular scientific news articles

1. Alger kan erstatte fiskeolje i fôr til oppdrettslaks, Forskning.no 22.09.2014
2. Jakter på nye kilder til omega-3. Kyst.no 17.03.2015
3. Omega-3 nivået i laksen halvert på 10 år. NRK Finnmark 24.06.2016
4. Laksen må klare seg på mindre omega-3 enn før, Forskning.no 23.06.2016
5. Manko på omega-3. Fiskeribladet Fiskaren 23.06.2016
6. Så lite marint omega-3 kan det være i fôret. Kyst.no 23.06.2016
7. Omega-3 i laks fra ny plante. Adresseavisen 25.04.2016, 24
8. Må finne mer omega-3 til oppdrettslaksen. Adresseavisen 22.04.2016, 28
9. GMO-raps kan bli ny oljekilde til laks. Kyst.no 01.12.2017
10. Oppdrettslaksen trenger marint omega-3. Kyst.no 04.05.2017

[Nofima] [4]

Institution: Nofima
Administrative unit: Nofima
Title of case study: Salmon farming in RAS
Period when the underpinning research was undertaken: 2015 – 2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2000 – 2017
Period when the impact occurred: 2015 - 2022

1. Summary of the impact (indicative maximum 100 words)

Nofima made Recirculation in aquaculture (RAS) part of the strategy during early 2000. To enable high quality research Nofima built the first RAS facility in 2010, in Sunndalsøra. The research done has filled in knowledge gaps regarding water quality requirements, water treatment methods, fish- and system performances that have been necessary for developing mainly Norwegian, but also international RAS industry besides contributing to research. In 2015, the focus on RAS also resulted in the funding of CtrlAQUA SFI, that has done research to develop technological and biological innovations to make closed containments to a reliable and economic sustainable technology. The focus on RAS has also resulted in the bi-annually conference “Smolt production in the future”, held in Sunndalsøra. Finally, the focus has resulted in expanding the infrastructure to meet the industrial needs for more research on RAS, and single-RAS units, where each tank has its own RAS system have been built in Sunndalsøra and Tromsø. This case will give three examples from CtrlAQUA research where the results have had high impact on the industry development.

2. Underpinning research (indicative maximum 500 words)

Carbon dioxide limits for Atlantic salmon:

The experiment was performed within the CtrlAQUA SFI during 2016-2017. The project was led by Nofima researcher Vasco Mota. The experiment aimed to contribute with RAS specific limit values for securing salmon health and welfare. Much established knowledge about water quality limit values originates from research performed in flow through (FT) systems, however the tolerance towards water quality in RAS and FT may differ. The hypotheses were therefore 1) Post-smolt salmon reared in RAS has a different CO_2 tolerance compared to general recommendations for salmonids and, 2) Post-smolts reared at high dissolved CO_2 levels have different physiological requirements compared to post-smolts reared at low dissolved CO_2 levels in RAS. In the experiment salmon post smolt were exposed to six CO_2 concentrations (5, 12, 19, 26, 33 and 40 mg/L) for 12-weeks (RAS phase) followed by non-CO_2 exposure period (< 5 mg/L) for 6-weeks (sea water phase). Fish exposed to 5-40 mg/l CO_2 showed no mortality, cataracts, nephrocalcinosis or signs of external injuries. However, skin dermis layer was significantly thinner in fish exposed to 40 mg/L of CO_2. Also, body weight and growth were significantly lower at CO_2 concentrations ≥12 mg/L. In matter of fact, growth showed a significant negative correlation with CO_2 levels from 5 mg/l CO_2, and the growth penalty followed the fish in the sea phase, even when CO_2 exposure had ceased. Additionally, blood parameters of stress and osmoregulatory properties were linearly related with water CO_2 concentration, and gill microarrays analysis showed 88 differentially expressed genes resulting from CO_2 exposure. The study concluded that post-smolt in RAS should be raised in CO_2 levels below the established recommendation of 15 mg/l CO_2.

Effects of Ozone on Atlantic Salmon Post-Smolt in RAS

This case contains of two experiments performed within CtrlAQUA, the first with Kevin Stiller in charge performed in 2018, and a second following up from the previous, led by Carlo Lazado during 2019 / 2020. Both researchers are from Nofima. Ozone is used as disinfection strategy in RAS systems, and from before it is known that ozone in freshwater improves water quality. It is desired to use ozone as a disinfectant also in saline water, however in saline water the

contact with ozone creates bromines that are toxic to fish. In the first short term study the aim was to define a safe level of ozone in brackish water that result in minimal welfare and health effects in salmon. Salmon were exposed to oxidation reduction potential concentrations of 250 mV, 280 mV, 350 mV, 425 mV and 500 mV. Fish exposed to 425 mV and higher showed ≥33% cumulative mortality in less than 10 days, while no significant mortalities were recorded in the remaining groups. Further, gill histopathology and genes for stress and inflammatory responses showed adverse effects of increasing ozone doses and the changes were more pronounced in the groups exposed to 350 mV and higher. The study concluded with a recommended ozone level in brackish water of ≤350 mV. With this recommendation as base, the second long term experiment took place, where salmon were exposed to 334 mV ozone for 45 days, with the aim to determine the health and welfare impacts on salmon post-smolts of continuous ozone application in brackish water RAS, using the safe threshold earlier identified. Besides low mortality, long term ozone treatment improved gill health in this experiment. Skin health and operational welfare were not affected. The second study thus verified the recommendation of safe levels of ozone in saline water.

Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt

The experiment, funded by CtrlAQUA (NFR) and FHF (Norwegian Seafood Research Fund) was a performed in RAS (2016), followed by a net pen sea phase (2017), and the fish were followed all the way to slaughter. This life-long experiment was led by Bendik Fyhn Terjesen and Trine Ytrestøyl, both from Nofima at the time of research. The experiment came up from a strong request from CtrlAQUA industry partners, because of the need to establish knowledge about how different relevant treatments during the RAS phase affect the fish on long term when they reach market size. The results showed that what might be the best protocol on short term, may not be the best in the end. The experiment tested a two-factorial design where fish were exposed to different light protocols (12h dark:12h light vs 24h light) and salinities (0 ppt vs 12 ppt). The fish were also transferred to net pens at different sizes; 100g, 200g and 600g. The fish group with the best growth in RAS was the one given 24h light and 12 ppt. However, at slaughter, the best growth was achieved by the fish given 12h dark:12h light during the RAS phase, while salinity had no effect. However, because of the head start of the 24h light group in RAS, the end weight at slaughter was higher for these fish. The smaller fish had better growth compared to the fish transferred to sea at 600 grams, however since the sea transfer happened at different times, these results are not regarded as solid.

This experiment has been followed up by additional life-long experiment within the CtrlAQUA umbrella, where duration of, and time for introducing winter signal were investigated, on request from the industry.

All three cases are examples of needed knowledge to further develop RAS industry, and they are examples of three key challenges. The first being that there is a need for RAS specific knowledge regarding environmental conditions such as water quality. Established recommendations that originate from research performed in flow through systems (FT) are still recommended also for RAS. Since conditions in RAS and FT differ, limit values for water quality variables may also differ. The dose-response study to find optimal CO_2 levels in RAS is one example of that fish perform better in concentrations lower than what is recommended. The next case is an example of knowledge needed for optimal disinfection of RAS systems. Pathogens and fouling are more potent in a RAS system because it is more closed compared to FT systems where foreign substances to a greater extent are washed out. Ozone have shown to improve water quality, but may have hazard effects on fish, especially in marine environments. Many RAS facilities are also aiming to keep fish longer on land and away from lice and the possibility to escape. The possibility for more control and manipulations in closed systems on land have resulted in that farmers and R&D have started to explore different protocols for smoltification, and other management procesures. Thus, there is a huge need to gain knowledge about how different protocols, such as smoltification protocol, what salinity to use and at what size the fish should be put in sea, affect fish performance, health and welfare at slaughter size, and quality of the product.

Carbon dioxide limits for Atlantic salmon

- Dr. Vasco Mota – Research scientist, joined Nofima in 2017
- Dr. Bendik Fyhn Terjesen – Senior research scientist, left Nofima autumn 2017

Effects of Ozone on Atlantic Salmon Post-Smolt in RAS

- Dr. Kevin Stiller - Research scientist, joined Nofima in 2018
- Dr. Carlo Lazado – Senior research scientist, Nofima
- Dr Åsa Maria Espmark - Senior research scientist, Nofima

Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt

- Dr. Bendik Fyhn Terjesen – Senior research scientist, left Nofima autumn 2017
- Dr. Trine Ytrestøyl – Senior research scientist, Nofima

3. References to the research (indicative maximum of six references)

Carbon dioxide limits for Atlantic salmon

Mota V.C., Nilsen T.O., Gerwins J., Gallo M., Ytteborg E., Baeverfjord G., Kolarevic J., Summerfelt S.T., Terjesen BF (2019). The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. Aquaculture 498, 578-586. https://doi.org/10.1016/j.aquaculture.2018.08.075

Mota V.C., Nilsen T.O., Gerwins J., Gallo M., Kolarevic J., Krasnov A., Terjesen B.F (2020). Molecular and physiological responses to long-term carbon dioxide exposure in Atlantic salmon (*Salmo salar*). Aquaculture 519, 734715. https://doi.org/10.1016/j.aquaculture.2019.734715

Effects of Ozone on Atlantic Salmon Post-Smolt in RAS

Stiller K.T. Kolarevic J., Lazado C.C., Gerwins G., Good C., Summerfelt S.T., Mota V.C., Espmark Å.M.O. (2020). The Effects of Ozone on Atlantic Salmon Post-Smolt in Brackish Water – Establishing Welfare Indicators and Thresholds. International Journal of Molecular Sciences, 21, 5109; doi:10.3390/ijms21145109

Lazado C.C., Stiller K.T., Reiten B-K.M., Osório J., Kolarevic J., Johansen L-H (2021). Consequences of continuous ozonation on the health and welfare of Atlantic salmon post-smolts in a brackish water recirculating aquaculture system. Aquatic Toxicology 238, 105935 . https://doi.org/10.1016/j.aquatox.2021.105935

Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt

Ytrestøyl T., Hjelle E., Kolarevic J., Takle H., Rebl A., Afanasyev S., Krasnov A., Brunsvik P., Terjesen B.F (2022). Photoperiod in recirculation aquaculture systems and timing of seawater transfer affect seawater growth performance of Atlantic salmon (*Salmo salar*). Journal of World Aquaculture Society 2022, 1-23. DOI: 10.1111/jwas.12880

4. Details of the impact (indicative maximum 750 words)

All three impacts described are examples of knowledge gaps that have been defined together with CtrlAQUA industry partners and that have been important for further development of RAS industry. All impacts have been disseminated alone and together with other CtrlAQUA results in different invited presentations where CtrlAQUA has been presented. For CtrlAQUA partners it has been important to have first access rights to results, and the described cases have been of special interest for farmers and product developers, that have implemented the results in their business. Through the CtrlAQUA midterm evaluation (2019), the user partners gave written statements confirming that results from “Carbon dioxide limits for Atlantic salmon” and “Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt” were implemented in their businesses. Later, during individual user partners meetings, the confirmation that results concerning safe use of ozone, and other disinfection protocols developed in CtrlAQUA, also have been implemented.

Carbon dioxide limits for Atlantic salmon
Fish farmers have taken the results into consideration, and search for methods to decrease CO_2 concentrations in their farms. However, since CO_2 is produced by the fish and biofilters, the only way to decrease CO_2 levels, is to remove it from the system with CO_2 stripping. CO_2 stripping is both expensive to install and also requires energy, that both farmers and product developers wish to reduce due to cost and environmental impact. Information from product developers is that decreasing CO_2 from 15 to 12 mg/l is cost demanding. However, the results have created many discussions between R&D and industry about the need and costs with decreasing CO_2. Besides being published, the results have been discussed with CtrlAQUA partners, and also disseminated externally in conferences (e.g. “Smolt production in the future, 2018 and Tekset 2020). The impact was led by Nofima, and has been a collaboration between R&D partners and industry partners within CtrlAQUA consortium, where Norce and Freshwater Institute, USA are the most important R&D partners.

Effects of Ozone on Atlantic Salmon Post-Smolt in RAS
Fish farmers that use marine RAS have hesitated to use ozone due to the risk for toxic effects of bromines, despite the need for disinfection. In addition, farmers have reported the need to be able to observe the fish in tanks, either with camara or manually, something that is not possible in high turbid RAS water. Ozone levels that have been reported as safe in other marine species are lethal for salmon (e.g. >500 Mv for turbot and Sea bass), thus to establish safe ozone limits for salmon have been crucial for the use in marine RAS, and it has not been done before. CtrlAQUA centre director has disseminated the results in several presentations for the farming industry, investment companies that have been interested in risks associated with RAS, in addition to R&D. Since the results in addition have research value they have also been published and presented at international conferences, amongst others at the World Aquaculture Society conference in Montpellier, France in 2018. The ozone research in CtrlAQUA has been in tight collaboration with R&D partner Freshwater Institute, USA, that specialise in freshwater RAS. They first established the safe ozone level in freshwater RAS, that is similar to marine RAS, and also provided with knowledge about the advantages for salmon health and welfare and water quality.

Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt
The need for running life-long experiments to see the whole picture of effects of management practices have been known for a long time, but the costs of running such experiment have made it difficult for most R&D environments. Within CtrlAQUA, the requests from the industry partners and the fact that the long lifetime of CtrlAQUA has enabled long term planning, made it possible to allocate project resources. Additional fundings from FHF finalised the possibility to run this experiment all the way to slaughter. The design was decided very much together with the industry partners, to make it commercially relevant. The industry partners have also requested regular updates on results as the experimental time has been long and divided in phases. The industry partners have participated in samplings, and a biotechnology partner of CtrlAQUA has used the results to investigate side effects of a vaccine developed by them, and they have used the results to develop a smolt test that is now patented. The results have been discussed in several fora where industry partners have been present, and CtrlAQUA farmers report that at some sites they now practice 24h light during the whole RAS phase. This practice is however not formally addressed as a recommendation, since health and welfare effects have not been fully investigated or understood. Besides heavily industrial involvement, the study is also in tight collaboration with Norce researchers and students, especially regarding smolt physiology. The results have been widely disseminated to industry and R&D, amongst others at the Aquaculture Innovation workshop in 2017, USA, hosted by CtrlAQUA partner Freshwater Institute.

5. Sources to corroborate the impact (indicative maximum of ten references)

Carbon dioxide limits for Atlantic salmon

CtrlAQUA partner Pure Salmon Kaldnes (contact person Frederic Gaumet; frederic.gaumet@puresalmonkaldnes.com), can corroborate on the importance of defining limit values for CO_2 in RAS. PSK has also been active in the discussions regarding reducing CO_2 from the recommended 15mg/l to 12 mg/l, due to the energy that this reduction will require.

In the Mid-term evaluation, Cermaq (Harald Takle; harald.takle@cermaq.com) wrote in the form D1 – Corporate partner assessment, that it was important to "run large experiments to address important biological questions related to production regimes of post-smolts e.g. transfer size of fish to sea, light regime, salinity, **CO_2**, density, water speed are examples of important research that the industry could not have done without the centre"

Effects of Ozone on Atlantic Salmon Post-Smolt in RAS

CtrlAQUA fish farmer partners can corroborate on the importance to have clear water in the RAS facility, and that ozone is facilitating this. Clear water is enabling the farmers to monitor their fish through camera and/or visual observations

FishGLOBE (Arne Berge; arne@fishglobe.no), is using the results from the impact to investigate the possibility to improve water treatment in FishGLOBE semi closed system at sea, and especially towards treatment towards bacteria causing wounds (e.g. Moritella viscosa).

Photoperiod in recirculation aquaculture systems and timing of seawater transfer of Atlantic salmon post-smolt

Grieg Seafood (contact person Knut Utheim; knut.utheim@griegseafood.com) reported during the Mid-term evaluation (2019) that they had dropped winter signal in RAS and ran the fish on 24h light.

In the same evaluation Pharmaq (contact person Rolf Hetlelid Olsen; Rolf.Hetlelid-Olsen@zoetis.com) confirmed that they from the experiment "… had the opportunity to follow antibody development after vaccination, in different production regimes (Benchmark) through the entire production cycle of farmed salmon".

In an interview with past Pharmaq Analytic contact person Siri Vike regarding SmoltVision (CtrlAQUA Annual report 2017 http://ctrlaqua.no/news/2018/04/06/annual-report-2017/), she said that "CtrlAQUA has developed the first commercial marker tests for use in closed containment aquaculture systems. Through the BENCHMARK project, they have developed "SmoltVision", which is a gene marker test for smoltification". Present contact person for Pharmaq Analytiq is Anne Katrine Reed; annekatrine.reed@zoetis.com

[Nofima] [5]

Institution: Nofima

Administrative unit: Nofima

Title of case study: Annual seafood industry analyses

Period when the underpinning research was undertaken: 2011-2021

One of the key strategic sources in Nofimas department – Industrial Economics (IE) - is a unique database with detailed longitudinal information on production, horizontal and vertical industry structure, and profitability in the marine value system at company, sector, and industry level. Efforts been made annually been made, and will be made, to further update and extend this database. This key research infrastructure is applied to develop new socio economic analyzes that are important for industry actors as well as public authorities involved in managing both wild marine resources and use of the coastal zone for aquaculture. In addition, IE has researchers that are in the international front in applying information technology and traceability instruments to understand how input material and product related information flow through food systems.

IE has expertise in evaluating how institutional instruments impact marine industries in terms of environmental, economic, institutional, and social sustainability. Accordingly, IE is strongly involved in documenting the claims made about IUU and mislabeling in seafood industry. An important output is to document how marine resources are utilized and impact on national, regional, and local value creation.

IE's effort to update and quality-assure a unique socioeconomic database provides contextual knowledge about new challenges and relevant research questions for the seafood industry. This also means that IE either receives direct questions from industry and/or public authorities about scientific explanations to why problems occur and suggestions for how such problems can be handled.

During the process of understanding why different problems arise, it is often revealed that explanations are linked to biology, technology and/or management. This means, among other things, that IE must collaborate with researchers from scientific areas related to biology, ecology and technology. At the same time, IE must be up to date on important institutional instruments and market conditions that are relevant to the problems studied.

In most of the projects that IE leads or participates in, this means that IE consists of researchers with many different professional backgrounds. It also means that the contributions from Nofima often improve understanding of how institutional frameworks affect the industry's performance along many dimensions and at the same time how institutional instruments can be adjusted to adapt them to new knowledge about biology and technological changes.

IE has four important groups of clients, and the Ministry of Trade, Industry and Fisheries (NFD) is the most important. The updated and quality-assured database, and annual industry analyses on structure and performance are important deliveries to the ministry. At the same time, IE has several projects exposed to competition for the ministry. In addition, IE's researchers participate at various levels in councils and committees appointed by NFD. Another important client for IE is The Norwegian Seafood Research Fund (FHF). All the projects managed for FHF are subject to competition - and the research group is one of the main suppliers to this fund in the social sciences area. In addition, IE has been active and successful in getting funding from the European Commission, achieved in strong competition with international research groups. Since the Horizon 2020 research program started in 2014, Nofima has had key roles (WP leadership) in 7 EU projects; 6 from Horizon 2020 and one from Horizon Europe which has just started. For Nofima, this has constituted from 2 to 4 Person

Years per year in the period mentioned. The fourth major client is the Research Council of Norway (NFR). IE has a relatively small part of its funding from the institute's basic grant from NFR, which means that most of the department's funds from NFR are open to competition. In addition, IE has several assignments from the industry and public authorities - both regional and locally.

IE has an annual project which aims to update and quality-assure a database that contains socioeconomic statistics in fishing fleets, sea farming, and the post-harvest industry. The database contains information at company level, sector level and industry level. The database contains financial accounting information and production statistics throughout the value chain in both the aquaculture industry and the wild catch industry. The database is unique in that it is detailed and adapted to study the seafood industry. It represents an unbroken time series that, among other things, contains financial accounting information at company-level in the post-harvest industry going back to 1976. This database is the most important research tool for Nofima to study the industry's performance from year to year. With the help of the base, we can measure developments in, for example, profitability, production, employment, greenhouse gas emissions in the entire industry or in various parts of the industry – nationally and locally.

Based on the database, IE provides frequently updates for NFD and industry on profitability and production in the industry. The database is also an important tool for evaluating the effect of various public measures and for assessing the consequences of various measures that are planned. Scientifically researchers are often invited to collaborate both nationally and internationally due to the database and their contextual knowledge (c.f. Form1).

Title: Annual seafood industry analyses

Period: 2011 -. Size: 16.5 Mill. NOK, Financed by The Ministry of Trade, Industry and Fisheries (NFD)

Objectives: Update, quality-assure and arrange economic data for analyzing and understand structure and profitability development in various parts of the Norwegian seafood industry.

Activities: Carry out annual profitability surveys, including updating an index that measures development in global competitiveness in the seafood sector. Make the data available for various studies on the effects of various management. Use these data in updated studies of economic and social sustainability in the seafood sector. Apply these data in multidisciplinary studies together with other research areas in Nofima. Based on this project NFD has decided to establish an agreement (rammeavtale) with Nofima to assist in producing relevant data and input to process documents NFD is preparing for government and/or parliament.

Nofima aims to develop knowledge through interdisciplinary research. The department has demonstrated partly by the mix of different social sciences represented among the employees. In addition, the researchers are often involved in projects where they collaborate with various parts of the institute with biological and technological scientific background.

Institutional frameworks are important for overall political goals to be achieved. The department is heavily involved in evaluating the effect of various public instruments, and at the same time central in developing a competence platform to assess how adjustments to institutional frameworks will affect the sustainability of the seafood industry and the ability to achieve overall goals for the industry. The Ministry of Fisheries is a major customer for the department and often ask the departments contribute in projects and in committees.

Through its insight into the industry's development, the department plays an important role in Nofima's search for relevant research questions. Through contextual knowledge, the department has an overview of barriers to overcome to improve different dimensions of

sustainability in seafood production. Thus, the department contributes to finding and prioritizing various relevant problems to focus on for researchers at the institute.

IE has contributed to contextual knowledge about the seafood industry's organization and performance along many dimensions of sustainability. IE has created a knowledge platform to understand how public authorities and institutional tools affect the industry's sustainability. The groups findings document how many goals in business policy often create tradeoff between objectives. Accordingly, an important task has been to create a knowledge base for prioritizing between the goals and designing business policy that mitigates the goal conflicts. In doing so, IE has contributed scientifically to how to solve conflict of interest in the coastal zone, how to solve conflict between different parts of a vertical integrated value system, how to deal with resource crime and resource waste, how to allocate licenses between actors and how to allocate resource rent between industry and society.

IE has distributed knowledge and findings in different ways, both locally and globally. Several of its findings are distributed globally in many EU-projects. In addition, socioeconomic knowledge is presented through membership in several ICES groups. The research group is member in the national fisheries management council and often member of different task forces appointed by the Ministry of fisheries.

Secretariat and scientific contribution to:

NOU 2014:16 Sjømatindustrien — Utredning av sjømatindustriens rammevilkår (Institutional framework for the Norwegian Post-harvest industry) (2013-2014):

til Nærings-og fiskeridepartementet 16. desember 2014 (NFD).

https://www.regjeringen.no/contentassets/b8395c5e287846c281e434173d733

Member of committee and scientific contribution to: Rapport, 2016, Forenklinger og forbedringer innen førstehåndsomsetningen av fisk (Improvements in the first-hand marketing of fish) (NFD)
https://www.regjeringen.no/no/dokumenter/forenklinger-og-forbedringer-innen-forstehandsomsetningen-av-fisk/id2524716/

Scientific contribution to NOU 2016: 26 Et fremtidsrettet kvotesystem (A system for future quota allocation) (NFD)
https://www.regjeringen.no/contentassets/3716cc15332f4cf683f01a50159d712a/no/pdfs/nou201620160026000dddpdfs.pdf

Member of committee and scientific contribution: Rapport 2016, Vurdering av leveringsplikten, bearbeidingsplikten og aktivitetsplikten (Assessment of the delivery obligation, the processing obligation and the activity obligation) (NFD)
https://www.regjeringen.no/contentassets/1ee88df85fb94e57949e0972fdd5f399/rapport---vurdering-av-leveringsplikten.pdf

Leader, member of committee, and scientific contribution to: NOU 2019: 21 Framtidens fiskerikontroll (Future resource control) (NFD)
https://www.regjeringen.no/no/dokumenter/nou-2019-21/id2680187/?ch=1

Member of committee and scientific contribution to: Rapport om grønn verdiskaping og økt bearbeiding i sjømatindustrien, 2022, (Report on added value in Norwegian post-harvest industry) (NFD)
https://www.regjeringen.no/no/dokumenter/rapport-8-mars-2022/id2898776/

Dreyer, B. (ed.) (2016): Mål, virkemiddel og effekt (Management objectives, means and impact) Special edition of Økonomisk Fiskeriforskning, September. (https://okonomiskfiskeriforskning.no/mal-virkemiddel-og-effekt/)

This special issue of Økonomisk Fiskeriforskning summaries scientific findings related to objectives and impact of different institutional instruments, how the industry actors adapt to them and what effect they have had on the seafood industry. The special issue covers the period from 2011-2016 and contains 46 different publications. Most of the authors are from the research group, part-time researcher at the department or students that were supervised by researchers at the department. It shows that the research group has extensive collaboration with various universities - nationally and internationally. The implications of the findings reported were important because several committees were set up by NFD to modernize the Norwegian fisheries management. As illustrated in Form 3. researchers from the group were active in those committees. At the same time, a book was published aimed to be a knowledge platform in the public debate about Norwegian fisheries management.

Industry-relevant research results are shared through: Nofima's report series.

Participation and presentations on business conferences.

Participation in committees where scientific input are given on various topics.

Information relevant for public authorities is shared through:

Separate reports are often published where public institutions are client. Such assignments are often linked to the evaluation of a specific public instrument.

Participation in public committees.

Research seminars for organized for employees in NFD.

Researchers are often asked to provide scientific input to consultation documents where industry is allowed to make comments on various public measures that are planned to be implemented.

As experts in public organizations that investigate various challenges in the seafood industry.

Academically relevant information is shared through:

International publication and as reviewers for international scientific journals.

FBA Impact case 1 Climate change and biodiversity

Institution: Nord University
Administrative unit: Faculty of Biosciences and Aquaculture
Title of case study: Effect of climate change on biodiversity
Period when the underpinning research was undertaken: 2019 to present
Period when staff involved in the underpinning research were employed by the submitting institution: 2020 to present
Period when the impact occurred: 2020 to 2022

1. Summary of the impact

The Ecology Group's Biogeography and Biodiversity unit research informed the 6th Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) Working Group II; the leading world scientific body that informs the UN Conference of the Parties to the Paris Agreement (COP) on the science behind climate change (UN SDG 13 Climate Action), a global crisis that needs urgent action. This included being first, lead and contributing AR6 authors, plus 21 peer-reviewed research papers (12 data analysis and 8 review papers). This contribution was recognised by being a co-recipient of 2022 "Gulbenkian Prize for Humanity".

2. Underpinning research

Since 2019, our continuing research has shown that (1) marine species have been shifting their distribution in concert with climate change for decades at global (with latitude) and regional (twice as many fish species now in Arctic seas since 1980s) scales, and since the last ice age, (2) terrestrial species endemic to mountains and islands were most at risk of extinction due to climate change (supported by the only two climate related extinctions), (3) how most fish species body size will shrink with climate warming but some Arctic species growth improves, (4) the parallels between the COVID pandemic and climate change crises, and (5) how the greatest risk to marine and freshwater biodiversity is current human impacts and not climate change. This research underpinned contributions to IPCC AR6, including qualifying and clarifying extinction risks to biodiversity and supporting earlier models that predicted global redistribution of marine species due to climate change which to date had only had evidence at regional scales.

This contributed to informing society and governments on the severity of climate change and how to respond under United Nations Sustainable Development Goal (SDG) 13 on Climate Action, while sections of the IPCC AR6 and associated publications covered many other SDG; from physical, chemical, biological, social sciences, to governance, with emphasis on 1 (poverty), 2 (hunger). 3 (health), 5 (gender), 6 (water quality), 7 (energy), 9 (industry), (inequalities – notably for indigenous communities), 11 (sustainable communities), 12 (responsible consumption), 14 (life underwater), and 15 (life on land).

Key Researchers involved:

Mark John Costello, Professor; Led authorship of the IPCC AR6 WGII Cross-Cutting Chapter on "Biodiversity Hotspots" [1]. Co-authored the Regional Chapter for Australasia [2], Glossary [3], Technical Summary for Policy Makers [4], and Cross-chapter Box on links between the COVID pandemic and Climate crisis [5]. Additional editorial contributions were made to the WG II Atlas [6] and Key Risks assessments [7]. In preparation of the report 12l supporting papers conducted new data analysis [8] and 8 critically reviewed the literature [9]. A public summary of the overall report was co-authored with all the IPCC AR6 WGII Norwegian authors [10] and a critique of the IPCC process [11].

Charles Lavin and **Francesco Gordo Vilaseca,** PhD candidates; led publication of research papers in the *Proceedings of the National Academy of Sciences a*nd two in the journal *Environmental Biology of Fishes*, plus two literature reviews.

3. References to the research

Gordó-Vilaseca C. Stephenson F, Coll M, **Lavin** C, **Costello** MJ. 2022. Three decades of increasing fish biodiversity across the north-east Atlantic and Arctic Oceans. *Proceedings of the National Academy of Sciences* in press.

Lavin CP, **Gordó-Vilaseca** C, **Costello** MJ, Shi Z, Stephenson F, Grüss A. 2022. Warm and cold temperatures limit the maximum body length of teleost fishes across a latitudinal gradient in Norwegian waters. Accepted 05 May 2022, *Environ Biology of Fishes*.

Lavin CP., **Gordó-Vilaseca** C., **Costello** MJ, Shi Z, Stephenson F, 2022. Warmer temperature decreases maximum size of six species of marine fish, crustacea and squid in New Zealand. *Environmental Biology of Fishes*, online.

Chaudhary C, Richardson AJ, Schoeman DS, **Costello** MJ. 2021. Global warming is causing a pronounced dip in marine species richness at the equator. *Proceedings of the National Academy of Sciences* 118 (15), online.

Kocsis, Á. T., Zhao, Q., **Costello**, M. J., and Kiessling, W. 2021. Not all biodiversity rich spots are climate refugia. *Biogeosciences* 18, 6567–6578. https://doi.org/10.5194/bg-18-6567-2021.

Manes S., **Costello** MJ, et al. 2021. Endemism increases species' climate change risk in areas of global biodiversity importance. *Biological Conservation* 257, 109070. 11 pp

4. Details of the impact (indicative maximum 750 words)

The research directly informed climate change science and policy through assessment of evidence, critical review of the literature, and new analyses of biodiversity data. This included looking at the distributions of species since the last glacial maximum (ice age) and last century, until the present year (references cited above plus [8]). The data showed that the number of marine species peaked at the equator during the last glacial maximum (c. 20,000 years ago). However, this peak had flattened after the ice age and now showed a dip at the equator. Further analyses of data since the 1950s showed this dip in number of species was deepening in line with ocean warming, such that the latitudinal gradient in marine diversity now peaks in the mid-latitudinal subtropics. These statistically significant empirical observations, adjusted for sampling effort, confirmed predictions that such shifts in marine species distributions would occur due to climate change, particularly ocean warming. The previous IPCC assessments relied on predictions. AR6 advanced this through accumulating observations of biodiversity confirming these predictions. Our work was cited in several WGII chapters because it was the first to show global scale responses of marine biodiversity.

To check the hypothesis that the areas most rich in species globally were past climate refugia, we compared the geographic variability of climate change across marine and terrestrial environments over time. We found that all regions, even those species-rich spots, were and would be affected by future climate warming. Thus, while the amount of warming varies geographically, there are no absolute refugia from climate change at regional spatial scales.

All our work was published into peer-reviewed journals and cited in IPCC WGII AR6. Although AR6 WGII was published in 2022, the bulk of the preparation was done in the previous three years with late 2021 and early 2022 being devoted to proof reading and addressing feedback from peer-reviews and UNFCCC countries representatives.

The IPCC is the scientific body commissioned by countries of the world to report on climate change science under the United Nations Framework Convention on Climate Change (UNFCCC). The IPCC reports directly informed the UNFCCC Conference of the

Parties (COP27) where participants prioritised measures to address climate change. These agreements have far reaching impacts for national economies and human well-being in the short and long-term.

Thus, the primary impact of our research was in directly (as an author) and indirectly (through our research) contributing to the IPCC AR6 WGII in 2022, which informed the UN COP the same year [1-7]. In addition, it contributed to an invitation to be a lead author of a section of a new State of the Ocean report by the Intergovernmental Oceanographic Commission of UNESCO on biodiversity [9].

5. Sources to corroborate the impact (indicative maximum of ten references)

[1] **Costello**, M.J., M.M. Vale, W. Kiessling, S. Maharaj, J. Price, and G.H. Talukdar, 2022: Cross-Chapter Paper 1: Biodiversity Hotspots. In: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 2123-2161. doi:10.1017/9781009325844.018. Supplementary Material at https://www.ipcc.ch/report/ar6/wg2/. https://doi.org/xxx.

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[5] van Aalst M, Cisse G, Ayanlade A, Berrang-Ford L, Bezner Kerr R, Biesbroek R, Bowen K, Caretta MA, Cheong SM, Chow W, **Costello** MJ, et al. 2022. Cross-Chapter Box COVID | COVID-19. In: Cissé, G., et al. 2022: Health, Wellbeing, and the Changing Structure of Communities. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, et al. (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 1041–1170, doi:10.1017/9781009325844.009.

[8]

Pelling M, Biesbroek R, Caretta MA, Cissé G, **Costello** MJ, Ebi KL, Gunn EL, Kerr RB, Parmesan C. Schuster-Wallace C, van Aalst MK, Woodward A, 2021. Synergies between COVID-19 and climate change impacts and responses. *Journal of Extreme Events*, online.

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[10] Benjaminsen TA, Buhaug H, Børsheim KY, **Costello** MJ, Eriksen SH, Skern-Mauritzen M, Stenseth NC. 2022. New report by the IPCC: climate adaptation is happening too slowly. March 9, 2022, Peace Research Institute Oslo (PRIO) Blogs.
[11] **Costello** MJ, Kelly KS. 2022. The pandemic shows that global climate and biodiversity science assessments need to be annual. *Climate Research* 87, 199-202. https://doi.org/10.3354/cr01695

APPENDIX: Supplementary information

These publications arose from this activity and are available through Google Scholar or IPCC website:

[6] IPCC, 2022: Annex I: Global to Regional Atlas [Portner, H.-O., et al. (eds.)]. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Portner, et al. (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 2811–2896, doi:10.1017/9781009325844.028. [member of editorial team]
[7] O'Neill, B., et al. 2022: Key Risks Across Sectors and Regions. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, et al. (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 2411–2538, doi:10.1017/9781009325844.025. ["Contributing Author"]

[8] Supporting data analysis published in peer-reviewed journals.

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[FBA] [Impact case 2: Sustainable Feeds]

Institution: Nord University
Administrative unit: Faculty of Biosciences and Aquaculture (FBA)
Title of case study: Sustainable feeds
Period when the underpinning research was undertaken: 2012 -
Period when staff involved in the underpinning research were employed by the submitting institution: Since 2009 permanent scientists at FBA have been performing this research to promote sustainable aquafeeds. New members recruited to the faculty in 2013 and 2017 have further strengthened the research on this theme
Period when the impact occurred: 2016 -

Summary of the impact
When we contemplate the increasing demand for food for the growing population, the type of food and its production criteria should be reconsidered globally from a sustainability perspective. FBA uses interdisciplinary approaches to educate the next generation of the workforce by inculcating appropriate knowledge on the importance of quality and suitability of novel sustainable feed ingredients/additives for livestock and fish used as food. Importantly, our research on sustainable feed components taps directly into the “mission1” in the long-term plan for research 2023-2032 of the Norwegian Government (Meld. St.5 2022-2023).

2. Underpinning research

The unique geopolitical situations have increased the uncertainties linked to supply chains and the demand for land, finite resources and food. Research at FBA considers anthropogenic climate change also as a factor that adds risk to food security. Based on this background, FBA has targeted novel feed components for livestock and fish production to generate knowledge for education and to be a catalyst for attaching sustainability to human food fish and animal production.

Milk, egg and fish fillet are essential sources of high-quality nutrients for humans. Protein supply (kg/capita/year) from dairy products has been reduced in most regions of the world[1]. FAO reports[2] that people around the world now consume more aquatic foods, which provide 17% of animal protein. However, the stagnating signs of wild fish harvest and the importance of preserving ecosystem services necessitates increased seafood production through aquaculture. Farmed animals and aquaculture products with high-quality proteins and other nutrients are necessary to feed the world population estimated to reach 10.4 billion in 2100[3], suggesting the increasing demand for food. Therefore, feed ingredients and feeding strategies will determine the sustainability of animal production for human food. For farming carnivorous fish as well as monogastric terrestrial animals, ideal feed ingredients should be derived from low trophic sources. Prominent among them are photosynthetic macroalgae and microalgae that capture carbon dioxide, inorganic nutrients and sunlight, elevating them as sustainable feed ingredients. Using insects grown on biowastes as feed components is also an alternate route to sustainability. Sustainability is not achievable without adopting preventive strategies to maintain the health of farmed animals; this includes manipulation of intestinal microbes through probiotic administration.

Based on the research performed during 2019-2022, the group of Prabhat Khanal has identified Norwegian seaweeds that possess anti-methanogenic activities in ruminants, an important result that can be exploited to establish a future sustainable livestock sector (1, 11). In addition, the research initiated (2020-) by Prabhat Khanal’s team found that mealworms reared on agricultural byproducts from mid-Norway have similar nutritional profiles and digestibility values as that of

[1] https://www.sciencedirect.com/science/article/pii/S1751731121001300?via%3Dihub

[2] https://www.fao.org/publications/sofia/2022/en/

[3] https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf

commercial soybean meal (unpublished results). Insects have a large potential as alternative feed ingredients, and they can recycle low-grade byproducts and biowaste sustainably (12). For a decade (2009-) the team led by Kiron Viswanath and Mette Sørensen has performed research on microalgae to reveal their suitability as feed ingredients for different fish species but primarily Atlantic salmon - either the whole biomass (13), or co-product from biofuel production (13, 2), whole cells or broken cells (21). In addition, the team has gathered evidence on the probiotic organisms-induced impact on salmon health (6, 22, 23). Thus, FBA's scientists have led many projects on sustainability in animal farming in collaboration with industry and research partners to provide meaningful and practical knowledge to nudge the aquafeed industry and start-ups to follow a sustainable path using next-generation feed ingredients and health-guarding additives.

Kiron Viswanath (KV), Professor in Aquaculture, 2004-: Four decades of experience in aquaculture research with focus on preventive health to devise better nutritional strategies aimed at improved health management and sustainability in aquaculture. For over 10 years his research targeted the application of microalga as aquafeed ingredient and has led/partnered several projects on this theme with funding from US Department of Energy – Large-scale production of fuels and feed from marine microalgae (Cornell Univ., 2013-2015). Marine Algae Industrialization Consortium (Duke Univ., 2017-2022), EU-COFASP/RCN-MarineAlgae4Aqua (2017-2020), RCN (Algae to Future 2017-2021). KV is also investigating algae-derived bioactive compounds for their health promoting benefits within the H2020 project Algae4IBD (2021-2025).

Prabhat Khanal (PK), Associate Professor in Animal Nutrition, 2018-: Main research area is animal nutrition and physiology with a special focus on lipid metabolism, nutritional programming, anti- methanotrophic ingredients, low-trophic feed ingredients with low CO_2 footprint. PK is the PI on several projects funded by the RFF Trøndelag: The application of an automated and integrated livestock management system for ruminants with seaweed-based novel feeding ingredients (2021-2022); Exploring the anti-microbial properties of brown seaweeds (2020-2021); From by-products to novel protein: Bio-resource recycling using insect larvae (*Tenebrio molitor*) (2020-2021); Seaweeds: an alternative and anti-methanogenic feedstuff for future livestock production (2019-2020); Insect-based diets improve the egg quality by altering gut health and metabolism in layers (2023-2024). PK is leading an international knowledge transfer project on sustainable feed development in Nepal: Establishing the Circular Economy-based livestock sector through collaborative Educational and Research activities (CEER) funded by Norwegian Ministry of Education and Research/Foreign Affairs, NORPART/DIKU (2022-2026).

Margarita Novoa-Garrido (MN), Associate Professor in Animal Nutrition, 2015-: The research field of MN is within applications of new environmentally friendly feedstuffs and additives to increase sustainability and self-sufficiency in food production. MN focuses on seaweeds and its products, their interaction with ruminal and intestinal microbiome, and its anti-methanogenic properties. MN has led several projects investigating the composition and nutritional value of different seaweed species and its products in feeds for livestock, as well as suitable preservation methods for seaweeds. The most recent project Ensiled cultivated macroalgae as sustainable ruminant feedstuff (EnMac) was funded by RFF-Nord targeting ensiling macroalgae and studying their nutritional effects on farmed animals.

Mette Sørensen (MS), Professor in Aquaculture, 2013-: The key research areas of MS are linked to novel feed ingredients, fish nutrition, alternative feed ingredients, feed manufacturing technology and effect of feed ingredients on fish health. She has extensive knowledge on nutritional quality of feed ingredients and is leading many projects including NON-Food Organic Resources-based feeds optimized for salmon until post-smolt stages (Non-Fôr), funded by Nordforsk/RCN (2021-2023). She is also a collaborative partner in the project: Norwegian-grown renewable pigment from microalgae for robust salmon, funded by the RCN (2021-2023). She is leading other ongoing collaborative projects with the aquaculture industry.

3. References to the research

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2. **Sørensen, M.**, Gong, Y., … **Kiron, V.**, 2017. *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. PLoS ONE July 2017. **(cited by 87).**
3. **Kiron, V.**, **Sørensen, M.**, … Gong, Y., … Palihawadana, A.M. 2016. Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Frontiers in Marine Science 17. **(cited by 82)**.
4. Gong, Y., … **Sørensen, M.**, **Kiron, V.** 2019. Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L). Aquaculture 501, 455-464. **(cited by 69).**
5. Yen, Y., Weisbjerg, M. R., … **Novoa-Garrido, M.** 2022. Improving fermentation of *Saccharina latissima* and *Alaria esculenta* silages with additives for preserving biomass and antioxidants. Journal of Applied Phycology, 34, 625-636. **(cited by 4)**.
6. Nimalan, N., Sørensen, S. L., … **Kiron, V.**, **Sørensen, M.** 2023. Supplementation of lactic acid bacteria has positive effects on the mucosal health of Atlantic salmon (*Salmo salar*) fed soybean meal. Aquaculture Reports 28, 101461. **(recent publication).**

4. Details of the impact

The interdisciplinary research environment at FBA has fostered sustainability-oriented studies on fish and farm animals, facilitating the establishment of a toolbox for, among others:

i) Assessing the effects of novel feed ingredients on animal health and performance and food fish quality,

ii) Evaluating anti-methanogenic effects of novel feed ingredients of both land-based (e.g., insects) and marine (e.g., seaweeds, microalgae) bioresources,

iii) Examining the life cycle assessment of novel feed ingredients in collaboration with project partners.

Our research outputs have been collated in review articles written along with our partners. The findings have been conveyed to the scientific community through our participation in aquaculture and industry-related conferences in Europe and beyond. The importance of incorporating microalgae and insect meals in aquafeeds has been discussed in radio and TV talks and with popular newspaper journalists by Mette Sørensen, Kiron Viswanath and Prabhat Khanal. In addition, sharing the information about our research on next-generation feed ingredients–through Nord webpages, forskning.no, NordForsk, Sjømat Norge and through open Research Days–has enabled us to reach more comprehensive sections of the society, even the Minister of Fisheries and Ocean Policy, noticed our efforts. The expertise we honed by focusing on sustainable feed components has attracted collaborations from different parts of the world. We have been invited as partners into RCN industry-oriented project consortiums, focusing on high-value components such as astaxanthin from microalgae and EPA and DHA from microbes. In another instance, our research presented at AlgaEurope 2019 led to us partnering in an EU project in the biomedical field. The infrastructure and knowledge generated through research funding linked to sustainable feeds have enabled us to team with prominent feed companies. Along with them, we are examining next-generation feed ingredients, a learning process for both parties to make an impact on the sustainable production of aquafeeds. Considering our expertise, microalgae-based start-ups in Norway invited the Nord team to test their products using the already developed toolbox. Nord's research activities along with a start-up company that produces microalgae-based products for aquafeeds, and a feed company have helped in directing the companies to consider new feed formulations with the novel feed component. Furthermore, our research has caught the attention of NCE Aquaculture Cluster, a key institution that helped bring in companies that are willing to carry out large-scale cultivation of cold-adapted microalgae that Nord has been studying for many years - a commercialization project is under consideration by RCN. Through international funding, companies in Portugal have already produced the cold-adapted microalga strain (studied at Nord) on a commercial scale and the biomass was incorporated into salmon feeds, which was eventually tested in a feeding study at Nord. Another interesting impact of our research on microalgae is that our efforts have been extended to the health benefits of algal bioactive compounds. This research is already funded by an ongoing EU project of Kiron Viswanath, and we are also collaborating with the Nordland Hospital on grant applications to anchor this research regionally. Thus, microalgae research at Nord has already made footprints not only in Norway and Europe but also in the US and in Asia. Although the group of Mette Sørensen is in the first phase of insect meal-based studies, the team

is poised to gather enough evidence to provide information to the aquafeed industry and further develop this line of research. On the other hand, the team of Prabhat Khanal has generated the know-how for insect culture and has succeeded in securing a project that aims to educate students from Nepal on sustainable feed production, demonstrating the international reach of our research. Finally, our research on microorganisms as feed additives and the long-term collaboration with the University of Veterinary Medicine and Pharmacy, Košice, Slovakia, has enabled us to file a patent application and submit a commercialization project to RCN. Our research on the application of microbes in feeds as an alternative approach to health management is expected to have a greater impact in the future.

Overall, our sustainable food production-oriented research has contributed to the development of knowledge for education and applied research, continuation of research outputs, development of new grant applications, widening network and collaborations, cooperation with aquafeed and microalgae companies, and the society at large, significantly augmenting the efforts of Norway to be self-reliant on animal feed ingredients.

5. Sources to corroborate the impact

Patent

7. https://search.patentstyret.no/Patent/20210585?caseIndex=0

Communication

8. https://www.trondelagfylke.no/en/nyhetsarkiv/spennende-samarbeidsprosjekt/
9. https://klimalandbruk.no/kan-tang-og-tare-erstatte-soyamel-i-forrasjonen-til-drovtyggere-og-samtidig-bidra-til-reduserte-metangassutslipp/
10. https://innocamp.no/1225-2/
11. https://site.nord.no/nonfor/communication/

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12. Pandey, D., Mansouryar, M., **Novoa-Garrido, M.,** Næss, G., **Kiron, V.**, … **Khanal, P.** 2021. Nutritional and anti-methanogenic potentials of macroalgae for ruminants. 2021, Burleigh Dodds Science Publishing Limited. **(cited by 4)**.
13. Adhikari, P., Aryal, N., Ghimire, A., **Khanal, P.** 2021. Sustainable biowaste recycling using insects, in Clean Energy and Resources Recovery. Elsevier. 399-420. **(cited by 1)**.
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Commercialisation project proposals to RCN

16. Commercialization project microalga Microalgae-based carbon capture utilization – a prototype development for deployment in coastal seas (AlgaeSeaCU)
17. Lactobacilli-based feeds for robustness and health (Lacthlth)

Other research articles

18. **Kiron, V.**, Phromkunthong, W., … De Scheemaker, G. 2012. Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquaculture Nutrition, 18(5), 521-531. **(cited by 190)**.
19. **Sørensen, M.**, Berge, G.M., Reitan, K.I., Ruyter, B., 2016. Microalga *Phaeodactylum tricornutum* in feed for Atlantic salmon (*Salmo salar*) - effect on nutrient digestibility, growth and utilization of feed. Aquaculture 460, 116-123 **(cited by 139)**.

20. Gong, Y., …. **Sørensen, M.**, **Kiron, V.** 2018. Digestibility of the defatted microalgae *Nannochloropsis* sp. and *Desmodesmus* sp. when fed to Atlantic salmon, *Salmo salar***.** Aquaculture Nutrition 24, 56-64. **(cited by 51).**
21. Gong, Y., … **Sørensen, M., Kiron, V.,** 2020. Approaches to improve utilization of *Nannochloropsis oceanica* in plant-based feeds for Atlantic salmon. Aquaculture 522, 735122. **(cited by 27)**.
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23. Gupta, S., … **Sørensen, M.**, … **Kiron, V.** 2019. *Lactobacillus* dominate in the intestine of Atlantic salmon fed dietary probiotics. Frontiers in Microbiology 9, 3247. **(cited by 57)**.
24. Liu, C., Palihawadana, A., … **Sørensen, M.**, **Kiron, V.** 2022. Utilization of *Nannochloropsis oceanica* in plant-based feeds by Atlantic salmon *(Salmo salar)*. Aquaculture. 738651.
25. Ferreira, M., Abdelhafiz, Y. A. A,… **Kiron, V.** 2022. *Gracilaria gracilis* and *Nannochloropsis oceanica*, singly or in combination, in diets alter the intestinal microbiota of European seabass (*Dicentrarchus labrax*). Frontiers in Marine Science 9:1001942.
26. Sørensen, S. L., Ghirmay, A., … **Sørensen, M., Kiron, V.** 2021. Growth, chemical composition, histology and antioxidant genes of Atlantic salmon (*Salmo salar*) fed whole or pre-processed *Nannochloropsis oceanica* and *Tetraselmis* sp. Fishes 6, 23. **(cited by 8)**.
27. Gupta, S., Lokesh, J., ... **Sørensen, M.**, ... **Kiron, V.** 2019. Macroalga-derived alginate oligosaccharide alters intestinal bacteria of Atlantic salmon. *Frontiers in Microbiology 10*, 2037. **(cited by 34)**.
28. **Khanal, P.**, Pandey D., … Overrein, H. 2022. Mealworms (*Tenebrio molitor*) as an alternative feeding resource: A comprehensive characterization of nutritional values and the larval gut microbiome**.** *(Under revision in the Journal of Cleaner Production-JCLEPRO-D-22-21919-R1).*
29. Pandey, D., … **Khanal, P.** 2023. Differential impacts of water blanching on the chemical composition, and carbohydrate profiling, and *in vitro* digestibility of two brown macroalgae (Ochrophyta, Fucales): *Ascophyllum nodosum* and *Fucus vesiculosus*. *(Submitted to Algal Research-ALGAL-D-22-00728).*
30. **Sørensen, M.**, Kousoulaki, K., … **Kiron, V.**, 2023. Mechanical processing of *Phaeodactylum tricornutum* and *Tetraselmis chui* biomass affects phenolic and antioxidant compound availability, nutrient digestibility and deposition of carotenoids in Atlantic salmon. **(***Submitted to Aquaculture, AQUACULTURE-D-22-02431 R1).*

[FBA] [Case 3: Epigenetics]

Institution: Nord Universitet
Administrative unit: Faculty of Biosciences and Aquaculture (FBA)
Title of case study: Development of epigenetic markers for improved growth of farmed Nile tilapia
Period when the underpinning research was undertaken: 2016-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2016-2021
Period when the impact occurred: 2019-2021

1. Summary of the impact

The EPIFISH team at the Genomics Division has been investigating the epigenetic basis of Nile tilapia domestication, with focus on growth-related genes. This has led to the discovery of novel biomarkers of growth and the development of affordable assays to identify them. These epigenetic markers (epimarkers) can cover a substantial part of the phenotypic variation that is currently missed by genetic selection. Therefore, they contribute towards increased sustainability and profitability of the aquaculture sector, which will have a major societal impact.

2. Underpinning research

Selective breeding of the major commercial fish species is essential to ensure sustainability of the rapidly expanding aquaculture sector, which is a major source of animal protein worldwide. The current molecular approaches used for selection of desired traits in farmed fishes are mainly based on genetic markers and genomic selection without considering the impact of epigenetics. However, epigenetic mechanisms are emerging as a new source of phenotypic variability and plasticity in response to environmental cues. Professor Jorge Fernandes was awarded an ERC Consolidator Grant in 2016 and led the EPIFISH team to investigate the role of epigenetics in fish domestication using Nile tilapia as a model species, since it is one of the most commercially important farmed fish worldwide.

Using reduced representation bisulphite sequencing to compare methylation profiles, we identified differentially methylated CpG sites in the muscle genome of wild Nile tilapia females compared to their offspring reared under farmed conditions (Reference 1). Many of these CpGs were found in genes involved in muscle growth, immunity, autophagy and diet response. This bottom-up approach showed that the phenotypic traits often related to domestic animals (e.g., higher growth rate and different immune status) may be regulated epigenetically and prior to artificial selection on gene sequences. We demonstrated for the first time that DNA hydroxymethylation in muscle differs significantly between wild fish and their progeny reared in captivity (Reference 2). Differentially hydroxymethylated cytosines were present mostly within gene bodies, which might indicate their functional role in epigenetic regulation, and were associated mainly with immune-, neuronal- and growth-related processes. Major gene expression changes occurred within a single generation of domestication and there was a consistently positive correlation between hydroxymethylation and gene expression levels. These findings help us to understand how animals can adapt so rapidly to captivity. We also published the first single-cytosine resolution study of methylation in fast muscle of Nile tilapia in the context of growth and the early domestication process in fish, reporting the identification of muscle-related genes that were differentially methylated between slow- and fast-growing fish (Reference 3). Our data demonstrated that the epigenetic regulation of growth in Nile tilapia most likely involves different gene networks in males and females. Another pioneer publication reported the ubiquitous presence of hydroxymethylation in tissues of the somatotropic axis, namely pituitary,

liver and fast muscle (Reference 4). DNA hydroxymethylation was found to be abundant within gene bodies and promoters of several growth-related genes, indicating that it may modulate growth through epigenetic regulation of the somatotropic axis.

During the implementation of an innovation grant from the European Research Council (ERC PoC), we evaluated the technical and commercial feasibility of these epigenetic markers of growth. A high-resolution melting qPCR assay was developed to determine differences in DNA methylation of the candidate epimarkers in small and large fish. Our research suggests that epigenetic markers have the potential to increase the profit of Nile tilapia farming by enhancing growth rates and thereby reducing the growth period. Employing a basic bioeconomic model developed by the United Nations' Food and Agriculture Organisation, we used a maximum and minimum predicted growth enhancement to extrapolate profit to a scenario in which breeders use these epigenetic markers.

3. References to the research

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2. Konstantinidis I., Sætrom P., Mjelle R., Nedoluzhko A.V., Robledo D. & Fernandes J.M.O. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. Epigenetics 15 (10): 1052-1067. https://doi.org/10.1080/15592294.2020.1748914 [research article]

3. Podgorniak T., Brockmann S., Konstantinidis I. & Fernandes J.M.O. (2019). Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication. Epigenetics 14(8): 818-836. https://doi.org/10.1080/15592294.2019.1618164 [research article]

4. Konstantinidis I., Anastasiadi D., Sætrom P., Nedoluzhko A.V., Mjelle R., Podgorniak T., Piferrer F. & Fernandes J.M.O. (2021). Epigenetic mapping of the somatotropic axis in Nile tilapia reveals differential DNA hydroxymethylation marks associated with growth. Genomics 113 (5): 2953-2964. https://doi.org/10.1016/j.ygeno.2021.06.037 [research article]

4. Details of the impact

The growth-related epialleles (methylation and hydroxymethylation) that we identified can have a major impact on aquaculture biotechnology, since they may enable the development and application of epigenomic selection as a new feature in future selective breeding programmes. These epimarkers have the potential to cover a substantial part of the unknown variability that is missed by current selection methods and will therefore provide a much more efficient selection for robust fish (e.g., disease resistant and high growth performance), which will translate into a tremendous gain in profitability and sustainability of the aquaculture sector. The results from EPIFISH directly contribute to the UN Sustainable Development Goals i) Zero hunger (SDG2), ii) responsible consumption and production (SDG12) and iii) Life below water (SDG14).

Our research results on epigenetic markers of fish growth have been published as scientific articles openly available to the scientific community and disseminated through a dedicated website, promotional video, e-leaflets and Twitter. We participated in the annual open science week events at Nord University and issued press releases for the general public (e.g, EU Research and Avisa Nordland). We have also written popular science publications and technical articles for the aquaculture sector that were published in specialised magazines (e.g., Fiskeribladet and Intrafish). Moreover, the EPIFISH team had direct interactions with policy makers, including the Research Council of Norway, Nordland County Council and the Norwegian ministry of Research and Higher Education.

We have worked closely with one of the major Nile tilapia producers (Genomar AS) and Nord Innovasjon AS to identify intellectual property assets during the project. A patent is being prepared for a multi-panel of epigenetic markers of improved growth. The wild founders and the first three generations of Nile tilapia undergoing domestication represent a unique resource, and will therefore be kept at Nord's research station as long as feasible for future studies related to growth and immunity epigenetics.

We estimate that a 10% increase in Nile tilapia growth rate through combined genetic and epigenetic selection would yield a +28.2% increase in profits, assuming no concomitant increase in operational costs. By reducing the time required to reach the profitable balance between enhanced growth and maintenance costs through shortening the production cycle, less resources would be required to achieve the commercial size, ultimately yielding a more profitable and sustainable sector. Our business development analysis suggests that collaborative research with an industrial partner offers a lucrative route to market, with higher revenues estimated for the fee-charging model than for the shared profit model.

5. Sources to corroborate the impact

1. R&D contact at Genomar Genetics AS (Norway)

2. DOFI from Nord Innovasjon AS (Norway)

3. Report from Konsert Strategy & IP (Sweden)

4. Report from Ttopstart B.V. (Netherlands)

NPI-FAVD case # 1

Institution: Norwegian Polar Institute
Administrative unit: Research Department
Title of case study: Global regulation of hazardous substances
Period when the underpinning research was undertaken: 2000 – 2022, and ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: Geir Wing Gabrielsen: 1984 – present Heli Routti: 2010 - present
Period when the impact occurred: 2011 – 2022 (and ongoing)

1. Summary of the impact (indicative maximum 100 words)

Arctic studies on legacy and emerging pollutants have played an important role in creating both awareness of pollution and providing scientific bases for international regulation. The Norwegian Polar Institute's (NPI) research on legacy and emerging pollutants in Arctic ecosystems has played an important role in providing input to the Stockholm Convention. NPI's research since 2010 (and earlier) has contributed to all four criteria for a compound to be listed under the Stockholm Convention: persistency, ability for long-range transport, bioaccumulation/biomagnification and toxicity. In recent years, the NPI's impact has been particularly strong in regulation process of perfluoroalkyl substances (PFAS).

2. Underpinning research (indicative maximum 500 words)

The main goal of NPI's Ecotox Section, established in ~2000, is to provide new knowledge on environmental pollutants in Arctic food webs and top predators, which gives input to national and international chemical regulation. One of the sections activities has been to conduct studies screening chemicals of emerging Arctic concern. These screening studies, on assignment from the Norwegian Environment Agency (NEA), have been produced in collaboration with high-quality laboratories Norwegian Institute of Air Research (NILU) and the Norwegian Institute of Water Research (NIVA). During the last 10 years, NPI, NILU and NIVA have collaborated on nominating candidates for screening studies in Norway (including the Arctic). The reports to NEA have provided important contributions to Norway's input to the Stockholm Convention as well as international publications on emerging pollutants in the Arctic. Documenting the presence of currently used chemicals in remote Arctic environments has also increased awareness of "chemicalization" of the world, which has been a spin-off for further research funding as exemplified for PFAS.

Early national screening studies and pan-Arctic studies indicated that polar bears from Svalbard have very high levels of PFAS (http://hdl.handle.net/11250/173168; (Smithwick et al., 2005). NPI's follow-up projects documented that PFAS biomagnify in Arctic marine food webs (Haukås et al., 2007), tissue levels increase during seasonal fasting periods (Aas et al., 2014) and some of them increase over time in ringed seals from Svalbard (Routti et al., 2016). Analyzing samples collected through NPI's extensive monitoring efforts revealed that emission changes dwarf the influence of feeding habits on temporal trends of PFAS in polar bears and arctic foxes from Svalbard (Routti et al., 2017). Combining information on movement data with PFAS concentrations proved that PFAS exposure in polar bears originates solely from long-range transport. Detailed studies on polar bears indicated that bears feeding on high trophic level sea ice-associated prey, are fasting and have small cubs are exposed to highest levels of PFAS (Tartu et al., 2017a). Large correlative studies linked PFAS exposure to altered lipid metabolism and hormone concentrations by measuring numerous parameters in polar bear females (Bourgeon et al., 2017; Tartu et al., 2017b). Potential of PFAS to disturb polar bear lipid metabolism was also shown by advanced molecular in vitro studies (Routti et al., 2019). NPI's research has also documented high levels of PFAS in different seabird species from the

Feltkode endret

Norwegian Arctic (e.g. Verreault et al., 2004; Miljeteig et al. 2009). Further research associated PFAS exposure with reduced reproductive success and alteration in metabolic rate in black-legged kittiwakes (Tartu et al., 2014; Blevin et al.2017), while experimental studies on kittiwakes and northern fulmars indicated hormone disruption following PFAS exposure (Nøst et al. 2012).

NPI has also been a driver for conducting research on interactions between climate change and pollutants in arctic food webs. Early studies documented that seasonal fasting of Arctic seabirds and migrating species in Arctic marine food webs leads to higher POP exposure (Hallanger et al. 2011, Bustnes et al. 2010 a,b). Later, the aspect of climate change has been part of pollutant fate, trend and effect studies conducted by NPI (Aas et al., 2014; Tartu et al. 2017; Routti et al., 2017).

Heli Routti, (senior) research scientist, 2010-
Geir Wing Gabrielsen, head of Ecotox section 1995-2022, senior research scientist 2022-
Sophie Bourgeon, post doc, 2013-2015
Sabrina Tartu, post doc, 2015-2017
Kjetil Sagerup, research scientist, 2009-2012

3. References to the research (indicative maximum of six references)

Seven key references are given, NPI authors are bolded:

Aas, C.B., Fuglei, E., Herzke, D., Yoccoz, N.G., **Routti, H.,** 2014. Effect of body condition on tissue distribution of perfluoroalkylated substances (PFASs) in Arctic fox (*Vulpes lagopus*). Environmental Science & Technology 48, 11654–11661. 10.1021/es503147n

Bourgeon, S., Riemer, A.K., **Tartu, S., Aars, J.,** Polder, A., Jenssen, B.M., **Routti, H**., 2017. Potentiation of ecological factors on the disruption of thyroid hormones by organo-halogenated contaminants in female polar bears (*Ursus maritimus*) from the Barents Sea. Environmental Research 158, 94-104. 10.1016/j.envres.2017.05.034

Routti, H., Aars, J., Fuglei, E., Hanssen, L., **Lone, K**., Polder, A., **Pedersen, Å.Ø., Tartu, S.,** Welker, J.M., Yoccoz, N.G., 2017. Emission changes dwarf the influence of feeding habits on temporal trends of per- and polyfluoroalkyl substances in two Arctic top predators. Environmental Science & Technology 51, 11996-12006. 10.1021/acs.est.7b03585

Tartu, S., Lille-Langøy, R., Størseth, T.R., **Bourgeon, S.,** Brunsvik, A., **Aars, J.,** Goksøyr, A., Jenssen, B.M., Polder, A., Thiemann, G.W., Torget, V., **Routti, H**., 2017b. Multiple-stressor effects in an apex predator: combined influence of pollutants and sea ice decline on lipid metabolism in polar bears. Scientific Reports 7, 16487. 10.1038/s41598-017-16820-5

Blevin, P., **Tartu, S.,** Ellis, H.I., Chastel, O., Bustamante, P., Parenteau, C., Anglier, F., **Gabrielsen, G.W**. 2017. Contaminants and energy expenditure in an Arctic seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with metabolic rate in a contrasted manner. Environmental Research 157: 118-126. 10.1016/j.envres.2017.05.022

Tartu, S., **Gabrielsen, G.W**., Blevin, P., Ellis, H., Bustnes, J.O., Herzke, D., Chastel, O., 2014. Endocrine and fitness correlates of long-chain perfluorinated carboxylates exposure in Arctic breeding black-legged kittiwakes. Environmental Science & Technology 48, 13504-13510. 10.1021/es503297n

Nøst, T.H., **Helgason, L.B.,** Harju, M., Heimstad, E.S., **Gabrielsen, G.W**., Jenssen, B.M., 2012. Halogenated organic contaminants and their correlations with circulating thyroid

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hormones in developing Arctic seabirds. Science of the Total Environment 414, 248-256. 10.1016/j.scitotenv.2011.11.051

4. Details of the impact (indicative maximum 750 words)

The Stockholm Convention on Persistent Organic Pollutants (POPs) is a global treaty to protect human health and the environment from chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in humans and wildlife, and have harmful impacts on human health or on the environment. Research conducted by NPI on levels, trends and effects of POPs in Arctic biota has contributed to the regulation of 12 out of the 19 POPs listed since 2009. NPI's research on PFAS in polar bears made a high contribution on the listing of perfluorohexane sulfonic acid (PFHxS) and related compounds. The listing was proposed by Norway, which was also responsible for drafting the risk profile. The profile (Persistent Organic Pollutants Review Committee, 2018) cited nine peer-reviewed papers led by NPI authors with focus on trends and effects in polar bears, and four out of six of the concluding statements referred to the research conducted by NPI. NEA invited H.Routti to review the draft several times, and she was also invited as an external expert to the POP review committee meeting held in Rome in 2018, where she held a presentation on PFAS in polar bears and other Arctic top predators. NPI's further research on PFAS has also given a strong contribution to Canada's proposal to list long-chain perfluorocarboxylic acids and related compounds under Stockholm Convention (Persistent Organic Pollutants Review Committee, 2021). Over 20 articles, that NPI had led or where NPI was strongly involved, on PFAS trends, levels and effects in Arctic wildlife are cited in the proposal.

Additionally, NPI's research has contributed to global regulation of several other compounds. NPI's research has been cited in risk profiles (only short reference given in the text) for decabromodiphenyl ether (UNEP/POPS/POPRC.10/10/Add.2, 2014), commercial octabromodiphenyl ether (UNEP/POPS/POPRC.3/20/Add.6, 2007), hexabromocyclododecane (UNEP/POPS/POPRC.6/13/Add.2, 2010), hexachlorobutadiene (UNEP/POPS/POPRC.8/16/Add.2, 2012), alpha and beta hexachlorocyclohexane (UNEP/POPS/POPRC.3/20/Add.8 and UNEP/POPS/POPRC.3/20/Add.9, 2007), pentachlorobenzene (UNEP/POPS/POPRC.3/20/Add.7, 2007), perfluorooctane sulfonic acid (PFOS) (UNEP/POPS/POPRC.2/17/Add.5, 2006), polychlorinated napthalenes (UNEP/POPS/POPRC.8/16/Add.1, 2012), short-chained chlorinated paraffins (UNEP/POPS/POPRC.11/10/Add.2, 2015), endosulfane (UNEP/POPS/POPRC.5/10/Add.2, 2009), commercial pentabromodiphenyl ether (UNEP/POPS/POPRC.2/17/Add.1, 2006).

Stockholm Convention has also addressed multiple stressors by developing a guidance document on how to assess the possible impact of climate change on the work of the Persistent Organic Pollutants Review Committee (POPRC-9/8, 2015). The final "recommendation"-type policy document is based on a longer assessment document developed by the POP Review Committee (UNEP/POPS/POPRC.9/INF/15, 2013). NPI's research referred in the assessment draft has been important to document interactions between climate change and pollutant exposure in Arctic ecosystems.

5. Sources to corroborate the impact (indicative maximum of ten references)

Persistent Organic Pollutants Review Committee, 2015. Guidance on how to assess the possible impact of climate change on the work of the Persistent Organic Pollutants Review Committee. POPRC-9/8, 2015
http://chm.pops.int/Convention/POPsReviewCommittee/LatestMeeting/POPRC9/POPRC9Documents/tabid/3281/Default.aspx

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Persistent Organic Pollutants Review Committee, 2015. Revised draft guidance on how to assess the possible impact of climate change on the work of the Persistent Organic Pollutants Review Committee.
http://chm.pops.int/Convention/POPsReviewCommittee/LatestMeeting/POPRC9/POPRC9Documents/tabid/3281/Default.aspx

Persistent Organic Pollutants Review Committee, 2018. Report of the Persistent Organic Pollutants Review Committee on the work of its fourteenth meeting - Risk profile on perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds. UNEP/POPS/POPRC.14/6/Add.1
http://chm.pops.int/theconvention/popsreviewcommittee/meetings/poprc14/overview/tabid/7398/default.aspx

Persistent Organic Pollutants Review Committee, 2021. Note by the Secretariat - Proposal to list long-chain perfluorocarboxylic acids, their salts and related compounds in Annexes A, B and/or C to the Stockholm Convention on Persistent Organic Pollutants. UNEP/POPS/POPRC.17/7.
http://chm.pops.int/TheConvention/POPsReviewCommittee/Meetings/POPRC17/Overview/tabid/8900/Default.aspx

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NPI-FAVD case # 2

Institution: Norwegian Polar Institute
Administrative unit: Research Department
Title of case study: SEATRACK - mapping seabird non-breeding distribution for better management and marine protection in the North Atlantic
Period when the underpinning research was undertaken: 2014 -
Period when staff involved in the underpinning research were employed by the submitting institution: Hallvard Strøm: 1999 - present Sebastien Descamps: 2010 - present Benjamin Merkel: 2015-2019 Francoise Amelineau: 2019-2022
Period when the impact occurred: 2016 -

1. Summary of the impact (indicative maximum 100 words)

Sustainable ocean management aims to conserve unique marine biodiversity while facilitating durable resource acquisition by humans. Such management is challenging and requires extensive knowledge of the distribution of marine organisms which are highly mobile and difficult to study. By using new and appropriate technology and through large-scale international collaboration since 2014, SEATRACK has provided such knowledge for seabirds in the North Atlantic. Societal impacts at international and national levels are: 1) designation of a new large marine protected area (NACES) in the North Atlantic by OSPAR, 2) providing knowledge basis for national policies on marine conservation, 3) international harvest plan for Brünnich's guillemot *Uria lomvia.*

2. Underpinning research (indicative maximum 500 words)

The world's oceans contain unique biodiversity, life forms and genetic resources that provide ecosystem services of enormous value to human societies. At the same time, impacts of human activities on the ocean are substantial, ubiquitous and rapidly growing. The High-level Panel for a Sustainable Ocean Economy consisting of 17 world leaders, have committed to sustainably manage 100% of the ocean areas under their national jurisdictions by 2025 and to protect 30% of the worlds' oceans as Marine Protected Areas (MPAs) by 2030. Maps of the spatial distribution of marine animals, especially those that are endangered and vulnerable, are powerful tools in ecosystem-based marine spatial planning of MPAs and in environmental impact assessments of human activities such as fisheries, shipping, oil and gas exploitation, and offshore wind farms.

Seabirds are one of the most threatened groups of vertebrates, with almost half of all species (47%) experiencing population declines. The North Atlantic supports some of the largest seabird populations in the world. Many seabird species undergo extensive seasonal migrations, often across large marine ecosystems or between marine areas under different national jurisdictions. The advances of electronic tracking, especially of the application of Global Location Sensors (GLS or geolocators), have made it possible to study the seasonal movements of seabirds throughout their entire annual life cycle. To take full advantage of this development, there is a need for large-scale and multi-species research programmes. The SEATRACK programm (2014 -) led by the Norwegian Polar Institute in collaboration with Norwegian Institute of Nature Research (NINA) and the Norwegian Environmental Agency aims with its 50 partners from 11 countries (Norway, Russia, Iceland, UK, Ireland, France, Poland, Denmark, Faroe, Greenland and Canada) to identify the year-round distribution and movements of seabirds breeding across the northern part of the North Atlantic (**R1**). Four main research themes have been defined: (1) the assessment of variation in migration strategies among individuals, populations and species (**R2,3**); (2) the linkage of migration strategies and winter distribution to demography and population dynamics (**R4**); (3) the linkage of non-

breeding distribution to contaminants (**R5**) and (4) the use of tracking data in marine spatial planning (**R6**). By 2023, 16 000 loggers have been deployed on 11 species in 57 seabird colonies, and data from 8300 retrieved loggers have been analyzed and compiled (**R1**).

Based on the positional (GLS) data, two spatial datasets have been developed: (i) kernel distribution maps for all 11 species and colonies showing the seasonal (autumn, winter, spring) distribution of tracked species and colonies (available at https://seatrack.seapop.no/map/) and (ii) a unique spatial dataset of the predicted monthly distribution of the 6 six most common pelagic seabird species, covering 23.5 million adult birds, constituting 87% of their combined breeding populations in the Northeast Atlantic (available at https://seatrack-e9bdc.web.app/). This dataset combines tracking data, data describing the physical environment and data on seabird population sizes. It consists of 4692 map layers, each predicting the densities of birds from a given species, colony, and month across the Northeast Atlantic (**R6**).

Both spatial datasets are now widely used for research (40+ peer-reviewed papers produced by 2023) and in management processes, including for example the identification of populations influenced by marine protected areas and human activities.

Hallvard Strøm, Research Scientist
Sebastien Descamps, Senior Research Scientist
Benjamin Merkel, PhD-student (2015-2019)
Francoise Amelineau, Post.doc (2019-2022)

3. References to the research (indicative maximum of six references) [NVI]

R1. Strøm H, Descamps S, Ekker M et al. 2021. Tracking the movements of North Atlantic seabirds: Steps towards better understanding of population dynamics and marine ecosystem conservation. *MEPS 676: 97-116,* https://doi.org/10.3354/meps13801

R2. Amélineau F, Merkel B, Tarroux A, et al. 2021. Six pelagic seabird species of the North Atlantic engage in a fly-and-forage strategy during their migratory movements. MEPS 676: 127-144, https://doi.org/10.3354/meps13872

R3. Merkel B, Descamps S, Yoccoz NG, et al. 2021. Strong migratory connectivity across meta-populations of sympatric North Atlantic seabirds MEPS 676: 173-188, https://doi.org/10.3354/meps13580

R4. Clairbaux M, Mathewson P, Porter W, et al. 2021 North Atlantic winter cyclones starve seabirds. Current Biology 31: 3964-3971, https://doi.org/10.1016/j.cub.2021.06.059

R5. Albert, C., Helgason, H.H., Brault-Favrou, M., et al. 2020. Seasonal variation of mercury contamination in Arctic seabirds: A pan-Arctic assessment. Science of The Total Environment, 750, 142201. https://doi.org/10.1016/j.scitotenv.2020.142201

R6. Fauchald P, Tarroux A, **Amélineau F** et al. 2021. Year-round distribution of Northeast Atlantic seabird populations: applications for population management and marine spatial planning. *MEPS 676: 255-276, https://doi.org/10.3354/meps13854*

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4. Details of the impact (indicative maximum 750 words)

Impact 1. OSPAR designation of the North Atlantic Current and Evlanov Sea basin Marine Protected Area (NACES MPA).

OSPAR is the mechanism by which fifteen governments and the EU cooperate to protect the marine environment of the North-East Atlantic. The NACES MPA is a vitally important area for seabirds covering approximately 600 000 km^2. By establishing this MPA in 2021 (**S1**), OSPAR reached the UN Convention for Biodiversity 2020 Aichi target of designating 10% of marine waters as MPAs, and it will be an important step for achieving the global target of protecting 30 % of the oceans by 2030

The designation of the NACES MPA was first proposed in 2016, with a workshop led by BirdLife International for seabird experts. This led to an effort to gather all available data, in which SEATRACK contributed significantly with seabird tracking data for the North Atlantic. The collaborative analysis of this dataset identified this seabird hotspot (**S2**) and it was officially designated by the OSPAR Commission on the 1st of October 2021 (**S1**), making it the first MPA on the High Seas to be identified from tracking data.

Impact 2. Knowledge basis for national politics on marine conservation

By providing data and results to the Norwegian Environment Agency and the Ministry of Climate and Environment, SEATRACK contributes significantly to the scientific basis for national policies on marine conservation. Specifically, SEATRACK provides annual progress reports and engages annually in progress meetings with the Norwegian Environment Agency and the Ministry of Climate and Environment. Norway has recently, through the membership and lead of the Ocean Panel, given its support to a global target of protecting 30 % of the oceans by 2030 through marine protected areas and other effective area-based conservation measures. In the governmental white paper 29 (2020–2021) to the Norwegian Parliament (**S3**), the Ministry of Climate and Environment presents its national plan for conservation of areas of special importance for marine biodiversity, stating that Norway's goal is to play a leading role in developing an integrated, ecosystem-based marine management regime protecting biodiversity while providing a sound basis for sustainable use of resources. The SEATRACK project is referred to in Chapter 2 as an important research activity for reaching this goal and for identifying areas of great importance for marine biodiversity in Norwegian waters: '*By tracking birds from populations subjected to monitoring of trends, reproduction and survival, new and revolutionary knowledge is provided about the species, e.g. about their habitat use, population origin, migration routes, wintering areas and how vulnerable populations are in Norwegian waters*'**.**

Impact 3. International harvest plan for Brünnich's guillemot

The Brünnich's guillemot is one of the most abundant Arctic seabirds, but several populations are declining (**S4, S5**). The species is subject to traditional harvest in some important wintering areas off West Greenland and Newfoundland. Until recently, knowledge on the species' winter distribution has been insufficient to assess the impact of this mortality source on specific breeding populations (**S6**). The SEATRACK project has significantly increased our knowledge of guillemot migration routes and non-breeding distribution, and thereby to better understand the potential threat represented by harvest on the wintering grounds (**S7**). By combining such tracking data with demographic data, it has for example been shown that hunting in Greenland affects breeding populations in Svalbard and Iceland. Such results stress the need for a coordinated harvest plan for Brünnich's guillemots – and a coordinated/comprehensive (flyway) management in general. Two workshops, organized by Aarhus University that gathered researchers and managers from Norway, Iceland, Greenland and Canada, took place in March 2021 and September 2022 to initiate such a process. The workshops were designed around the concept of adaptive management, with the goal of developing an international harvest plan. SEATRACK data are the core data source in the assessment of the connectivity between the different guillemot populations in the North Atlantic and the overlap between guillemot at-sea distribution and harvest grounds.

5. Sources to corroborate the impact (indicative maximum of ten references)

S1. OSPAR Commission (2021) OSPAR Decision 2021/01 on the establishment of the North Atlantic Current and Evlanov Sea basin Marine Protected Area. OSPAR 21/13/1, Annex 23

S2. Davies, T. E., A. P. B. Carneiro; M. Tarzia et al. 2021. Multi-species tracking reveals a major seabird hotspot in the North Atlantic. Conservation Letters 14: e12824.

S3. Klima- og miljødepartementet 2021. Heilskapleg nasjonal plan for bevaring av viktige område for marin natur. Meld. St. 29 (2020 –2021).
English translation: Ministry of Climate and Environment 2021. Norway's integrated plan for the conservation of areas of special importance for marine biodiversity. Meld. St. 29 (2020–2021) Report to the Storting (white paper).

S4. Descamps, S., Strøm, H., Steen, H. 2013. Decline of an arctic top predator: synchrony in colony size fluctuations, risk of extinction and the subpolar gyre. Oecologia 173(4): 1271-1282. https://doi.org/10.1007/s00442-013-2701-0

S5. Frederiksen M, **Descamps S**, Elliott KH et al. 2021. Spatial variation in vital rates and population growth of thick-billed murres in the Atlantic Arctic. MEPS 672: 1-13, https://doi.org/10.3354/meps13823

S6. Frederiksen, M, **Descamps, S**, Erikstad, KE et al. 2016. Migration and wintering of a declining seabird, the thick-billed murre *Uria lomvia*, on an ocean basin scale: Conservation implications. Biological Conservation 200, 26-35. https://doi.org/10.1016/j.biocon.2016.05.011

S7. OSPAR. Status Assessment 2020 - Thick-billed murre or Brünnich's guillemot. OSPAR Assessment - Biodiversity and Ecosystems. https://oap.ospar.org/en/ospar-assessments/committee-assessments/biodiversity-committee/status-assesments/thick-billed-murre/

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NPI-FAVD case # 3

Institution: Norwegian Polar Institute (NPI)
Administrative unit: Research Department
Title of case study: Monitoring and research on polar sea ice informing decision and policy makers and society
Period when the underpinning research was undertaken: 2010-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2010-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact

The impact of research and monitoring of polar sea ice (Arctic and Antarctic) and the accumulated expertise at the unit informs processes that contribute directly to knowledge transfer for decision and policy makers and for informing the broader public. This expertise and knowledge is accumulated over longer periods of time which is also in line with the unit's mandate towards the owners, which includes that our research and knowledge is used towards environmental management by our government. Researchers contribute to national and international assessments (AMAP, IPCC, etc.) in the subject lending their expertise to these products.

2. Underpinning research

The core mandate of the institute (and thus researchers at the unit) is to carry out long-term observations and monitoring, upkeep expertise in the subject through current literature, and attract external funding to support recruitment positions to augment the permanents staff's capacity to carry out research. The primary aim of the monitoring and research and knowledge gathering is to support environmental management of Norway's territories and representation of Norway in many international bodies and working groups, which means a somewhat different approach and time use of research staff than in academic organisations. Direct assignments from the owners also entails synthesising research knowledge not only from our own unit but by anyone in the respective field.

Broadly the work carried out on sea ice can be divided into two aspects;

- Long-term sea ice monitoring in Fram Strait (since 1990s), around Svalbard and in Svalbard fjords (since 1966/2003/2006, site depending) and in the Southern Ocean/coastal waters of Dronning Maud Land (Antarctica, since 2005), and
- Sea ice process studies including role of sea ice in biogeochemical cycles in the framework of major national and international multidisciplinary projects such as N-ICE2015, MOSAiC, the Nansen Legacy and CIRFA SFI.

The former provides longer term records of Arctic sea ice climate and have underpinned many findings on the evolution and changes in sea ice thickness over the years, showing the dramatic reduction in ice thickness on both regional and pan-Arctic scales. It's also been the basis of better understanding of the changes in the sea ice thickness distribution and the volume of ice exported from the Arctic. This is accomplished by sustained use of upward looking sonars on ocean moorings, measuring sea ice draft autonomously all year around, sea ice thickness surveys from ground and helicopter, and in situ sea ice and snow thickness surveys during expeditions. Some of this work is also supported by satellite remote sensing and provides validation products for satellite missions used in both national and international collaborative projects.

The latter has more recently (2015 onwards), especially from the N-ICE2015 expedition, provided understanding on the underlying processes that affect the Arctic sea ice cover, especially through effects from snow cover and winter storms and warming events in the Atlantic sector of the Arctic from field observations. This inspired work on the larger scale connections of sea ice, snow and atmospheric patterns, accomplished through analysis of field observations, buoy, reanalysis and satellite data and different level of numerical modeling.

The research at the unit is typically interdisciplinary, and the sea ice work has always included components of sea ice biogeochemistry and ecology. This work has recently highlighted e.g. the role sea ice ridges as possible ecological hotspots, and the interplay of sea ice, snow and ice dynamics on pelagic primary production. Process understanding is also developed through use of numerical ice-ocean-ecosystem models (regional and one-dimensional).

While the majority of the recruitments positions (post docs) at the unit related to this research theme are externally funded to do project-related research, it is largely the permanent staff that, given the institutes mandate, contribute to the use of research and knowledge towards assessments, evaluations, reviews and direct government assignments to benefit decision and policy making. List of key researchers (permanent staff only) at the administrative unit:

Scientists (permanent staff)	Job title	Period being permanent staff
Sebastian Gerland	Section head/res. Scientist (sea ice)	2002 to present
Dmitry Divine	Research scientist (paleo and sea ice)	2015 to present
Agneta Fransson	Research scientist (sea ice, ocean bgc)	2018 to present
Mats Granskog	Research scientist (sea ice and ocean)	2008 to present
Philipp Assmy	Research scientist (marine ecology)	2014 to present
Stephen Hudson	Research scientist (atmosphere)	2013 to present
Laura de Steur	Research scientist (ocean)	2014 to present
Haakon Hop	Research scientist (marine ecology)	1995 to present
Olga Pavlova	Research scientist (sea ice and ocean)	1999 to 2022
Edmond Hansen	Research scientist (sea ice)	2000 to 2013
Gunnar Spreen	Research scientist (sea ice remote sensing)	2012 to 2015

3. References to the research

The unit's permanent staff members are highlighted in **bold**, and temporary positions (post docs, graduate students) are indicated in *italics*.

Hansen, E., Gerland, S., Granskog, M. A., Pavlova, O., *Renner, A. H. H*., Haapala, J., Løyning, T. B., & Tschudi, M. (2013). Thinning of Arctic sea ice observed in Fram Strait: 1990-2011. Journal of Geophysical Research: Oceans, 118(10), 5202–5221. https://doi.org/10.1002/jgrc.20393

Fransson, A., Chierici, M., Skjelvan, I., Olsen, A., **Assmy, P.**, Peterson, A.K., **Spreen, G.** and Ward, B. (2017): Effects of sea-ice and biogeochemical processes and storms on under-ice water fCO2 during the winter-spring transition in the high Arctic Ocean: implications for sea-air CO2 fluxes. J. Geophys. Res. Oceans, 122, 5566–5587, https://doi.org/10.1002/2016JC012478

Graham, R. M., Cohen, L., Petty, A. A., Boisvert, L. N., Rinke, A., **Hudson, S. R.**, Nicolaus, M., & **Granskog, M. A.** (2017). Increasing frequency and duration of Arctic winter warming events. Geophysical Research Letters, 44(13), 6974–6983. https://doi.org/10.1002/2017GL073395

Pavlova, O., Gerland, S. and **Hop, H.** (2019): Changes in sea-ice extent and thickness in Kongsfjorden, Svalbard, and related ecological implications. Advances in Polar Ecology. In: Hop H., Wiencke C. (eds.) The Ecosystem of Kongsfjorden, Svalbard. Advances in Polar Ecology 2, 105-136. Springer, Cham. https://link.springer.com/chapter/10.1007/978-3-319-46425-1_4

Merkouriadi, I., Liston, G. E., *Graham, R. M*., & **Granskog, M. A.** (2020). Quantifying the Potential for Snow-Ice Formation in the Arctic Ocean. Geophysical Research Letters, 47(4), e2019GL085020. https://doi.org/10.1029/2019GL085020

Spreen, G., **de Steur, L.**, **Divine, D., Gerland, S., Hansen, E.,** & Kwok, R. (2020). Arctic Sea Ice Volume Export Through Fram Strait From 1992 to 2014. Journal of Geophysical Research: Oceans, 125(6). https://doi.org/10.1029/2019JC016039

4. Details of the impact

Sea ice research at the unit broadly contributed to assessments, tasks and assignments that are given to us due to the institute's mandate to provide research-based knowledge for environmental management in Norway. Outcome of this activity is reported directly to the ministry.

Sea ice data and observations are published annually in several national portals, IceWatch (icewatch.met.no) and ASPeCt databases. MOSJ (https://mosj.no/en/) is an official system monitoring the environment on Svalbard and Jan Mayen. Here, Barents Sea and Fram Strait sea ice extent is presented (https://mosj.no/en/indikator/climate/ocean/sea-ice-extent-in-the-barents-sea-and-fram-strait/), as well as Fram Strait sea ice thickness (https://mosj.no/en/indikator/climate/ocean/thickness-of-sea-ice-in-the-arctic-ocean-measured-in-the-fram-strait/). Information on sea ice in Barents Sea, Fram Strait and in Svalbard fjords are provided as a indicator to the monitoring of environmental status of Norway's polar areas at miljostatus.miljodirektoratet.no.

Reports with input from our unit that contribute to Norwegian environmental management have been published, usually in Norwegian (e.g. Jepsen, et al. 2019 (http://hdl.handle.net/11250/2600003); Arneberg et al. 2020 (https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2020-13)), occasionally in English (e.g. Lowther et al., 2018; Hanssen-Bauer et al. 2019). Knowledge of the Barents Sea marginal ice zone (MIZ) contributed to the environmental management and resource exploration regulations developed by the Norwegian government: See also https://www.npolar.no/en/themes/the-marginal-ice-zone/, and von Quillfeldt et al. (2018, NPI brief report 047, http://hdl.handle.net/11250/2563226). Contributions were made to a white paper to the Norwegian parliament Stortingsmelding #20 (2019-2020, in Norwegian) in the context of environmental management of Barents Sea MIZ and Stortingsmelding #9 (2020-2021) on national policy for northern Norway and Arctic. Work until 2021 also supported advice for specific questions about marine exploration in the Northern Barents Sea, e.g. assessment for the planned Wisting-oilfield, https://www.npolar.no/nyhet/horingssvar-tilleggsutredning-for-wisting (in Norwegian).

AMAP is a working group under the Arctic Council, and the scientific findings from AMAP work is summarized in Summary for Policy Makers reports which is delivered to the Arctic Council ministerial meetings. Contributions from our unit includes the AMAP SWIPA (Snow, Water, Ice and Permafrost in the Arctic) reports from 2011 and 2017 (Meier et al.,2011; Barber et al. 2017), plus a climate update published in 2021, represent assessments of the status and changes of snow, water, ice and permafrost in the Arctic, with connections across disciplines, e.g., impacts of changes for the ecosystem. This work also resulted in peer-review publications (e.g., Meier et al. 2014, Gerland et al. 2019).

NOAA Arctic report card (ARC) updates are published annually since 2006, summarizing the state of the Arctic environmental system relative to historic records on both NOAA web-site (https://www.arctic.noaa.gov/Report-card) and in journal articles (e.g. Meier et al. 2021). The ARC targets a broad audience on the most recent development of Arctic climate. Scientists from our unit have contributed regularly to the ARC sea ice essays since 2012.

IPCC reports disseminate status and development of climate change to society/stakeholders/policymakers. Scientists from our unit contributed to IPCC AR5 (2013) and AR6 (2019-22, Gulev et al. 2021) processes in roles as lead author, contributing author and expert reviewer. Research background with knowledge about status and changes of sea ice and climate was a basis for nominations of work. Several of the monitoring and research studies of our research unit have been cited in recent IPCC reports (e.g., Hansen et al. 2013; Fransson et al. 2017; Pavlova et al. 2019; Spreen et al. 2020). Beneficiaries are countries that use IPCC reports in their decision making, and the society is benefitting for example from

regulations limiting greenhouse gas emissions. The Paris agreement 2015 can be seen as a process directly related to findings published in IPCC AR5.

Contributions to the Expert Group on Biogeochemical Exchange Processes at the Sea-Ice Interfaces (BEPSII), a SCOR (Scientific Committee on Oceanic Research) working group since 2012, and results were published in Lannuzel et al. (2020) and a policy brief.

While our work is often based on field studies, sea ice observations have often also been used for satellite or airborne validation (ESA, NASA), and thus contributed towards improvement of satellite data products on several occasions in the period of interest.

We have also actively engaged with media to document our research work. This has resulted in dissemination through National Broadcaster (NRK), newspapers, and internationally through BBC, National Geographic and others. For example. several reports were made by NRK, BBC and National Geographic from the N-ICE2015 expedition in 2015. An exhibition based on this expedition circulated around the world in Norway's embassies for several years. We share our research work to a broader audience through social media (especially Twitter and Instagram especially with the researcher-led @oceanseaicenpi channel) to educate the larger public.

5. Sources to corroborate the impact

Here we list sources of impact that are in English and easily accessible. The numerous direct contributions to Norwegian government/management are more diffuse, in Norwegian and not always public (a number of those are noted in section 4 above).

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NPI-FAVD case # 4 Marine mammals

Institution: Norwegian Polar Institute (NPI)

Administrative unit: Research Department

Title of case study: NPI marine mammal research and societal impacts

Period when the underpinning research was undertaken: 2011-2021

Period when staff involved in the underpinning research were employed by the submitting institution: 1990s – onward

Key researchers:
Kit Kovacs, Biodiversity Section Leader, 1996 - present
Christian Lydersen, Research Scientist, 1994 - present
Jon Aars, Research Scientist, 2002 - present
Andrew Lowther, Research Scientist, 2013 - present
Magnus Andersen, Research Scientist, 1999 - present
Jade Vacquié-Garcia, Post Doc, 2015-2019
Charmain D. Hamilton, MSc – Post Doc 2011-2019

All researchers were employed in the Biodiversity Section of the Norwegian Polar Institute Research Department. The main focus of the section has been to study the ecology of Arctic and Antarctic ecosystems, to evaluate the human impact on these systems and give science based advise to management authorities.

Period when the impact occurred: 2011-2021

1. Summary of the impact (100 words)

Marine mammal research and monitoring at NPI over the past decade has provided knowledge that has been vital to the management and conservation of marine ecosystems at local, regional, circumpolar, and global scales. Our data and expertise are underpinning protected area planning in both the Arctic and the Southern Ocean and providing the basis for assessments of populations (for OSPAR, NAMMCO etc.) and Red List assessments (Artsdatabank, IUCN Europe and Global), as well as contributing to multinational status assessments (e.g., CAFF's State of the Arctic, World Commission on Protected Areas (IMMAs), IPCC (Cryosphere Report), particularly in the context of climate change.

2. Underpinning research (indicative maximum 500 words)

NPIs marine mammal research team has produced most of the existing knowledge for endemic Arctic seals, whales, and polar bears in the European High Arctic. Creation of data over the last decade has been extensive and has involved both short-term, intensive studies (often funded by NRC) and long-term monitoring in a wide variety of fields including behavioural ecology, physiology, acoustics, toxicology/health, genetics and demography as well as population and sightings surveys. Spatial ecology is a key field of study within the group that allows for assessment of distribution, ecosystems interactions, and habitat use (and change through time), which is vital within the context of global warming. NPI researchers and their project partners have been instrumental in developing methodology and technology to facilitate marine mammal studies – particularly in logistically challenging environments (i.e., sea ice) in terms of capture and handling methods, but perhaps most importantly facilitating the design of tracking devices (with commercial and university partners) - animal-borne CTD "tags", long-term tracking devices that can withstand walrus lifestyles, and swim-dive recorders for polar bears (adapted from instruments designed for seals), to name a few.

Some of the baseline work that we have accomplished recently enables assessments of population "boundaries" and status. We have studied population structure of all the endemic whales (genetically)(white whale, narwhale, bowhead whale), documented distributions and

habitat use and have also done the first population estimates for all three species in the Barents Region with ecologically appropriate study design and methodology. Polar bear abundance data was also updated (in 2015 - producing the first trend estimate for the region). All these data were immediately incorporated into status assessments for Conservation of Flora and Fauna and the 2021 Norwegian National Red List assessments. They are also contributing to the 2023 European and Global IUCN mammal assessments. Large compilation and review efforts lead by NPI have produced circumpolar hot-spot analyses in the North and a foundation for planning MPAs in Dronning Maud Land.

Our broad ecological studies have significantly advanced understanding of the impacts of climate change, replacing general theoretical predictions with data-driven projections based on knowledge of habitat use in concert with IPCC abiotic predictions. We have documented distributional, behavioural and dietary trends in ringed seals, polar bears, and white whales and many other Arctic species while in parallel documenting loss of sea ice, tidewater glacier retractions and concomitant community change - using acoustics data, tracking data, long-term sighting records and local harvest sampling (for ringed seals) and considerable cross-disciplinary engagement with glaciologists, sea-ice scientists and oceanographers. Some key results include: polar bears have increasing genetic metapopulation structure associated with sea ice reductions; adult ringed seal have displayed behavioural plasticity in using terrestrial haul-out sites for the first time in Svalbard and yet have retracted into Arctic refugia in front of tidewater glaciers, reducing individual range sizes by 75%; polar bears have dramatically increased use of terrestrial prey (reindeer/ground-nesting birds) without ice to hunt on in summer.

3. References to the research (indicative maximum of six references)

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4. Details of the impact (indicative maximum 750 words)

1) Marine Protected Areas – NPIs marine mammal data is used to help designate appropriate protected (and human-activity) areas at local scales in the Svalbard Archipelago. Recent assessments (done by NPI on behalf of the Governor of Svalbard) include west coast Spitsbergen protected area designation, no-go zones for snow-mobile traffic in spring and the Nedre Adventdalen (a marine/estuary system near Longyearbyen) planning process. NPI marine mammal research and monitoring also contributes heavily to regional assessments – recent processes include identification of especially vulnerable marine areas in the Barents Region (SVO) and the status of Norwegian Seas (Barents Sea, North Sea, Greenland Sea - https://www.regjeringen.no/en/topics/climate-and-environment/biodiversity/innsiktsartikler-naturmangfold/forvaltningsplaner-for-havomrada/id2076485/). Participation by NPI marine mammal scientists (and management staff) in Arctic Council work has contributed to the development of advanced planning documents for a network of Arctic MPAs – e.g. PAME's Framework for a Pan-Arctic Network of Marine Protected Areas) and it has also fed into non-governmental organisations' planning processes – such as WWFs Arctic Ocean Network of priority areas for Conservation. In the Southern Ocean NPI marine mammal scientists have for decades been active participants in MPA planning and initiation processes within CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) and have served as conservation-oriented advisors to the Polar Ambassador. Currently, Norway co-leads the Weddell Sea-Dronning Maud Land MPA process, which is expected to be completed/designated in 2023-24, and have in this process been responsible for background scientific reviews as well as actively facilitating planning "tools". Occurrence of animals from top trophic levels, including whales and seals, play a large role in protected area designations and NPIs tracking data has been instrumental in identifying key-habitat areas. NPI marine mammal scientists have also served as reviewers for Southern Ocean IMMAs (Important Marine Mammal Areas) for the Marine Mammal Protected Areas Task Force.

2) NPI marine mammal data and expertise are routinely used in species status assessment by OSPAR, NAMMCO and Arctic Council groups (as well as IUCN – see pt 3). The most recent assessments done for OSPAR include blue whales and bowhead whales. NPI scientists lead NAMMCO's current bearded seal assessment processes and CAFF's white whale status assessment, which was completed last year.

3) Red List Assessments – NPI marine mammal scientists have leading roles in Red List assessments at the national, European and global levels, where NPI data have been providing most of the information available for the endemic High Arctic species. The most recently completed example is the 2021 Norwegian national Red List update (https://artsdatabanken.no/rodlisteforarter2021/Artsgruppene/Pattedyr). In this process all three of the Arctic endemic cetaceans moved from the Data Deficient category Endangered for both bowheads and white whales and Vulnerable in the case of narwhals). NPI marine mammal scientists are also engaged with European Red List Assessments and KK leads the Global assessments for seals world-wide in her position as the IUCN Chair for the Pinniped Specialist Group. Our polar bear scientists (MA and JA) also serve on the IUCN Polar Bear Specialist Group. These assessments reach the highest political levels for biodiversity conservation – with the Species Survival Commission

(for which specialist groups serve) being one of the most influential conservation organisations in the world.

4) Multinational/multispecies processes related to climate change – marine mammal scientists at NPI have done research on climate change impacts on marine mammal in one of the Arctic hot-spots for decades. Our work has contributed to the Arctic Council's State of the Arctic Marine Biodiversity Report (SAMBR) in 2015 and NPI has led the first taxonomic group update of this report (Marine Mammals 2021 Technical Report)– in a particularly politically challenging environment. NPI marine mammal scientists have also frequently provided input in assessment processes related to climate change since the first IPCC report came out in the late 1990s –and broader ocean community interactions, in ACIA, AACA, SWIPA and most recently the 2021 Oceans and Cryosphere Report from IPCC. NPI also is a member of the IUCNs Climate Change Specialist Group, providing input relevant for the development of guidelines and criteria for dealing with conservation in a time of climate change, assessing species vulnerabilities, as well as helping to produce a great number of outreach products.

5. Sources to corroborate the impact (indicative maximum of ten references)

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[NTNU_IBI] [Case number 1]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: **Quantitative criteria for assessment of environmental risks to biological diversity**
Period when the underpinning research was undertaken: 2011-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact (indicative maximum 100 words)

An outcome of the research in the unit has been the development of quantitative methods for analysing dynamical processes in nature. These approaches have been included in development of criteria for risk assessment of alien species in Norway operated by Norwegian Biodiversity Information Centre. These criteria have been used to develop different levels of blacklisting of species, which can serve as objective tools for prioritizing funds for management actions. Such a list of black-listed species is now regularly produced every 5- 6 years based on evaluation by several expert panels. Our models have also been used to develop strategies for reducing the impact of Chronic Wasting Disease on the wild reindeer population at Hardangervidda. CBD has also been involved by the Norwegian Environment Agency to develop criteria for assessing the vulnerability of environmental changes on ecosystems.

2. Underpinning research (indicative maximum 500 words)

A central aim of the research of the unit has been the development of a theoretical framework for analysing ecological and genetical dynamics in natural populations. A characteristics of these models is the focus of on stochastic processes, which facilitate risk analyses. These models have been developed during 2011-2021 by Steinar Engen, Russell Lande and Bernt-Erik Sæther. During the period 2011-2013 this approach was used to develop a quantitative risk classification system of alien species in Norway. This system was based on two dimensions: categories for the risk for colonization and establishment of new species along one axis and an assessment of the potential ecological and genetical impact of the species on Norwegian ecosystems on the other axis. The practical implementation of this system was done during 2012-2014 by researcher Hanno Sandvik and Bernt-Erik Sæther and is summarized in Sandvik , H., B. E. Saether, T. Holmern, J. Tufto, S. Engen, and H. E. Roy. 2013. Biodiversity and Conservation **22**:37-62. Two national lists of black-listed species are now produced by expert panels using these criteria to assess different alien species. An important aspect of this approach is that it also enables assessment of which species to prioriterize for making management interventions, see Sandvik, H., et al. 2020. Ecological Solutions and Evidence 1 and Sandvik et al. 2019.. Biological Invasions **21**:2803-2810.
Another application of the modelling framework developed at the unit has been developing proposal for harvsting the wild reindeer population at Hardangervidda to maintain genetic diversity but at the same time changing the structure and size of the population in a way that prevents further spread of the Chronic Wasting Disease to other Norwegian reindeer populations. Researcher Thomas Kvalnes and professor Bernt-Erik Sæther developed models for how different harvesting regime to reduce the prevalence of ChorniC Wasting Disease will affect the random genetic drift. These caculations strongly affected the choice of management strategies by the Norwegian authorities. These analyses were based on methods developed in Engen, S., B. -E. Sæther, T. Kvalnes, and H. Jensen. 2012. Journal

of Evolutionary Biology **25**:1487-1499 and were published in Flagstad, Ø., Kvalnes, T., Røed, K. H. Våge, J., Strand O., & Sæther B.-E. 2022. NINA Rapport 2176.
An important challenge in the management of biological diversity is that the number of species in area is diffucult to asses because of low detection proababilities. Based on community models using lognormal species abundance distribution (Sæther and Engen 2013 Journal of Animal Ecology (2013) , professor Sæther proprosed to Norwegian Environment Agency a new set of rules to evaluate the impact of environmental changes on species diversity (see CBD Reports) and through provide quantitive criteria for assessing the vulerability of ecosystem to global changes.

3. References to the research (indicative maximum of six references)

Engen, S., Saether, B.E., Kvalnes, T. & Jensen, H. Estimating fluctuating selection in age-structured populations (2012). *Journal of Evolutionary Biology,* **25,** 1487-1499. https://doi.org/10.1111/j.1420-9101.2012.02530.x

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Sandvik, H., Hilmo, O., Finstad, A.G., Hegre, H., Moen, T.L., Rafoss, T.,..., Gederaas, L. Generic ecological impact assessment of alien species (GEIAA): the third generation of assessments in Norway. (2019) *Biological Invasions,* **21,** 2803-2810. https://doi.org/10.1007/s10530-019-02033-6

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Sæther, B.-E. Prediksjonsmodellering av arter og samfunn i forvaltning av biologisk mangfold: utfordringer og muligheter (2021). *CBD Report*, pp. 1-84.

Sæther, B.-E., Engen, S. & Grøtan, V. Species diversity and community similarity in fluctuating environments: parametric approaches using species abundance distributions (2013). *Journal of Animal Ecology,* **82,** 721-738.

Sæther, B.-E., Herfindal, I., Solbu, E.B. & Norberg, A. Prediksjonsmodellering av samfunn i et endret miljø: praktiske eksemple (2022)r. *CBD Report*. Trondheim.

4. Details of the impact (indicative maximum 750 words)

Quantitative approaches have a long tradition in the management of vulnerable and threatened, for which Red Lists strongly influence management practices of vulnerable species all over the world. The risk categories involved in the classification of species are often based on different levels of the probability of extinction within a given period. For alien species no such quantitative scheme for classification was available even some of these alien species could have large economic and ecological impact in Norway. Beginning in 2011 we developed a new set of criteria which in principle were based on the reverse principle of those involved in risk classification of vulnerable species. Instead of extinction as overall measure, we proposed that the probability of establishment should form as the key classification parameter. We further indicated a set of characteristics as indicator of such an ability for a species to colonize and establish itself. In addition, we suggested that the potential for affecting important aspects of ecosystem structure and functioning also should form as an assessment criterium. This two-dimensional risk classification scheme provides a fundament for grouping different alien and

doorstep-species into different risk categories. This approach has been used two times by the Norwegian Bioinformation Center to assess by means of expert panels the risk categorization of different alien species in Norway. These lists of black-listed species have received huge public attention and resulted in several management actions to reduce the invasion of harmful alien species into Norway.

Wild reindeer is a species which Norway takes particular responsibility for ("ansvarsart). In one of the Norwegian populations Chroniic Wasting Disease was found, which led to a governmental decision to exterminate the whole population to prevent spread to other wild reindeer populations and semi-domesticated reindeer herds owned by the Sami people. Unfortunately, the disease was then recently found to be present in the largest reindeer population in Norway, located at Hardangervidda. In this area the reindeer hunt is of great cultural and economic importance, and extermination of the herd would be extremely controversial. CBD was involved, starting in 2021, on behalf of Norwegian central authorities to develop harvest strategies that reduce the rate of disease transmission while at same time reduces the impact on the rate of loss of genetical diversity. These analyses have probably influenced decisions made by Norwegian government of the choice of harvest practices of this population.

Norway is rapidly losing biological diversity, mainly due to loss and fragmentation of important habitat types. CBD has since 2020 been involved in developing for the Norwegian Environment Agency criteria for assessing the vulnerability of communities to environmental change. Furthermore, based on analyses of species distribution models researcher at CBD have evaluated how well remote sensing data can be used to predict the occurrence of rare and vulnerable bird species. Such information is crucial when imposing on restrictions on land use.

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5. Sources to corroborate the impact (indicative maximum of ten references)

Artsdatabanken 2018
https://www.artsdatabanken.no/Pages/239659/Risikokategorier_og_kriterier)

Grøtan, V. and Engen, S. poilog: Poisson lognormal and bivariate lognormal distribution. – R package ver. 0.4, http:// cran.r-project.org/web/packages/poilog/

Ims, RA, N.G.. Yoccoz, B.-E. Sæther and NC. Steinseth (2020) Naturovervåkningen i Norge må trappes kraftig opp. Aftenposten 20.3.2020

Ovaskainen, O. & Abrego, N. (2020) *Joint Species Distribution Modeling with Applications in R*.Cambridge: Cambridge University Press.

Salguero-Gómez, R. & Gamelon, M. (2021) *Demographic Methods across the Tree of Life*. Oxford: Oxford University Press.

Sæther, Bernt-Erik. (2021) Jul uten multekrem. NRK Ytring .29.12. 2021

Sæther, B.-E., Herfindal, I., Solbu, E.B. & Norberg, A. Prediksjonsmodellering av samfunn i et endret miljø: praktiske eksemple (2022)r. *CBD Report*. Trondheim.

[NTNU_IBI] [Case number 2]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: AfricanBioServices and other research in the South
Period when the underpinning research was undertaken: 2011-2020
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 – 2022
Period when the impact occurred: from 2011 until present

1. Summary of the impact (indicative maximum 100 words)

The Department of Biology has a long tradition for building research capacity in the South. From 2010- to 2013 the department was engaged in a project related to the construction of a new road in northern Serengeti. In this project we built up capacity of Tanzanian researchers and students to independently assess impacts of such a road in a vulnerable area surrounding a national park. **AfricanBioServices** is another example of a research driven capacity building project. The project generated and collated, analysed, synthesized, and disseminated unprecedented amounts of data from the Greater Serengeti Mara Ecosystem.

2. Underpinning research (indicative maximum 500 words)

In AfricanBioServices we by use of satellite images produced a description of the ecosystem and land cover maps for the entire Serengeti-Mara region. This described fine-scale spatial land cover changes during the past 40 years. We developed future scenario models with inputs from stakeholders (local farmers and pastoralists, rangers and conservancy leaders, and policymakers). Unfortunately, the papers associated with the future scenario workshops are not yet published because the pandemic prevented us to go back in early 2020 fdor follow up meetings as planned. We furthermore made more traditionally ecological studies providing more knowledge about the system and training students and researchers. One example is the use of GPS collars showing movement of wild dogs. Wild dogs did not rely on the protected areas as much as expected but were having a much larger home range and may even be jeopardized by an increasing population of lions in the Serengeti National Park. We also studied behaviour, stress, and movement of impala and concluded that the environmental variation in the landscape were a stronger driver of stress hormone levels in the impala than fear created by human or other predator encounters. An ambitious masters project in collaboration with Tanzania National Parks and Michael Anderson from Wake Forest University, USA, described the preferred plant species of the reintroduced black rhino and how these preferred plant species respond to fire that is used as a management tool prescribed in most of Serengeti as a management tool. We could show how fire actually decreases the food source for the rhino and that rhinos avoid areas that has been burned for many years after. We also carried out biodiversity measurements with eDNA sampling from streams, and guided a study on the use of local land users' information on species occurrence and abundance. We repeated countings of animal density along transects from other earlier studies to be able to track increases or decreases in species densities over the past decade, and could show that most mammal herbivores were doing well in the Serengeti park. Another such repeated study was on carnivores where by use of call-in stations we could demonstrate that lion populations had increased in the Western Serengeti protected area while it completely collapsed just as the vulture populations dramatic decrease outside the park. The vegetation part described in detail the importance of rainfall and other regional variation and of grazing pressure by livestock outside the park versus by the wildlife inside the park to species composition, plant productivity and decomposition. Despite big differences in species composition and grazing pressure temporal patterns, the primary productivity and decomposition outside the park appeared still well functioning in the Serengeti region. A Bayesian Belief Network (BBN) for land cover changes and projection was developed based on consultation with stakeholders. Part of the data gathering

was done at the future scenario workshops where also playing a board game with stakeholders on different levels simulated development of local communities.

Fieldwork activities were intense, and many institutions contributed and generated much synergy in the work. Local community facilitators participated in workshops in Tanzania and Kenya and were instrumental to the fieldwork. During the fieldwork, close contact between researchers and locals helped a two-way transfer of knowledge on important ecological and anthropogenic processes. The land cover maps and the BBN were done to show the development in land use and potential future land development scenarios. Such maps and tools are important resources for informed policy decisions in land management strategies.

In the AfricanBioServices project we developed a database that is accessed by conservation managers in East Africa. The work involved moving data from a repository (in Groningen), and into the database. NTNU hosted the work and is still in control of the database until sufficient facilities and human capacity are in place in Tanzania and Kenya. The training of technicians at advanced stage has been continuing until we are sure of ensuring sustainability of the commenced important database on biodiversity and social economic datasets in Tanzania and Kenya. The database work was led by Dr. Peter Sjolte Ranke and Dr. Gine Roll Skjærvø from NTNU.

Other projects associated with AfricanBioServices or running before this project was carried out in East Africa and other parts of Africa. These has been mostly on animal behaviour and vegetation responses to various anthropogenic impacts.

During the period 2011-2020 the group produced 13 Tanzanian PhDs, and 14 Masters. In addition, the group produced 5 PhDs from other African and Asian countries as well as 15 Masters from other southern countries. Many of these students possess currently senior positions in the Conservation and Education sector. Three of the former students are having top positions (VC, principles) at three universities in Tanzania, while many have top positions in the conservation sector. One student is currently the national rhino conservation coordinator in Tanzania. Though the research group has avoided being involved in political decision-making processes in Africa and Asia, the impact has been indirect due to the capacity built during their studies at NTNU.

The NTNU researchers involved in this work were Prof Eivin Røskaft (coordinator of AfricanBioServices and researcher, who has been working in Africa for more than 30 years) and active in Africa from 2010-2021), prof Bente J Graae (WP2 leader in AfricanBioServices and researcher in vegetation, active in Africa from 2011-2021), The Kenyan guest researcher Dr. Mohammed Saíd (researcher in land use mapping, employed at NTNU three months during 2018), Dr. Stuart Smith (Post doc and researcher in vegetation, 2015-2019), Dr. Gine Roll Skjærvø (assistant coordinator and researcher in AfricanBioServices and active in Africa btw 2010-2020). In addition, Dr Bård Gunnar Stokke (Researcher 2015-2020), Dr. Frode Fossøy (Researcher 2015-2020) and Dr. Mimi Stith (Researcher 2018-2019) and the PhD students Louis Hunninck (working on impala), Emmanuel Masenga (wild dogs), Emmanuel Clamsen Mmasssy (kori bustard), Wilfred Marealle (Giraffes), Monica Shilereyo (small rodents), Richard Lyamuya, Moses Kyando, Flora Manyama and Franco Mbise (human-carnivore conflict). Several master students from Tanzania were also enrolled in the project, eg. Philbert Ngoti (2015-2017 on rhinos), Joana Awuha (2015-2017 on ecosystem carbon response to fire)

3. References to the research (indicative maximum of six references)

Hunninck, L., May, R., Jackson, C., Palme, R., Røskaft, E., & Sheriff, M. (2020). Triiodothyronine (T3) levels fluctuate in response to ambient temperature rather than nutritional status in a wild tropical ungulate. Conservation Physiology, 8(1), coaa105.

Jackson, C., Maddox, T., Mbise, F., Stokke, B., Belant, J., Bevanger, K., Durant, S., Fyumagwa, R., Ranke, P., Røskaft, E., May, R., & Fossøy, F. (2020). A dead giveaway: foraging vultures and other avian scavengers Ecology and Evolution, 219443182.

May, R., Jackson, C., Bevanger, K., & Røskaft, E. (2019). Servicescape of the Greater Serengeti-Mara Ecosystem: visualizing the linkages between land use, biodiversity and the delivery of wildlife-related ecosystem services. Ecosystem Services, 40, 101025.

Smith, S. W., Speed, J. D. M., Bukombe, J., Hassan, S. N., Lyamuya, R. D., Mtweve, P. J., Sundsdal, A., & Graae, B. J. (2019). Litter type and termites regulate root decomposition across contrasting savanna land-uses. Oikos, 128, 596–607.

Kija, H. K., Ogutu, J. O., Mangewa, L. J., Bukombe, J., Verones, F., Graae, B. J., ... & Nzunda, E. F. (2020). Spatio-temporal changes in wildlife habitat quality in the greater Serengeti ecosystem. Sustainability, 12(6), 2440.

Anderson, T., Ngoti, P., Nzunda, M., Griffith, D., Speed, J., Fossøy, F., . . . Graae, B.J. (2020). The burning question: Does fire affect habitat selection and forage preference of the black rhinoceros Diceros bicornis in East African savannahs? Oryx, 54(2), 234-243. doi:10.1017/S0030605318000388

4. Details of the impact (indicative maximum 750 words)

With our scientific studies we have been able to contribute in great details to the knowledge about the nature and its threats in Africa. Far most of our studies have been carried out in Tanzania with professor Eivin Røskaft as leader. Røskaft has built an extensive network with various Tanzanian universities and institutions which facilitates both the logistics and the quality of the studies. This approach has been challenging because collaboration with institutions in developing countries requires much aligning with our research tradition. Nevertheless, it also enrich the research in terms of realism an opportunities for including indigenous knowledge and it serves the important purpose of contributing to what we anticipate as sustainable development in the developing countries. With the high number of supervised students, Røskaft contributed significantly to developing the research in ecology and in human wildlife conflicts in Tanzania.

Overall, our many studies in the Tanzanian ecosystems have shown the drivers of change in biodiversity and ecosystem services. In AfricanBioServices we could demonstrate the impact of management, the intactness of the fauna and flora within the park and the drastic changes in land use and land cover, vegetation composition and animal density outside the park since 1975. By involving the locals in our studies, we were able to discuss the reasons for the ecological changes and the anthropogenic drivers, including climatic, cultural, economic and psychological drivers. These discussions both elevated our research credibility and acceptance and the knowledge of the driving forces and their sustainability for the locals and policymakers. Our results generated discussions among the researchers within the AfricanBioServices but also in some previous projects and political/ideological drivers often will have a great impact on how researchers may advice the stakeholders on nature management and sustainable development. In our group, we decided that our most important role is to provide the African researchers with up-to-date knowledge and tools for making informed decisions, and not for us to recommend how to manage their land that is a highly political issue. Therefore, it has been important for us to have local researchers and other stakeholders involved in our projects but avoid too many recommendations and what may be seen as political activism or even neocolonialism.

We are therefore proud to have contributed to the capacity building for both academics and local stakeholders. Many scientific papers resulted from AfricanBioServices and surrounding projects, but the societal impact is likely more gained through the capacity building and discussions we had with stakeholders at various levels. These are though hard to evaluate and document.

An indicator of contribution to the African academia and natural resource management and conservation sector can though be demonstrated by the success of our graduated students as mentioned above.

We disseminated our research on the ***AfricanBioServices*** blog, in scientific journals and at conferences. We also presented our results to local stakeholders in the 12 villages we worked with. Finally, we developed poster scenarios and the board game. The game has further been played at various universities and is now included in ecology curriculums at universities in Norway, Denmark, and Sweden. This is to further teach students in the Western world about human-wildlife conflicts and dilemmas in an innovative way and based on our experiences in Tanzania and Kenya. The game is also sitting at the embassies of Denmark and Norway in Tanzania (https://maasaimarascience.org) as well as in the 12 villages we visited and with regional policymakers in both countries.

5. Sources to corroborate the impact (indicative maximum of ten references)

http://africanbioservices.eu/

http://www.savannalife.no

https://www.tandfonline.com/doi/abs/10.1080/14660466.2018.1491754?journalCode=uevp20

Dybas, C. L. (2014). *Is the Serengeti safe? Ask an elephant*. http://africageographic.com/blog/is-the-serengeti-safe-ask-an-elephant/

Fyumagwa, R. D., Mfunda, I., Ntalwila, J., & Røskaft, E. (Eds.). (2017). *Northern Serengeti Road Ecology*. Fagbokforlaget.

Røskaft, E. (2014a). Capacity in biodiversity conservation. *Pan European Networks: Science & Technology*, *12*, 287.

Røskaft, E. (2014b). Threats to Africa's biodiversity. *Pan European Networks: Science & Technology*, *12*, 248-249.

Røskaft, E. (2019). Linking biodiversity, ecosystem functions and services in the Serengeti-Mara Region, East Africa: Drivers of change, causalities and sustainable management strategies. *The Project Repository Journal - PRJ*, *3*, 42-45. http://edition.pagesuite-professional.co.uk/html5/reader/production/default.aspx?pubname=&pubid=fdadc48b-a0c9-49a3-b12f-fadaebf0aa07

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Røskaft, E., & Warwicker, M. (2013). *African elephants prefer Serengeti National Park*. http://www.bbc.co.uk/nature/21279321

[NTNU_IBI] [Case number 3]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: **Research on emerging pollutants contributes to public awareness and regulation**
Period when the underpinning research was undertaken: 2007- 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2007 - ongoing
Period when the impact occurred: 2011 – 2021 (ongoing)

1. Summary of the impact (indicative maximum 100 words)

At IBI we study both exposure and effects to different types of pollutants in the natural environment. The growing amount of emerging pollutants Is enormous and most of them are not regulated. Our recent research on poly- and perfluorinated substances (PFASs) has received significant governmental. media and public attention. The Norwegian Environmental Agency has used our research in preparations for the regulation of PFASs in both firefighting foams (AFFFs) and skiwax within Europe. Our research on emerging pollutants and plastic pollution is also used for evaluation and inclusion in global regulations such as the Stockholm Convention (SC) and in the regulation of microplastics in Europe as well as in the ongoing negotiations of the global plastic treaty.

2. Underpinning research (indicative maximum 500 words)

Research on emerging organic pollutants, including PFASs, has been a main research focus at the ENVITOX group at IBI, including field observations in Norway, Europe and the Arctic, as well as exposure studies at NTNU. PFASs are used in many consumer products because of their water and fat repellant properties. Examples include goretex, Teflon pans, firefighting foams and skiwax. The Research Council of Norway (RCN) and NTNU funded fellesløfte project NewRaptor (RCN 230465, 2014-2018) found that PFASs in Norwegian raptors were the most prominent pollutants in these birds and that concentrations were higher around Trondheim than Tromsø. Higher human density and ski activity in Trondheim were suggested to play a role. In addition, exposure studies on chicken eggs showed that exposure to PFASs significantly lowered embryonic heart rate before hatching and that hatchlings exposed to a high dose of F-53B (an emerging PFAS compound) had a significantly enlarged liver (8%). Another exposure study on flame retardants, showed that within the incubation period, quail embryos were able to biotransform a chlorinated phosphorous flame retardant (TDCIPP), but not the chlorinated Dechlorane plus, which seems to be very persistent and could present a health risk. These results are used to inform restrictions in Europe and for the SC (see below).

In two other projects funded by RCN (two PhD-fellowships, RCN 268419, 268258, 2017-2022), we focused on bioaccumulation and biomagnification of PFAS and flame retardants in freshwater and marine ecosystems in Norway and the Arctic (Svalbard), and on thyroid related effects in gulls in the Arctic (Svalbard). The results documented that AFFFs (leaking from airports and paper factories) are significant sources for exposure in ecosystems, resulting in levels above safe guideline threshold for ecological effects and human food safety. Emerging flame retardants were reported in Arctic gulls. The results are important in documenting presence and effects of emerging contaminants that are candidates for inclusion in the SC.

Further work on PFASs was done in a NTNU-financed PhD project (2016-2021). Soil, earthworm and bank vole samples were collected from the Granåsen skiing area in Trondheim

(as a potential point source of PFASs contamination) and from a reference area with no skiing activities (Jonsvatnet, Trondheim). The summarized PFAS concentrations were significantly higher in bank voles from the skiing area, and 35% higher in earthworms from the skiing area, compared to the reference area. The perfluorocarboxylic acid (PFCA) profile in samples from the skiing area resembled that of the previously analyzed commercial ski waxes, dominated by long chained PFCAs, while the samples from the reference area were dominated by short-chained PFCAs. This indicates that animals inhabiting skiing areas are exposed to higher PFAS concentrations than animals inhabiting areas with no skiing activities, and that these PFAS most likely are derived from fluorinated ski wax. The bank voles from the skiing area had significantly higher brain dopamine concentrations, compared to the reference area. In addition, reduced testosterone concentrations were detected in the muscle tissue of male bank voles from the skiing area. The liver PFAS concentrations reported in the bank voles from the skiing area, were within the range of concentrations reported in the plasma of professional waxing technicians, which raised concerns for human health.

Ongoing work on plastic pollution includes characterizing the human and environmental toxicity of nano- and microplastics as well as of the chemicals present in plastics. We have assessed the environmental risks of nano- and microplastics using meta-analyses. We have estimated species sensitivity distribution using toxicity data from the published literature to derive toxicological thresholds for Norwegian policymakers (VKM) and for the State of California. These can be applied by regulators in multiple contexts (see below). Working with an international group of experts, we have also provided the first scientific definition of plastic debris in nature that has been used by regulators across the globe. Lastly, our work on chemicals in everyday plastic products has established that the plastics contain many more toxic chemicals than previously thought. This knowledge has contributed to making this aspect part of the international negotiations of the plastics treaty.

PhD students involved:

- Jenny Bytingsvik (RCN 175989, 2007-2012)
- Nathalie Briels, PhD student working on NewRaptor (RCN 230465, 2014-2019)
- Mari Løseth, PhD student working on NewRaptor (RCN 230465, 2014-2019)
- Randi Grønnestad (2016-2021)
- Håkon Langberg (RCN 268258, 2017-2021)
- Åse-Karen Mortensen (RCN 268419, 2017- ongoing)
- Essa Ahsan Khan (RCN 248840, 2016-2021)

Permanent staff at ENVITOX involved:

- Veerle Jaspers, project leader NewRaptor (RCN 230465), supervisor of Briels, Løseth, co-supervisor of Grønnestad
- Augustine Arukwe, supervisor of Grønnestad, Khan
- Bjørn Munro Jenssen, supervisor of Bytingsvik, Langberg, Mortensen, co-supervisor Grønnestad, Løseth
- Åse Krøkje , co-supervisor Grønnestad
- Martin Wagner, research on plastic pollution
- Tomasz M. Ciesielski, co-supervisor Briels, Mortensen

3. References to the research (indicative maximum of six references)

1. Briels N., Torgersen L.N., Castaño-Ortiz J.M., Løseth M.E., Herzke D., Nygård T., Bustnes J.O., Ciesielski T.M., Poma G., Malarvannan G., Covaci A., Jaspers V.L.B. (2019). Integrated exposure assessment of northern goshawk (*Accipiter gentilis*) nestlings to legacy and emerging organic pollutants using non-destructive samples, Environmental Research 178: 108678. https://doi.org/10.1016/j.envres.2019.108678

PFASs were the compounds found in the highest concentrations in blood plasma, suggesting that the nestlings were recently and continuously exposed to PFASs, likely through dietary intake.

2. Briels N, Løseth ME, Ciesielski TM, Malarvannan G, Poma G, Kjærvik SA, Léon A, Cariou R, Covaci A, Jaspers VLB (2018). *In ovo* transformation of two emerging flame retardants in Japanese quail (*Coturnix japonica*). Ecotoxicol Environ Saf. 149:51-57. DOI: 10.1016/j.ecoenv.2017.10.069

   In this in ovo experiment it was found that Dechlorane plus could not be biotransformed by the embryo.

References 1-2 and others were cited in Persistent Organic Pollutants Review Committee (POPRC) recommendations to nominate PFCAs and Dechlorane plus to the SC.

3. Bytingsvik J, van Leeuwen SP, Hamers T, Swart K, Aars J, Lie E, Nilsen EM, Wiig O, Derocher AE, Jenssen BM (2012). Perfluoroalkyl substances in polar bear mother-cub pairs: a comparative study based on plasma levels from 1998 and 2008. Environ Int. 15;49:92-9. https://doi.org/10.1016/j.envint.2012.08.004

Reference 3 and other relevant papers were cited in Risk profile on perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds. UNEP/POPS/POPRC.14/6/Add.1

4. Briels, N., Ciesielski, T. M., Herzke, D., & Jaspers, V. L. B. (2018). Developmental Toxicity of Perfluorooctanesulfonate (PFOS) and Its Chlorinated Polyfluoroalkyl Ether Sulfonate Alternative F-53B in the Domestic Chicken. Environ Sci Technol, 52(21), 12859-12867. doi:10.1021/acs.est.8b04749

Reference 4 and others are cited in the European Chemicals Agency (ECHA) restriction proposal on PFASs in firefighting foams.

1. Grønnestad, Randi; Vazquez, Berta Perez; Arukwe, Augustine; Jaspers, Veerle; Jenssen, Bjørn Munro; Karimi, Mahin; Lyche, Jan Ludvig; Krøkje, Åse. Levels, patterns, and biomagnification potential of perfluoroalkyl substances in a terrestrial food chain in a Nordic Skiing Area. Environmental Science and Technology 2019 ;Volum 53.(22) s. 13390-13397. DOI: 10.1021/acs.est.9b02533

   This study was cited in the Miljødirektoratet report: PFAS in the treatment of skis — Use, Emissions and Alternatives, M-2032 | 2021.

2. Grønnestad, Randi; Schlenk, Daniel; Krøkje, Åse; Jaspers, Veerle; Jenssen, Bjørn Munro; Coffin, Scott; Bertotto, Luísa Becker; Giroux, Marissa; Lyche, Jan Ludvig; Arukwe, Augustine. Alteration of neuro-dopamine and steroid hormone homeostasis in wild Bank voles in relation to tissue concentrations of PFAS at a Nordic skiing area. Science of the Total Environment 2021;Volum 756:143745. s. 1-8. DOI: 10.1016/j.scitotenv.2020.143745

References 5-6 intensified the discussion about the regulation of PFASs in skiwaxes for professional competition in 2021, which has been postponed.

3. Skåre, J.U., Alexander, J., Haave, M., Jakubowicz, I., Knutsen, H.K., Lusher, A., Ogonowski, M., Rakkestad, K.E., Skaar, I., Sverdrup, L.E., Wagner, M. et al. (2019) Microplastics; occurrence, levels and implications for environment and human health related to food. Scientific opinion of the Scientific Steering Committee of the Norwegian Scientific Committee for Food and Environment. 2019. VKM Report (2019:16).

4. Mehinto, A. C., Coffin, S., Koelmans, A. A., Brander, S. M., Wagner, M., Thornton Hampton, L. M., Burton Jr, A. G., Miller, E., Gouin, T., Weisberg, S. B., Rochman, C. M. (2022) Risk-based management framework for microplastics in aquatic ecosystems. Microplastics and Nanoplastics, 2, 17. DOI: 10.1186/s43591-022-00033-3

References 7 and 8 present species sensitivity distribution to estimate safe levels of nano- and microplastics in the aquatic environment.

4. Details of the impact (indicative maximum 750 words)

The published results of PFASs in both wildlife and effects found in exposure studies raised cause for concern for human health as well. In 2019, Krøkje and Grønnestad were approached by journalists from Dagbladet as they were making an investigation and large case on PFASs in skiwax. The research related to the findings found in ski areas was subsequently largely covered by the public media, national and internationally (15 media outlets registered in Cristin, including NRK, The Telegraph and Daily Mail) and Grønnestad was invited to give several talks regarding her findings from 2019-2021. The Norwegian Environmental Agency (Miljødirektoratet) also started looking into the studies and published a report in 2021 (M-2032). There was a plan to ban PFASs from all skiwax for professional competition in 2021, but this regulation was postponed. However, the current aim is to put a European wide legislation on PFASs into place by 2025 through REACH and Norway is a key player in the preparations towards the restriction report (to be delivered to ECHA in January 2023). The societal impact is already evident with several non PFASs alternatives now on the market and consumers being aware and asking about non PFASs skiwax when they buy skiwax. Our research, carried out and mainly drive by people at IBI (ENVITOX), has thus made a major impact on both the attention of regulators and the public attention towards PFASs in skiwax.

In a global context, both Dechlorane plus and long-chain PFCAs are currently under review for being listed on the Stockholm Convention for persistent organic pollutants. The Norwegian Environment Agency is routinely checking research articles and has also employed many of our studies to include for making proposals to ECHA and the Stockholm Convention (see references for some examples). As a results, in 2019 and 2022, two specific PFASs were added to the Stockholm Convention, PFOA and PFHxS, respectively. PFHxS was also one of the compounds found higher in the voles from the ski area as compared to the reference area by Grønnestad et al. 2019. Also the results from IPY project BearHealth (RCN 175989, 2007-2012) were presented for the SC in 2018 (POPRC-14) and contributed to regulations of PFHxS in the SC in 2022 (SC-10/13). Our research has made important contributions to these regulations, but this has to be seen as a part of the puzzle within the international research community that in total leads to a global impact.

Regarding the results on thresholds of nano- and microplastics in the environment: This research is being used by the Californian government to manage plastic pollution in their water bodies. The definition of plastic debris (recommendations formulated by the research group of Martin Wagners) has been used in ECHA's restriction proposal for intentionally used microplastics. Multiple other national regulations reference Wagner's work but are difficult to track down (1000+ citations). Research on plastic chemicals has been used to include the issue of hazardous chemicals in plastics in the ongoing negotiations of the global plastic treaty.

In summary, our research at IBI (ENVITOX) has contributed to many important regulations and is used in many proposals for new regulations. Regulations and restrictions of harmful chemicals are for the benefit of the global biosphere, including human health and society. Especially global regulations are of essence as chemical pollution crosses national and international borders.

5. Sources to corroborate the impact (indicative maximum of ten references)

Risk assessment and regulation:

1. Miljødirektoratet/ Norwegian Environment Agency PFAS in the treatment of skis — Use, Emissions and Alternatives - M-2032 | 2021
   PFAS in the treatment of skis - Use, Emissions and Alternatives - Miljødirektoratet (miljodirektoratet.no)

2. Restriction under REACH – proposal for a European wide regulation on PFASs:
   https://www.miljodirektoratet.no/ansvarsomrader/kjemikalier/reach/restriksjoner-under-reach/forbud-mot-pfas/

3. Persistent Organic Pollutants Review Committee (POPRC) recommendations UNEP-POPS-POPRC.17-7 (Canadian proposal for long chain PFASs) and UNEP-POPS-POPRC.17-13 (Risk profile for Dechlorane plus)
   http://www.pops.int/TheConvention/POPsReviewCommittee/Recommendations/tabid/243/Default.aspx

4. Perfluorohexane sulfonic acid (PFHxS), its salts and PFHxS-related compounds listed in 2022 on Annex A without specific exemptions (decision SC-10/13) and perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds in 2019 on Annex A with specific exemptions (decision SC-9/12)
5. http://www.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspxA MAP, 2018. AMAP Assessment 2018: Biological Effects of Contaminants on Arctic Wildlife and Fish. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. vii+84pp

6. Dietz R, Letcher RJ, …, Ciesielski TM, … Jenssen BM, et al (42 authors) 2022. A risk assessment review of mercury exposure in Arctic marine and terrestrial mammals. Science of the Total Environment 829: 154445.
   DOI: 10.1016/j.scitotenv.2022.154445

7. Definition of plastic debris: European Chemicals Agency (2019) ANNEX XV RESTRICTION REPORT PROPOSAL FOR A RESTRICTION
   https://echa.europa.eu/documents/10162/05bd96e3-b969-0a7c-c6d0-441182893720

8. Plastic chemicals: UNEP Preparation of an international legally binding instrument on plastic pollution, including in the marine environment - Plastics science, UNEP/PP/INC.1/7
   https://www.unep.org/about-un-environment/inc-plastic-pollution

International media:

9. Wax on skis could be harming the environment. The Telegraph (telegraph.co.uk) [Avis] 2019-11-06

10. Ski wax is being EATEN by animals at winter resorts and infiltrating the food chain at potentially toxic rates, scientists warn. Daily Mail (dailymail.co.uk) 2019-11-06

[NTNU_IBI] [Case number 4]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: **Improved interaction between business, upper secondary school, university, and public administration in aquaculture – "Bridgehead Aquaculture - 2050"**
Period when the underpinning research was undertaken: 2012- Ongoing
Period when staff involved in the underpinning research were employed by the submitting institution: 2018 - Ongoing
Period when the impact occurred: 2012 - 2022

1. Summary of the impact (indicative maximum 100 words)

"Bridgehead Aquaculture" (Brohode Havbruk in Norwegian) is a multi-stakeholder regional knowledge platform for efficient and mutual exchange of theoretical and practical knowledge within the aquaculture sector. It assembles key resources from aquaculture producing and supplying companies and the public sector, professors, students and researchers, teachers and pupils from secondary schools. The vision is to enhance the aquaculture sector's capacity to implement resilience, sustainability and competitiveness.

Based on the platform activity new study programs have been developed, key competence resources from the aquaculture industry have been used in teaching activity in regular university courses and in supervision of students' theses. In addition, mobility between academia and industry has been promoted to increase mutual understanding between stakeholders.

2. Underpinning research (indicative maximum 500 words)

The goal of Bridgehead Aquaculture is to enhance the aquaculture sector's capacity to implement resilience, sustainability, and competitiveness, with regional stakeholders as key enablers. A knowledge gap analysis undertaken in 2018 showed the sector's challenge to recruit technology and engineering competence. The aquaculture sector also misses opportunities by not maximizing the potential of high-tech solutions as steppingstones towards the above-mentioned goals. An understanding of the impact of new methods for sustainable farming of aquaculture species and its environment is hereby critical. Bridgehead Aquaculture 2050 has contributed to the capacity of the aquaculture businesses to apply new technologies, whilst setting biology in the centre, leading to smarter, greener and more efficient processes.

Since the signing of cooperation agreement between NTNU Oceans and the secondary "blue" school of Guri Kunna (Hitra/Frøya) in 2012, an annual Bridgehead conference has been organized at Frøya. This has become a unique meeting place for aquaculture stakeholders and academia, where students fulfill a key role as representatives of their capacity building abilities. The agreement was renewed in 2021.

In addition to the annual conference, a series of Bridgehead events are organized throughout the year. These vary in form, ranging from experience exchange seminars, company site visits, business presentations, workshops, student competitions and/or speed date sessions. The events stimulate stakeholders in the region to increase their network and to implement student and/or research interactions into their business strategies. The project specifically accentuates the underexploited capacity of interdisciplinarity across engineering and technology, biology and other natural and social sciences, and humanities. By stimulating interactions between

stakeholders, a common understanding of the various processes along the aquaculture value chain is established.
The Bridgehead aquaculture 2050 toolbox helps introducing research-based competence through different pathways. It has introduced a considerable number of companies to the opportunities provided by an industry PhD and has assisted many of them in applying. It has also engaged industry stakeholders in part-time positions at the university, promoted mobility of professors through short-term stays in a company and organized visits for researchers to different types of aquaculture businesses. These interactions have additionally contributed to increase the societal relevance of education and research among academic partners, and to attract candidates towards a sector where Norway has a global responsibility.

Finally, Bridgehead aquaculture 2050 adds value to regional and international aquaculture industry meeting places, such as:

- AquaNor and Nor-Fishing
- TEKSET and TEMAR
- NCE Aquatech Cluster

In these meeting places interactive student related events are organized.

The Norwegian Research Council's mid-term evaluation in 2021 highlighted the progress that had already been made in the creation of a knowledge capacity-building legacy in the region of Mid-Norway.

Key researchers (positions) joined:

- Alexandra Neyts, Project leader - Bridgehead aquaculture 2050
- Yngvar Olsen, project collaborator and head of the Bridgehead conference programme committee
- Anna Solvang Båtnes, project collaborator - Bridgehead aquaculture 2050
- Rolf Erik Olsen, programme leader of bachelor aquaculture engineering programme
- Bjørn Egil Asbjørnslett, leader of “minor in aquaculture” programme
- Martin Føre, project collaborator - Bridgehead aquaculture 2050
- Kjell Olav Skjølsvik, project collaborator - Bridgehead aquaculture 2050

3. References to the research (indicative maximum of six references

1. Olavsen T, Winther U, Skjermo J Olsen Y .
   DKNVS and NTVA Report: Value created from productive oceans in 2050 2012
   ISBN 978-82-7719-074-3
   The report makes prediction of future development of the seafood sector in Norway and conclude that the value created in 2050 can be 550 billion NOK, five times higher in 2050 than in 2010. The report has had a tremendous impact on society and policy formation in the seafood sector, but also on the scientific community. The report is generally simply mentioned as the “2050 report” and is very frequently sited. It is still after 10 years among the planning documents used by Government, and an implementation plan is worked out after request of the Department responsible for fishery. if requested by RCN or the evaluation secretariate.
2. Neyts, A.
   Which professional profiles does the aquaculture industry need and which educational paths are missing or need to be improved?.
   2022
   Aquaculture Europe '22; 2022-09-27 - 2022-09-30

3. Neyts, Al., Vedal, T.,
Årsrapport Brohode havbruk 2050 - fjerde år.
2022
4. Neyts, A., Heggstad, T., Fallmyr, J.,
2018
Kartlegging av kompetansebehov i Midt-Norge.
5. Akslen Emblem, H. L., Halstenrud, K. B.
NTNU Brohode Havbruk. En kvalitativ analyse av kommunikasjon og forventninger i et samarbeid mellom havbruksnæringen og akademia. Prosjektoppgave i MV3010 Forskningsoppdrag for bedrift.
2021

4. Details of the impact (indicative maximum 750 words)

In 2018, a consortium of academia (NTNU, SINTEF Ocean), industry (NCE Aquatech Cluster, Blue Competence Centre), and public sector (Trøndelag County Authority) was formed and the Norwegian Research Council granted a 6-year collaborative capacity building project "Bridgehead Aquaculture 2050". Its goals are to increase business relevance in higher education, strengthen recruitment to the marine sector, and reinforcing research-based expertise in the seafood industry. Through this Bridgehead Aquaculture 2050 project, a toolbox is developed to close the gap between students, researchers and aquaculture stakeholders. New study programs have been developed (see below), aquaculture relevant student cases and assignments have been promoted, and mobility efforts between academia and industry have contributed to a larger mutual understanding. The annual Bridgehead conferences strengthen the collaborative culture in aquaculture across stakeholder groups with a specific emphasis on students and their knowledge building capacity.

Recommendations by and dialogue with the sector resulted in the generation of a unique aquaculture engineering bachelor programme in 2020. It prepares the students for a professional career in the producing and supplying industry, providing them with knowledge and insights on how technology and operational choices impact the efficiency and sustainability in aquaculture. A pilot programme package for civil engineers, called "Minor in aquaculture" was established in 2021. This allows the students to combine their technological expertise with a basic understanding of biology and aquaculture operations, thus introducing new types of knowledge into the sector. An increasing share of students is performing bachelor and master assignments and PhD theses in aquaculture related topics.

In 2022, more than 150 student publications were delivered, across more than 20 different study programmes. Upskilling of existing staff was also targeted by developing and offering continued education courses at Master level. So far, three courses have been established: on recirculating aquaculture systems, on safety management and risk analysis and on project management and engineering in aquaculture.

In 2016 the project *"Taskforce salmon lice" – cooperation between university and aquaculture industry to solve salmon lice challenges"* – was established as a direct result of this impact case. "Taskforce salmon lice" is a research platform that was established as a project at NTNU, mainly funded by the aquaculture industry. Through cooperation with the industry, the research group (researchers, PhD students, master students and supervisors) are investigating topics related to the parasitic salmon louse, that are currently relevant to the industry. The results from the research are used by the industry to handle the sea lice challenges more efficiently and thus in

a more environment friendly way. The platform also enables students to work with highly relevant research in cooperation with companies, and to make an impact after ending their education.

"Bridgehead Aquaculture" is at the core of several United Nations sustainable development goals (SDGs), particularly;

- Goal 3 - Ensure healthy lives and promote well-being for all at all ages
- Goal 4 – Quality education, ensuring inclusive and equitable quality education and promote lifelong learning opportunities for all
- Goal 8 - Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
- Goal 9 - Build resilient infrastructure, promote inclusive and sustainable industrialization, and foster innovation
- Goal 11 - Make cities and human settlements inclusive, safe, resilient, and sustainable
- Goal 14 - Life below water. Conserve and sustainably use the oceans, seas and marine resources for sustainable development
- Goal 17 - Strengthen the means of implementation and revitalize the global partnership for sustainable development

5. Sources to corroborate the impact (indicative maximum of ten references)

Brohode Havbruk - NTNU
Studentoppgaver: Projects | Bridge NTNU
NTNU Brohode – Minor i havbruk – NTNU
Bachelor i ingeniørfag, havbruk - NTNU
NTNU Brohode - Brohodekonferansen - NTNU
Taskforce sea lice - NTNU Oceans - NTNU

[NTNU_IBI] [Case number 5]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: **Cellular communication tools show success as novel therapeutics**
Period when the underpinning research was undertaken: 2012-2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2012-2022
Period when the impact occurred: 2015 and 2022

1. Summary of the impact (indicative maximum 100 words)

Hyperproliferation is a characteristic of many pathologic processes, such as cancer and chronic diseases. Understanding the molecular mechanisms regulating normal physiologic processes and how these mechanisms change when pathology is activated, is crucial. The Lipid signalling group has identified and investigated important nodes controlling cellular communication regulating the very neat balance between pro and anti-inflammatory cellular conditions. These studies were enabled and successfully accomplished by development and application of powerful molecular tools carrying node high selectivity and sensitivity. Upon later testing these tools as candidate therapeutics, PoC for treatment of psoriasis was accomplished in 2015, and PoC for Aktinic keratosis was accomplished in 2022.

2. Underpinning research (indicative maximum 500 words)

In basic research performed for understanding fundamental biological process as life and death at the molecular and cellular level, a range of tools are needed like genetic and chemical ones. Our effort on understanding cellular communication mechanisms regulating hyperproliferation and programmed cell death and the balance between them, we were among the first worldwide to determine a very central role of an enzyme modifying membrane phospholipids, namely the phospholipase A2α (cPLA2α) enzyme. This is a member of a large PLA2 enzyme family comprising more than 25 different members. We have shown that two PLA2 enzymes are sequentially activated in cells when cytokine, f.ex TNFα, bind to its receptor that in parallel to initiating its intracellular classical kinase cascade eventually leading to activation and nuclear translocation of the very important transcription factor, NF-κB; a parallel kinase cascade is initiated by extracellular activation of a secretory PLA2 enzyme leading to production of minute amounts of leukotriene B4 (LTB4), LTB4 translocate to the outside of the cell and bind to G-protein coupled receptor and initiate a kinase cascade including atypical PKC and MAPkinase p38, leading to activation of cPLA2α and production of massive amounts of LTB4. Thus, generating an autocrine PLA2-driven kinase loop mediated by LTB which is obligatory for the cytokine induced nuclear translocation of NF-κB (2xAnthonsen, Johansen et.al. J.Biol.Chem., 2001 aug and sept). This research was performed in skin keratinocytes which created the understanding that cPLA2α could act as a therapeutic target in psoriasis? After these findings, basic research was expanded into other cellular models representing fibrosis and cancers (balance between hyperproliferation and programmed cell death) and research identifying cPLA2α as critical regulator of such processes, was and is, performed in collaboration with international experts like Prof Joseph V Bonventre,

Harvard University and Prof Ed A Dennis, UCSD, USA. Professor Dennis is the founder of the NIH-supported Lipid Maps Consortium in the US, where we enjoy very successful collaborations. At Harvard University in collaboration with Prof Bonventre, we enjoy access to highly relevant mouse models.

As part of elucidation of the specific role of the cPLA2α, we together with internationally renowned synthetic chemists (Prof Lars Skattebøl, UiO and Prof George Kokotos, U of Athens, GRE), developed highly selective and sensitive chemical compounds that can enter cells, bind selectively to the cPLA2α enzyme active site, and most successfully inhibit its activity! These molecular tools have helped us and others to understand fundamental biological process at molecular level. After achieving this, we speculated that such molecular tools might even have therapeutic potential in diseases characterized by hyperproliferation and delayed programmed cell death? We have now shown by several successful phase IIA clinical trials in patients that there are proof of concept (PoC) for treatment of psoriasis and actinic keratosis. A new clinical trial for skin cancer (BCC) is underway. Furthermore, these treatments are with less adverse events compared to currently used therapeutics, and therefore more sustainable. Indeed, the independent safety committee following the psoriasis clinical phase IIA trials concluded that the treatment was remarkably safe. Planning of testing this inhibition strategy as novel therapeutics is underway also for leukemia.

Key personnel involved in research, where PhD-students are supervised by Postdocs:

Phd-students:
Randi Sommerfelt, defended thesis 2015
Hanna Maja Tunseth, defended thesis
Eirini Tsirvouli, to defend thesis 2023
Nur Mahammad, to defend thesis 2023

Post.docs:
Dr Astrid J Feuerherm
Dr Felicity Ashcroft

Professor, PI:
Dr Berit Johansen

3. References to the research (indicative maximum of six references)

Huwiler A, Feuerherm AJ, Sakem B, Pastukhov O, Filipenko I, Nguyen T, **Johansen B**. The ω3-polyunsaturated fatty acid derivatives AVX001 and AVX002 directly inhibit cytosolic phospholipase A(2) and suppress prostaglandin E(2) formation in mesangial cells. *Br. J. Pharmacol.* **167:**1691–1701, 2012.

SA Moestue, MT Grinde, E Marangoni, T Sørlie, O Engebråten, GM Mælandsmo, **B Johansen**, TF Bathen. Cytosolic phospholipase A2 (cPLA2) as a therapeutic target in basal-like breast cancer. *Cancer Research* 73:24, P6-04-08, 2013.

Randi M. Sommerfelt, Astrid J Feuerherm, Trine Skuland, and **Berit Johansen**. Cytosolic phospholipase A2 modulates TLR2 signaling in synoviocytes. *PLoS One,* 10;4 (EMID:92836968d9e74a43, 2015.

Feuerherm, AJ., Dennis, EA. and **Johansen, B**. AVX001 and AVX002 inhibitors of cytosolic group IVA phospholipase A2 ameliorate collagen induced arthritis. *Arthritis Research and Therapy,* Jan 21;21(1):29, 2019.

Tunset, Hanna Maja; Euceda, Leslie Romelia; Feuerherm, Astrid Jullumstrø; Rao, Shalini Vasudev; and **Johansen, Berit**; Moestue, Siver Andreas. New insight into anti-metastatic properties of cytosolic phospholipase A2 alpha inhibition: Regulation of migration, transcriptome, and protein networks. *Internat. J. Mol. Sci,*20.19 //doi.org/10.3390/ijms20194800, 2019.

Nur Mahammad, Felicity J. Ashcroft, Astrid J. Feuerherm, Samah Elsaadi, Esten N. Vandsemb, Magne Børset, **Berit Johansen**. Inhibition of cytosolic phospholipase A2a induces apoptosis in multiple myeloma cells. *Molecules*, Dec 9;26(24):7447. doi: 10.3390/molecules26247447, 2021.

4. Details of the impact (indicative maximum 750 words)

The basic research performed identified and documented a novel regulatory node, the cPLA2α enzyme, in intracellular signalling cascades controlling the balance between hyperproliferation and programmed cell death. Such fundamental biological processes are of highest importance also in regulation between physiologic and pathologic conditions, therefore the cPLA2α may be exploited as a novel therapeutic target. During our investigations several molecular, cellular and animal tools, like commercial inhibitors against kinases, antibodies for ELISA or westerns or microscopy, primers for pcr, siRNA and animal KO models were employed. To pinpoint the unique physiological role of the cPLA2α enzyme, highly selective and sensitive inhibitors targeting the active site of the enzyme were developed by us in collaboration with internationally renowned synthetic chemists. These inhibitor molecules were very successful in pinpointing the role of the enzyme in intracellular signalling, AND would it also be beneficial as therapeutics? That was something we asked ourselves, and we made contact to biotechnological entrepreneur experts. In collaboration with these we established the biotech company Avexxin AS, that was successful in attracting about a total of 350 millNOK in capital both private and governmental to support development, preclinical safety testing and successful clinical testing all through phase IIA for psoriasis. The funds were primary made available from Norwegian government supported investment funds like SARSIA Seed and LEN Nyskaping and a large number of private investors in Mid-Norway like Sparebanke1 and others. A part of this funding was also from NFR and Skattefunn. Avexxin existed until 2019 when it was decided to enter the stock exchange, be listed. Then we needed to change name to Coegin Pharma (www.coeginharma.com), and in 2020 we were successfully listed on NGM in Stockholm. The listing enabled access to more private and governmental, international capital including Almi Invest supported by the Swedish government, and the company was valued about 400 millSEK. In several rounds of financing the company has lifted more money and successfully completed the clinical phase IIA testing of AVX001 cPLA2α inhibitor as treatment of Actinic Keratosis. The core company Coegin Pharma is now the platform company developing and performing preclinical safety and formulation work of the different molecular tools that were developed against cPLA2α, and when a new indication is decided a daughter company is established in order to recruit money for clinical testing. Until now, Avexxin Oncology is established for testing AVX420 in leukemia and Reccura Therapeutics is established for testing of AVX001 inhibitor to treat basal cell carcinoma, BCC.

The company strategy has attracted highly competent businesspeople with former international pharma competence. The business model is to license the technologies to big pharma after clinical phase II, for further clinical phase III development and market entry of therapeutic. Such licensing discussions are already ongoing with big pharma.

5. Sources to corroborate the impact (indicative maximum of ten references)

George Kokotos, Astrid J. Feuerherm, Efrosini Barbayianni, Ishita Shah, Mari Sæther, Victoria Magrioti, Thuy Nguyen, Violetta Constantinou-Kokotou, Edward A. Dennis and **Berit Johansen**. Inhibition of Group IVA Cytosolic Phospholipase A_2 by Thiazolyl Ketones *In Vitro*, *Ex Vivo*, and *In Vivo*. *J. Medicinal Chemistry*, 57(18):7523-35, DOI: 10.1021/jm500192s, 2014.

Kim, E., Tunset, HM., Cebulla, J., Vettukattil, R., Helgesen, H., Feuerherm, AJ., Engebråten, O., Mælandsmo, GM., **Johansen, B.,** Moestue, SA. Anti-vascular and molecular effects of cytosolic phospholipase A2 inhibition in a patient-derived basal-like breast cancer model. *BMC Cancer,* 16:191, 2016.

Omland SH, Habicht A, Damsbo P, Wilms J, **Johansen, B**, Gniadecki R. A randomized, double-blind, placebo-controlled, dose-escalation first-in-man study to assess the safety and efficacy of topical phospholipase A2 inhibitor AVX001 in patients with mild to moderate plaque psoriasis. *Eur. J. Derm. Venerol.* Jan 20. doi: 10.1111/jdv.14128, 2017.

Chiorazzo, Michael G., Tunset, Hanna Maja, Popov, Anatoliy V., **Johansen, Berit**, Moestue, Siver, Delikatny, Edward, J. Detection and Differentiation of Breast Cancer Sub-Types using a cPLA2 Activatable Fluorophore - DDAO arachidonate. *Scientific reports*, Apr 16;9(1):6122, 2019.

Ashcroft, FJ., Mahammad, N., Flatekvål, HM., Pinõl, M., Feuerherm, AJ. and **Johansen, B.** cPLA2a enzyme inhibition attenuates keratinocyte inflammation and proliferation. *Biomolecules*, Oct 2;10(10):1402. 2020.

Tsirvouli E, Ashcroft F, **Johansen B**, Kuiper M. Logical and experimental modeling of cytokine and eicosanoid signaling in psoriatic keratinocytes. iScience. 2021 Nov 15;24(12):103451. doi: 10.1016/j.isci.2021.103451. eCollection 2021.

Ortner, Vinzent K; **Johansen, Berit**; Kilov, Kim; Mondragon, AC; Kihl, Jesper; Ashcroft, Felicity; Feuerherm, Astrid Jullumstrø; Laugesen, CP; Espersen, MLM; Manole, I; Isberg, IP; Andersen, AD; Rakvaag, Elin; Zibert, JR; Hædersdal, Merete. The Copenhagen Actinic Keratosis study (COAKS). A decentralized clinical trial to evaluate tolerability, safety and efficacy of daily field-directed topical treatment with cytosolic phospholipase A2 inhibitor, AVX001, in participants with actinic keratosis: Protocol for a randomized controlled phase I/IIa trial. *BMJ Open* Oct 5;12(10):e061012; doi: 10.1136/bmjopen-2022-061012, 2022.

Johansen, Berit; Naini, Said Movahedini; Selvik, Linn-Karina M.; Feuerherm, Astrid Jullumstrø; Ashcroft, Felicity; Wang, C; Yu, S; Yin, WQ; Alevizploulos, Konstantinos; Quehenberger, Oswald; Dennis, EA; Bonventre, Joseph V. Group IVA cytosolic phospholipase A2 (cPLA2α): pharmacological 2 inhibition attenuates kidney inflammation and fibrosis. *JASN, in review* 2022.

NTNU_IBT [1]

Institution: Norwegian University of Science and Technology
Administrative unit: Department of Biotechnology and Food Science
Title of case study: Spin-off Syngens AS
Period when the underpinning research was undertaken: 2016 – 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2009 – …
Period when the impact occurred: 2020

1. Summary of the impact

The establishment of a spin-off Syngens AS in November 2020 that operates at the intersection of artificial intelligence and synthetic biology.

2. Underpinning research

The impact presented in this document is the result of research conducted in Rahmi Lale's group within the Molecular Biotechnology (MB) division. Historically, the MB division has been strong in bacterial gene expression. For instance, research conducted at Svein Valla's (deceased in 2013) research group led to the founding of Vectron Biosolutions in 2008 with patents also shared by several of the MB division members among others including Ingrid Bakke, Trygve Brautaset and Rahmi Lale. Lale is the head of an interdisciplinary lab that combines biophysics, computational, and synthetic biology. Understanding and controlling transcriptional and translational regulation in microorganisms has been a focus of Lale's research group.

The research conducted between 2016 and 2020 yielded the following results, among others:

- In addition to their significance in translation, 5'-untranstalted regions (UTRs) contain DNA sequence characteristics involved in transcriptional control (Lale et al. 2011, Le et al. 2020).
- Artificial promoters can be constructed from scratch and be functional without containing sequence motifs that mimic the patterns seen in the genomic host of interest (Lale et al. 2019/2022).
- The DNA sequence composition of promoter, 5'-UTRs, and coding sequences are context-dependent and affect both transcriptional and translational processes (Tietze and Lale 2021).
- The use of several expression hosts improves the chances of successful gene expression of target products such as industrial enzymes (Fast-X-Press).

The following grants have been critical that led to the impact:

- 2016 – 2017, NTNU-Biotechnology Program, Bluesky Microfinance Project. *Protein Expression and Codon Restriction Á La Carte*.
- 2016 – 2020, EU-H2020. *Advanced toolbox for rapid and cost-effective functional metagenomic screening – microbiology meets microfluidics.*
- 2017 – 2018, NTNU-Discovery Pilot Project. A key enabling expression platform.
- 2017– 2021, The Engineering and Physical Sciences Research Council (EPSRC) UK, Design the Future 2: Thinking Soils: Engineered bacteria as computational agents in the design and manufacture of new materials and structures.
- 2017 – 2021, NTNU Enabling Technologies, Biotechnology, *A New Enabling Technology Platform for Recombinant Protein Production.* Ph.D. project

- 2018 – 2019, NTNU-Discovery Main Project, *SUPERAP for protein production at industrial scales.*
- 2019 – 2020, FORNY2020, The Research Council of Norway. *Fast-X-Press, Fast track for efficient protein production in novel hosts.*

Syngens was founded by two people: Rahmi Lale, and Gurvinder Singh Dahiya, an AI-researcher who is not an NTNU employee. Lale and his colleagues together with Dahiya integrated AI with synthetic biology methodologies, resulting in the development of a machine learning-based DNA design platform.

The platform can be used to:

- *De Novo* 5' regulatory sequence (promoter and 5' UTR) design across variety of bacterial hosts;
- Optimisation of expression;
- Pathway engineering;
- Novel CDS optimisation;
- Promoter prediction.

The platform's algorithms have not been disclosed and are being kept as know-how, which is the key IP underpinning Syngens. Syngens has entered into a licencing arrangement with NTNU-TTO in 2021.

Rahmi Lale's positions held at IBT:

- 2009 – 2012 Postdoctoral researcher (100%)
- 2012 – … Researcher (60%)
- 2016 – … Adjunct Assoc. Prof. (20%)

3. References to the research

Lale R, Berg L, Stüttgen F, Netzer R, Stafsnes M, Brautaset T, Aune TEV and Valla S. Continuous control of the flow in biochemical pathways through 5'-UTR sequence modifications in mRNA expressed from the broad-host-range *Pm* promoter. 2011 Appl. Env. Mircobiol. http://dx.doi.org/10.1128/AEM.02091-10

Lale R, Tietze L, Fages-Lartaud M, Nesje J, Onsager I, Engelhardt K, Wong CFA, Akan M, Hummel N, Kalinowski J, Rückert C, Hohmann-Marriott MF. A universal approach to gene expression engineering. 2019 Preprint at bioRxiv https://www.biorxiv.org/content/10.1101/644989v1, 2022 in OUP Synthetic Biology https://doi.org/10.1093/synbio/ysac017

Le SB, Onsager I, Lorentzen JA, **Lale R**. Dual UTR–A novel 5' untranslated region design for synthetic biology applications. 2020 OUP Synthetic Biology. https://doi.org/10.1093/synbio/ysaa006

Tietze L, **Lale R**. Importance of the 5′ Regulatory Region to Bacterial Synthetic Biology Applications. 2021 Microbial Biotechnology. https://doi.org/10.1093/synbio/ysaa006

4. Details of the impact

The impact is the establishment of the NTNU spin-off Sygens AS in November 2020. The major research that led to the impact was the molecular biology studies described above in section 2, as well as the AI expertise provided by the co-founder Dahiya.

Syngens' in collaboration with NTNU leads to further impact:

- **Achieving excellence for future exploitation.** With Lale's research group is tightly collaborating with Syngens' team. With this unique combination they both pursue collaborative efforts in publications and EU and national grant applications.
- **Mobility and training of researchers.** The early-career researchers employed both at Syngens and NTNU advance their scientific career in an interdisciplinary environment. The tight collaboration with the NTNU researchers lead to a unique opportunity to improve their skills and gain valuable experience in an interdisciplinary environment that neither organisation could provide alone. This provides them with a competitive skill set in the challenging scientific job market.
- In terms of societal impact, Syngens contributes to the UN's sustainable development goals:
    - **#8, Decent Work and Economic Growth.** Syngens lead to increase employment opportunities, promoting safe and secure working environments, and improve access to financial services to ensure sustained and inclusive economic growth;
    - **#9, Industry, Innovation and Infrastructure,** by ensuring that Norway has the required skills in fast developing fields of AI and synthetic biology.
    - **#12, Responsible Consumption and Production.** Syngens' technology contributes to the growing bioeconomy with sustainable production using microorganisms as cell factories that aids the green transition.

5. Sources to corroborate the impact

- Syngens received Adolf Øien's prestigious start-up award in 2021: https://oienfond.no/prosjekter/naeringsutvikling-og-innovasjon/
- Commercialisation grant from the Innovation Norway (not available online).
- NTNU-TTO license agreement (not available online).
- https://syngens.ai

[NTNU_IBT] [2]

Institution: NTNU
Administrative unit: Department of Biotechnology and Food Science
Title of case study: ConCordix – chewable dosage for neutral and pharmaceuticals
Period when the underpinning research was undertaken: whole evaluation period
Period when staff involved in the underpinning research were employed by the submitting institution: whole evaluation period
Period when the impact occurred: whole evaluation period

1. Summary of the impact (indicative maximum 100 words)

ConCordix (CCx) is a patented chewable delivery technology developed by our research group for the oral administration of nutra and pharmaceuticals with a family of specific formulations manufactured by Vitux AS (formerly ProBio AS and Ayanda AS). Since 2016 Vitux AS have focused their business solely on products based on CCx technology, which are covered by a family of 20 published WO/PCT patent applications. ConCordix dosages with varying formulations and active ingredients are marketed in over 25 countries worldwide. Vitux A/S employs personnel equivalent to 70 man-labour years at their headquarters in Oslo, and manufacturing sites in Andenes, Norway and Windsor, Canada. Annual turnover is 200 mill NOK.

2. Underpinning research (indicative maximum 500 words)

The research that resulted in the ConCordix technology is underpinned by long established expertise in biopolymer materials, emulsions, biopolymer-based emulsion stabilisation including for tissue models) and drug delivery, alongside a clear understanding of the technological challenges for dosage forms and the market need for user friendly, chewable and easily swallowed dosage forms that also provide appropriate conditions to maintain stability of the active ingredient(s). Research directly related to the impact began in 2005 as a research collaboration with ProBio AS (a manufacturer of soft gelatin capsules, now Vitux AS) that developed the original ConCordix formulation (a gelled emulsion chewable dosage form). Research focussing on the functional properties of gelatin established its ability to provide both interfacial stabilisation and the gelling agent for the aqueous phase, where the gelling provides further stabilisation of the emulsion, and the result is a stabile solid emulsion that can be utilized as a chewable dosage form. The structure of the gelled emulsion provides excellent taste-masking, which is an important consideration for patient acceptance and compliance. In this original formulation the oil phase was composed of omega 3 oils (DHA, EPA), and the emulsion structure provided enhanced bioavailability of when compared to traditional soft gelatin capsules containing omega 3 oils. This combination formed the basis of the first patent application (in 2007) and was the first product to reach the market (in 2011). Since 2011 further research into the physiochemical properties of gelled emulsion matrices has enabled further development of the CCx technology for the delivery of both oil and water-soluble active agents, for combination dosages containing both oil and water-soluble nutraceuticals (in separate matrix compartments to avoid unwanted interactions), and for delivery of insoluble molecular aggregates again providing excellent taste masking, an example of this being research and development of CCx formulations for ibuprofen, an analgesic with a pronounced off taste (~2014-2018).

Another central aspect of research underpinning the impact has been the investigation of tailored matrices utilizing plant based gelling agents in place of gelatin (from 2018). This is of great importance in the market as gelatin, as an animal product, has certain restrictions on its acceptance among patients / consumers. The development of plant based CCx technology is significant, both in terms of product design in applied biopolymer science and in terms of meeting market need.

Kurt I. Draget, Researcher / Professor (whole evaluation period); Ingvild J. Haug, Researcher (pre 2011-2012), Magnus N. Hattrem, Industrial PhD (pre 2011-2015), Morten J. Dille Engineer / PhD (2012-2021); Tuna Baydin, Industrial PhD (2019-2022)

3. References to the research (indicative maximum of six references)

- IJ Haug, LB Sagmo, D Zeiss, IC Olsen, KI Draget and T Seternes (2011) Bioavailability of EPA and DHA delivered by gelled emulsions and soft gel capsules, European Journal of Lipid Science and Technology, 113, 137-145 doi.org/10.1002/ejlt.201000450
- Hattrem, M. N., Dille, M. J., Seternes, T., & Draget, K. I. (2014). Macro- vs. micromolecular stabilisation of W/O/W-emulsions. Food Hydrocolloids, 37(0), 77–85. doi.org/10.1016/j.foodhyd.2013.10.024
- MJ Dille, MN Hattrem and KI Draget (2016) Bioactively filled gelatin gels; challenges and opportunities. Food Hydrocolloids, 76, 17-29 doi.org/10.1016/j.foodhyd.2016.12.028
- MJ Dille, MN Hattrem and KI Draget (2017) Soft, chewable gelatin-based pharmaceutical oral formulations; a technical approach. Pharmaceutical Development and Technology, //doi.org/10.1080/10837450.2017.1332642
- MN Hattrem, MJ Dille, T Seternes, T Ege and KI Draget (2018) The Relative Bioavailability of Ibuprofen After Administration With a Novel Soft Chewable Drug Formulation. Clinical Pharmacology in Drug Development, 7, 168-176 doi: 10.1002/cpdd.357
- T Baydin, SW Arntsen, MN Hattrem, KI Draget (2022) Physical and functional properties of plant-based pre-emulsified chewable gels for the oral delivery of nutraceuticals. Applied Food Research, doi.org/10.1016/j.afres.2022.100225

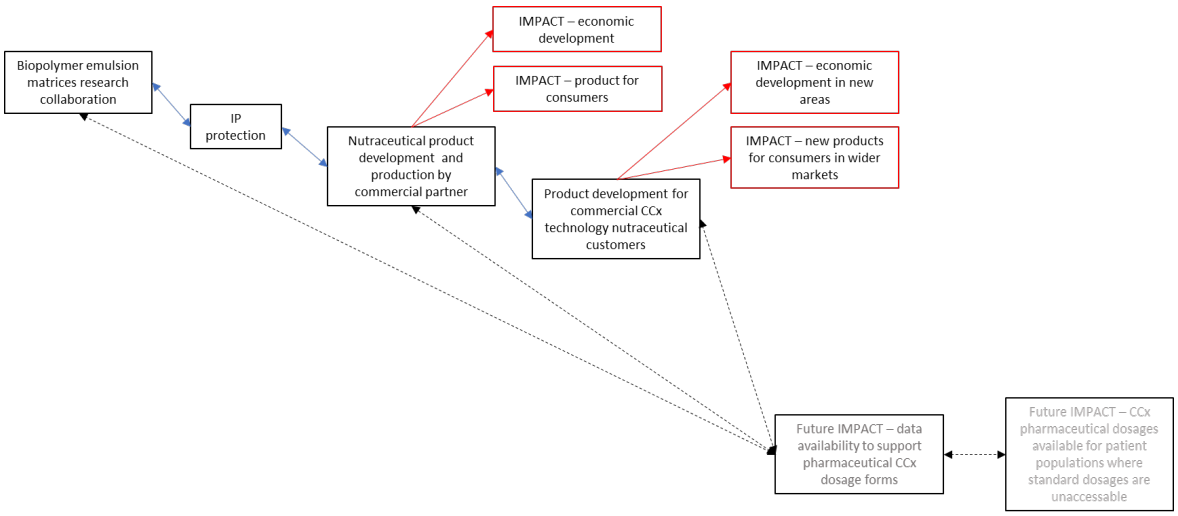
4. Details of the impact (indicative maximum 750 words)

The ConCordix technology and its commercialisation: beneficiaries as of 2022

- the Norwegian economy (Vitux AS. Status 2022, 200 mill. NOK turnover, 70 person years employment)
- commercial customers of Vitux AS (new products in new markets, economic benefit)
- end user customers in Norway and beyond (access to novel, consumer / patient friendly products)

Research and development is ongoing, both internally at our research group and via our commercial collaborators, and it is envisioned that future beneficiaries will include pharmaceutical companies (alternative patient friendly / increased compliance formulations of existing APIs, particularly for low compliance segments such as small children and the elderly) and patients who struggle with traditional dosage forms (e.g. tablets). Research has included testing with an API used in the treatment of Alzheimer's disease, a clinical indication where patient swallowing is often impaired, increasing the need for chewable dosage forms.

The flow of information and impact map is shown below.



Impact map showing current (red) and future (grey) impacts and collaborative research and development (blue arrows)

The research collaboration between ProBio AS (now Vitux AS) and our researchers that resulted in the ConCordix technology began in 2005. The first patent application, relating to the delivery of omega 3 oils (EPA, DHA) was filed in 2007 and this resulted in the first product in the market in 2011 in Norway. In 2016 Vitux AS sold its traditional soft capsule production capacity to focus exclusively on products based on ConCordix Technology developed by our researchers. Continued scientific and product development and company expansion has led to an annual turnover of 200 mill. NOK in 2022 with international expansion to establish production facilities in North America (Windsor, Canada).

Our continuous established research collaboration has, since 2005, resulted in a family of 20 WO/PCT patent applications the most recent of which, filed in 2021 and 2022, address pharmaceutical applications, in this case with cannabinoid drugs (GELLED OIL-IN-WATER EMULSION COMPRISING AT LEAST ONE CANNABINOID, LUNDHAUG KAMILLA [NO]; SIWEK ANDRZEJ [NO]; DRAGET KURT [NO] Published as: WO2021099792A1) and plant based (vegan) chewable drug dosage forms (Compositions (Agar based chewables) DILLE MORTEN J [NO]; BAYDIN TUNA [NO]; HATTREM MAGNUS [NO]; DRAGET KURT INGAR [NO] Published as WO2022219358A1).

ConCordix products have significant technological advances over traditional gummies including greater loading capacity and the ability to include more than one active ingredient with compartmental separation, improving stability and bioavailability. The value of the ConCordix technology has been noted also internationally with an US industry award (Best childrens omega 3 product) for a Vitux AS customer (Childlife Essentials, based in El Segundo, CA) for their children's chewable DHA product, which utilises ConCordix Technology.

Beyond the economic and customer impact this research and its commercialisation story has been used as a case study in the education of students at NTNU for example during the course "Biological and commercial barriers to getting drugs into patients".

5. Sources to corroborate the impact (indicative maximum of ten references)

EU-startups https://www.eu-startups.com/directory/vitux-group/

Environmental accreditation https://friendofthesea.org/norwegian-nutraceutical-manufacturer-vitux-sa-achieves-certification-soft-chews/

Industrial growth in Andenes https://siva.no/2021/09/tilrettelegger-for-a-fortsette-industrieventyret-pa-andenes-2/

White paper https://chewivits.com/uploads/images/whitepaper_CCX_2019.pdf

Award for ConCordix from Nutra Ingredients USA https://www.nutraingredients-usa.com/Article/2021/12/22/Winning-NIU-award-helped-open-doors-for-chewable-delivery-technology-developed-by-Norwegian-firm

Soft chews for brain health Fun for children and helpful for seniors: Vitux launches soft chews for brain health (nutritioninsight.com)

Better compliance with CCx technology https://www.nutraingredients-usa.com/Article/2022/09/02/vitux-says-research-shows-its-concordix-smart-chew-tech-poised-to-reinvigorate-omega-3s-category

[IBT] [3]

Institution: NTNU - Norwegian University of Science and Technology
Administrative unit: Department of biotechnology and food science
Title of case study: *Biomasses from the Norwegian fishery and aquaculture sector for human foods, feed ingredients, and nutraceuticals*
Period when the underpinning research was undertaken: 2011-2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2022
Period when the impact occurred: 2011-2022

1. Summary of the impact

The case study describes *the impact of using biomasses from the Norwegian fishery and aquaculture sector for human foods, feed ingredients, and nutraceuticals*. Over the decades, the food science division has provided research and education to improve the handling and processing of seafood to obtain commercial processes, maintain product quality and safety, and improve product shelf life. Additionally, extensive effort has been put into optimizing the respective side streams to improve the seafood sector's sustainability. The impact has been disseminated to the industry through joint research activities and close collaboration in BSc, MSc, and Ph.D. projects.

2. Underpinning research

The IBT's food science division has contributed to the scientific topic representing the case through decades. However, the underpinning research is represented by a body of work produced from 2010 up to the present.

The number of people in the world is increasing rapidly, and providing enough food, especially high-quality protein, and lipids, is challenging. ***Actions improving marine food products' quality, safety, and shelf-life impact society by reducing wasted foods and nutrients***. During the last decade, our impact within this field has been significant through several projects, e.g., *Safefishdish*[1]*, OPTiMAT*[2], *SGS-Concept*[3], *Prohealth*[4], and *Smart sjømat*[5]. Moreover, >40 MSc and five Ph.D. candidates completed relevant work between 2011 and 2021, beneficial to the Norwegian seafood industry by offering their knowledge by taking relevant jobs in the sector. Despite the industries' efforts to use marine raw materials directly for human consumption, a significant part of the biomass is underutilized or used for low-value products. ***Our division has been/is a partner in several projects looking for ways to extract valuable components from marine materials and incorporate them in products for human consumption***. Relevant projects are e.g., *OPTiMAT*[2], *SUPREME*[6], *PROMAC*[7], and OMEGA[8]. Moreover, >50 MSc and four Ph.D. candidates have completed relevant work between 2011 and 2021, beneficial to the Norwegian ingredient and nutraceutical industry by offering their knowledge by taking relevant jobs in the sector.

The underpinning research has been coordinated and co-led by a group of academic staff in the Food science division at IBT. ***Professor Turid Rustad*** is working with seafood processing and marine side-streams. In addition to being the principal supervisor of >30 MSc and six Ph.D. students (2014, 2014, 2015, 2019, 2021), she has coordinated or been responsible for work packages in *Safefishdish*[1] (2015-2018), *Prohealth*[4] (2016-2019)*, and PROMAC*[7] (2015-2018). ***Professor Jørgen Lerfall*** (associate until 2021) is working with seafood processing and quality, safety, and shelf-life extension (at NTNU since 2016). In addition to being the principal supervisor of >20 MSc (2016-2021) and two Ph.D. students (2020, 2021), he has coordinated projects such as *OPTiMAT*[2] (2016-2023), *SGS-Consept*[3] (2019-2023), and *Smart sjømat*[5] (2015-2018). ***Associate professor Anita Nordeng Jakobsen*** is working with food safety, seafood processing, and shelf-life extension (at NTNU since 2016). In addition to being the principal supervisor of >15 MSc (2016-2021) and one Ph.D. student (2018), she has been responsible for work packages in *OPTiMAT*[2] (2016-2023), *SGS-Consept*[3] (2019-2023), and *Smart sjømat*[5] (2015-2018). ***Associate professor Eva Falch*** is working with seafood side

streams and bioeconomy (at NTNU since 2016). In addition to being the principal supervisor of >10 MSc (2016-2021) and one Ph.D. student (2021), she has coordinated or been responsible for work packages in SUPREME[6] (2019-2022), OMEGA[8] (2020-2024), *OPTiMAT*[2] (2016-2023), and *Smart sjømat*[5] (2015-2018).

A significant part of the underpinning research can be classified as applied, performed in close collaboration with the industry. The work conducted between 2011 and 2021 has been highly valuable for the group. The number of funded projects has increased significantly, and currently >10 Ph.D. students and three post doctors are working within subjects related to the presented case.

3. References to the research

Chan, S. S., Roth, B., Skare, M., Hernar, M., Jessen, F., Løvdal, T., Jakobsen, A. N., & Lerfall, J. (2020). Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: From whole fish to cold-smoked fillets of Atlantic salmon (Salmo salar). *Aquaculture*, *526*, 735381. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.735381

Cropotova, J., Mozuraityte, R., Standal, I. B., Grøvlen, M. S., & Rustad, T. (2019). Superchilled, chilled and frozen storage of Atlantic mackerel (Scomber scombrus) fillets – changes in texture, drip loss, protein solubility and oxidation. *International Journal of Food Science & Technology*, *54*(6), 2228-2235. https://doi.org/https://doi.org/10.1111/ijfs.14136

Hoel, S., Vadstein, O., & Jakobsen, A. N. (2017). Species Distribution and Prevalence of Putative Virulence Factors in Mesophilic Aeromonas spp. Isolated from Fresh Retail Sushi. *Frontiers in microbiology*, *8*, 931. https://doi.org/10.3389/fmicb.2017.00931

Kaale, L. D., Eikevik, T. M., Rustad, T., & Kolsaker, K. (2011). Superchilling of food: A review. *Journal of Food Engineering*, *107*(2), 141-146. https://doi.org/http://dx.doi.org/10.1016/j.jfoodeng.2011.06.004

Lerfall, J., Roth, B., Skare, E. F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M. A., & Rotabakk, B. T. (2015). Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon (Salmo salar L.) during ice storage. *Food Chemistry*, *175*, 157-165. https://doi.org/http://dx.doi.org/10.1016/j.foodchem.2014.11.111

Rustad, T., Storrø, I., & Slizyte, R. (2011). Possibilities for the utilisation of marine by-products. *International Journal of Food Science and Technology*, *46*(10), 2001-2014. https://doi.org/https://doi.org/10.1111/j.1365-2621.2011.02736.x

4. Details of the impact

Measuring the underpinning research's specific impact in a single context is challenging. However, a couple of highlights will be addressed to show the work's impact on achiving excellence for future exploitation, education, mobility and training of researchers, and social impact. Moreover, the work's impact on specific thematic topics such as sustainable utilization of marine raw materials, food safety, quality and shelf-life, and human health can be pointed out.

Using the project OPTiMAT[2], and more specifically the Ph.D. thesis of Sherry Stephanie Chan (2021) as an example, highlights a potential shift in logistics of Atlantic salmon from land to the storage and transport of gutted salmon in RSW thanks. The Ph.D. work was conducted in close collaboration with Nofima AS and Hav Line AS. Hav Line AS is the owner of the world's first slaughtering vessel for Atlantic salmon. Chan's thesis demonstrated that chilling Atlantic salmon and storing it in RSW provide good quality products throughout the value chain. With the global increase in demand for food to meet the rising population, more food will come from aquaculture, which calls for better logistics in storage and transport. Fishing vessels using RSW systems is a common storage method practiced for fisheries. Nonetheless, the slaughter vessel introduced in this thesis is by far the first of its kind. Other similar vessels are also simultaneously under planning and construction, suggesting the potential of this slaughter method and a possible shift in the direction of more sustainable logistics from land to the

storage and transport of gutted Atlantic salmon in RSW tanks. The work has gained significant national and international interest through good citations and from the media (e.g., with an article in Global Aquaculture Advocate[9].

OPTiMAT[2], through the Ph.D. thesis by Veronica Hammer Hjellnes and the beforementioned projects OMEGA[8], SUPREME[6], PROMAC[7], and Prohealth[4], have increased the knowledge on strategies and technologies to utilize better marine lipids, proteins, and the production of ingredients from marine resources. Nutriceuticals of marine origin generally have significant potential for human health, highlighting this work's impact. In addition to project work, many MSc theses and several Ph.D. projects have been completed on this topic. The impact of this work has resulted in close collaboration with, e.g., Nutrimar AS. Nutrimar AS is currently funding a gift professorship and a Ph.D. at IBT. The professorship started in 2020 and will run until 2025.

Through projects such as OPTiMAT[2], SGS-Concept[3], Smart sjømat[4], and the Ph.D. thesis by Sunniva Hoel[10] (2018, Associate professor at the food science division from 2019) impact on food safety, food quality, and strategies to extend the shelf-life of perishable seafood has been obtained. To be mentioned the work on food safety has, e.g., resulted in a significant project, portfolio focusing on the challenge of *Listeria monocytogenes* in the Atlantic salmon industry.

The presented case, highlighting the work on ***the improved utilization of marine raw materials for human foods,*** was conducted by academic staff in the food science division at IBT, NTNU. However, most of the research was carried out in collaboration with research institutes such as Nofima AS and SINTEF and in close relationship with the industry. The presented collaborative work will lead to further impact on the following:

Achieving excellence for future exploitation: The underpinning work has improved the division's excellence by enabling high-impact publications and national and international grant applications. Both the impact of and the number of publications related to the chosen case and the number of externally funded projects have increased significantly during the last five years.

Education, mobility, and training of researchers: Both MSc and Ph.D. students and young researchers benefit from the presented activity through improved possibilities to have thesis and projects impacting the society by meeting the criteria of UN's sustainable development goals nr. 2, 3, 9, 12, 13, and 14. Moreover, collaboration and networking create a unique opportunity to improve skills and gain valuable experience in an interdisciplinary environment. This gives students and young researchers a competitive skill set in the challenging scientific job market.

Social impact: The presented case's social impact can be highlighted by the underpinning research contribution to the UN's sustainable development goals.

UN's goal #2 – Zero hunger: The underpinning research leads to sustainable management of marine food resources.

UN's goal #3 – Good health and well-being: Protein, lipids, and nutraceuticals of marine origin benefit good health and well-being. The presented work has developed novel strategies and knowledge supporting improved utilization of rest raw materials of marine origin.

UN's goal #9 – Industry, innovation, and infrastructure: Our work ensures that the Norwegian industry has the required skills in the fast development of infrastructure and innovative technology to meet the sustainability criteria of the future.

UN's goal #12 – Responsible consumption and production: The underpinning research leads to more responsible seafood and marine ingredients production.

UN's goal #13 – Climate action: Improved logistics, actions improving the food shelf-life, and reducing food loss has the potential to reduce the sector's CO_2 footprints.

UN's goal #14 – Life below water: Improved utilization of marine food resources after harvest will reduce wasted marine resources, e.g., having the potential of improved stock management.

5. Sources to corroborate the impact (indicative maximum of ten references)

1. *Safefishdish (2015-2018)* https://www6.angers-nantes.inrae.fr/secalim_eng/Expertise-Projects/Microbial-ecology/SAFEFISH-DISH/SAFEFISH-DISH
2. *OPTiMAT (2016-2023)* https://www.ntnu.edu/ibt/research/optimat, og https://app.cristin.no/projects/show.jsf?id=562088
3. *SGS-Concept (2019-2023)* https://www.ntnu.edu/ibt/research/food-safety/projects#SGS-Concept
4. *Prohealth (2016-2019)* https://www.ntnu.edu/ibt/research/food-safety/projects#Prohealth
5. *Smart sjømat (2015-2018, Innovative technological solutions to improve shelf-life of lightly processed seafood)* https://www.ntnu.edu/ibt/research/food-safety/projects#Shelf-life
6. *SUPREME (2019-2022)* https://www.sintef.no/projectweb/supreme/
7. *PROMAC (2015-2018)* http://promac.no/
8. *OMEGA (2020-2024)* https://www.sintef.no/en/projects/2020/omega-fish-oil-microencapsulation-generating-fortified-food-products-for-improved-human-health/
9. *Global Aquaculture Advocate (2020) https://www.globalseafood.org/advocate/effect-of-superchilling-on-atlantic-salmon-quality-through-the-value-chain/*
10. *Sunniva Hoel Ph.D. thesis (2018) https://ntnuopen.ntnu.no/ntnu-xmlui/handle/11250/2564421*

SINTEF AS_BTN, case number: 1

Institution: SINTEF AS
Administrative unit: Biotechnology and Nanomedicine (BTN)
Title of case study: Nanomedicine research for accelerated translation
Period when the underpinning research was undertaken: 2005 - 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2005 – 2021 (some persons shorter, see details below)
Period when the impact occurred: 2011- 2021

1. Summary of the impact

BTN has established research along the whole value chain within nanomedicine, including formulation, full preclinical characterization and regulatory issues. The research in BTN has been translated into health and economic benefits via industrial use, a spin-off company, input to regulatory frameworks and policies as well as through information to the public. The research enabled the following impacts:

- Establishment of a spin-off company within cancer treatment based on a nanoformulation technology platform (PACA).
- Significant contribution to translation of nanomedicines through our involvement in the H2020 infrastructure EUNCL and international projects REFINE and B-SMART.
- Influence on the public opinion towards covid-19 vaccines with extensive public dissemination for increased awareness and consumer knowledge in Norway, based on our research activities and competence building within RNA therapeutics and vaccines.
- Significant contribution to bringing attention to the necessity for and possible impact of developing nanomedicines at European level through our involvement and management in European organizations like the European Technology Platform on Nanomedicine. Education of nanomedicine students in Norway through lectures and supervising bachelor, master and PhD students at Norwegian University of Science and Technology (NTNU).

2. Underpinning research

In 2005, BTN initiated a strategic focus on nanomedicine research lead by Vice president marketing Dr Ruth Schmid. The same year Dr Schmid also participated in the creation of the European Technology Platform on Nanomedicine (ETPN) (https://etp-nanomedicine.eu/), an initiative led by industry to address the application of nanotechnology in healthcare. The research in BTN focused on delivery of drugs through polymeric nanoparticles and was the starting point for building a broad nanomedicine research activity in the department. In 2013, nanomedicine was defined as a strategic priority area in BTN. Senior research scientist Dr Yrr Mørch joined the unit in 2012 and developed together with Schmid and other researchers a patented drug delivery platform based on poly(cyanoacrylate) (PACA) nanoparticles (See **[R1, R2]**). The PACA technology is especially versatile for delivery of hard-to-deliver hydrophobic small molecule drugs and allows for multiple administration routes including intravenous and localized treatment. This enables potent cancer treatment drugs to be delivered more focused and in higher doses at target site, than the pure drug itself, avoiding the severe side effects stemming from normal administration of these drugs.

The research on nanomedicine drug delivery triggered a need for establishment of advanced methods for characterization, not only in BTN and SINTEF, but also on European level. BTN possessed advanced infrastructures for mass spectrometry-based analysis and robotic *in vitro* characterization, and strategic internal projects were used to establish competence and knowledge and position BTN within preclinical characterization in Europe. In the H2020 project European Laboratory for Nanomedicine Characterization (EUNCL, GA 654190, H2020-INFRAIA-2014-2015, 2015-2021, https://www.euncl.org/), standard operating procedures for preclinical characterization were developed and validated by the consortium. SINTEF was a core partner in EUNCL, and Senior Research Scientist Dr Sven Even Borgos and Senior Research Scientist Dr Geir Klinkenberg were assay group leaders for chemical characterization and *in vitro*

cytotoxicity. In total 35 standard operating procedures were developed and validated for preclinical characterisation in EUNCL. The validated SOPs were made publicly available through the project web site (See **[S3**]). Several research papers with SINTEF staff as main contributors were published based on the method development and validation work as well as results from studies of user cases in EUNCL (See **[R3,S3,S4**]). Furthermore, SINTEF (by Schmid, Mørch, Borgos, Klinkenberg and coworkers) participated in the H2020 project "REFINE" (GA 761104, 2018-2022, http://refine-nanomed.eu/) aiming to establish a new regulatory framework for nanomedicines and nanoenabled medical devices. The research performed in REFINE with BTN personnel in leading roles laid a foundation for a new set of methods and standard operating procedures, as well as a decision support system for preclinical characterization and assessment of nanomaterials in medical products [**R4, R5, R6**].

As part of our nanomedicine initiative, BTN by Dr Borgos and coworkers extended the physico-chemical characterization to therapeutic RNA in the B-SMART project (EU H2020 GA 721058, 2017-2022 https://b-smart-project.eu/). This project concerned development of delivery platforms for siRNA across the blood-brain barrier. Project results included successful targeting of the brain and delivery of siRNA. BTN developed methods to perform characterization and quality control of the nanoparticle systems, including analytical methods for quantification of nanoparticles and their components in tissue for use in biodistribution studies. In 2019, BTN participated in one of the first EU projects (EXPERT, H2020 GA 825828, 2019-2024, https://www.expert-project.eu/) specifically aimed at therapeutic mRNA. The EXPERT project develops nanoparticle delivery platforms (synthetic and biologically derived) for mRNA, primarily for cancer, in collaboration with European industry. BTN has contributed research on physicochemical characterization of the candidate formulations, quantitative analysis of biodistribution, and regulatory assessment (Borgos, Research scientists Fanny Caputo and Jeremie Parot). BTN by Borgos and coworkers has since 2019 built a complete competence chain on mRNA therapeutics, including design, synthesis, their encapsulation in nanoparticles, advanced characterization and cell- and animal preclinical testing. In 2020, SINTEF started an internal 13MNOK project, still ongoing, to specifically build competence and capacity in the above fields. This has been highly successful and has enabled collaborative projects with Norwegian industry (SMEs) and academic groups, besides several international projects, both with public (EU, Dutch Research Council) funding, and direct projects with European industry. The characterization of the lipid nanoparticle systems used for mRNA delivery built heavily on the competence established through EUNCL and similar projects (above) but was rapidly expanded into mRNA design and synthesis, as well as nanoformulation. A large, international and interlaboratory comparison of advanced analytical methods for mRNA delivery systems was led by BTN and is currently being published.

Key researchers and positions they held at the administrative unit at the time of the research, Roles: S: Strategic work, P: PACA technology, C: Characterization, I: Industrial research.

Trond E. Ellingsen, Research director (1981-present), Role: S, I
Ruth Schmid-Baumberger, Vice President Marketing (1981-present), Role: S, P, C
Heidi Johnsen, Research Manager (1997-present), Role S, P
Ýrr Mørch, Senior Research Scientist (2012-present), Role: S, P, I
Andreas Åslund, Research Scientist (2018 – present), Role: P, I
Sven Even Borgos, Senior Research Scientist (2006-present), Role: S, C, I
Geir Klinkenberg, Senior Research Scientist (1997-present), Role: S, C, I
Jérémie Parot, Research Scientist (2020- present), Role: C
Fanny Caputo, Research Scientist (from 2019-2021), Role: C
Håvard Sletta, Research Manager (1990 – present), Role: C, I

3. References to the research

All SINTEF authors are in bold. Citations (Web of Science) per Jan 30 2023 given in brackets.

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2. Fusser, M., Øverbye, A., Pandya, A.D., **Mørch, Ý., Borgos, S.E**., Kildal, W., **Snipstad, S., Sulheim**, **E.**, Fleten, K.G., Askautrud, H.A., Engebraaten, O., Flatmark, K., Iversen, T.G. Sandvig, K., Skotland, T., Mælandsmo, G.M. *Cabazitaxel-loaded Poly(2-ethylbutyl cyanoacrylate) Nanoparticles Improve Treatment Efficacy in a Patient Derived Breast*

Cancer Xenograft. Journal of Controlled Release **293**, p. 183-192 (2019). https://doi.org/10.1016/j.jconrel.2018.11.029 *(28)*

3. Fernández, Y., Movellan, J., Foradada, L., Giménez, V., García-Aranda, N., Mancilla, S., Armiñán, A., **Borgos, S.E., Hyldbakk, A.,** Bogdanska, A., Gobbo, O.L., Prina-Mello A., Ponti J., Calzolai L., Zagorodko O., Gallon E., Niño-Pariente A., Paul A., Schwartz Jr S., Abasolo I. and Vicent M.J. *In Vivo Antitumor and Antimetastatic Efficacy of a Polyacetal-Based Paclitaxel Conjugate for Prostate Cancer Therapy*. Advanced Healthcare Materials, **11(7)**, p. 2101544 (2022, published online 2021). https://doi.org/10.1002/adhm.202101544 *(3) See appendix 1*

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*although published in 2022 this special issue describes research performed and results obtained in the REFINE project (GA 761104, 2018-2022) prior to 2022.

4. Details of the impact

BTN's research and competence covering the entire value chain within nanomedicine has made us an attractive research partner and allowed us to amplify our impact on translation of nanomedicines.

Industrial impact for Norwegian companies

Underpinning research on nanomedicine at BTN has been crucial for the growth and commercial activities of our industry partners. During the last years, we have contributed to development of nanobased products at customers Biosergen and Algipharma. Our research in PACA technology formed the basis for the establishment of a spin-off company NaDeNo (decided in 2021), focussing on unleashing the potential of hard-to-deliver hydrophobic small molecule drugs. The lead candidate is targeting peritoneal cancer, where preliminary results show the potential of curing patients with a currently very low survival rate. See **[S1]** for statements and **[S2]** for patent.

International impact by contributing to shaping the nanomedicine landscape and translation of nanomedicines in Europe

BTN has contributed significantly to enhanced translation of nanomedicine through development and standardisation of methods for preclinical characterization of nanomedicines and through preclinical characterization of nanomedicine drug candidates for the European research community. Strategic internal funding was used to develop competence in nanomedicine characterization in the period from 2010 to 2015 (e.g. Strategic internal project “Eucaryotic cell”) and to position BTN and its unique infrastructure on the European research arena. In the period from 2015 to 2021, BTN was a core partner in the European Nanomedicine Characterization Laboratory (EUNCL, GA 654190, H2020-INFRAIA-2014-2015), which is a H2020 trans-European, transdisciplinary characterization infrastructure founded in 2015, providing a comprehensive and integrated set of preclinical characterization assays for the nanomedicine formulations, including physical, chemical, *in vitro* and *in vivo* biological testing. EUNCL supported European stakeholders to advance the translation of their products into the clinic, e.g. advancing from TRL 3 to TRL4 or higher, through an in depth safety and quality assessment of their lead formulations, as well as by providing technical and regulatory advice. The infrastructure was encompassing six leading European partner laboratories and the Nanotechnology Characterization Laboratory (NCL) in the United States (henceforth 'NCI-NCL' for clarity), funded

by the National Cancer Institute (NCI). EUNCL established a “Core expert team” (CET) with expertise in relevant characterization steps in preclinical characterization of nanomedicines. BTN had two members in the CET (Borgos, Klinkenberg) and coordinated the work on chemical characterization and *in vitro* cytotoxicity. EUNCL set up an assay cascade and developed and validated 35 standard operating procedures for preclinical characterization that were used in EUNCL and made available to the general research community (See **[S3], [S4]**). During the operational years from 2016 to 2020, EUNCL evaluated and gave comprehensive feedback to 35 users of the infrastructure (including big pharma companies, SMEs and academic groups) and made an in-depth characterization of 7 user samples of which 3 progressed into *in vivo* testing. One of the candidates evaluated by EUNCL is currently in clinical trials for treatment of haematological malignancies.

Furthermore, SINTEF has through the H2020 project “REFINE” (GA 761104, 2018-2022) contributed to establishment of a regulatory framework for nanomedicines and nanoenabled medical devices. The European Commission's Innovation Radar, highlighting excellent innovations in EU projects, identified results from REFINE as innovative **[S5]**. Through REFINE, BTN together with partners, actively influenced public awareness and policies on regulatory requirements and preclinical characterization of nanomedicines. Communication with regulatory bodies such as the European Medicinal Agency and the Food and Drug administration was actively pursued in the EUNCL and REFINE projects and the European Commission's Joint Research Centre was actively involved in both these projects.

Impact related to mRNA-based therapeutics and vaccines

Our work within research on RNA therapeutics, including design, synthesis, their encapsulation in nanoparticles, advanced characterization and cell- and animal preclinical testing has led to dissemination of standard test methods guiding the translation of mRNA research into innovation. Results from B-SMART ("New methods for Mass Spectroscopy of nanobodies and LNP carriers" were identified as an innovation of high market potential by the European Commission's Innovation Radar **[S5]**. BTN is working with international regulatory and standardization bodies and public laboratories (FDA, NIST, ASTM International, LNE, JRC) on standard test methods for characterization of lipid nanoparticles and is chapter leader in a Standards Guide (ASTM International) on characterization of mRNA. Standardization of methods and regulatory requirements, and development of reference materials, have been identified by industry and regulatory authorities as some of the key requirements for efficient translation of mRNA medicines. Thus, SINTEF’s contribution carries impact well beyond the academic community.

Public dissemination mRNA as medicine has been a priority, and the activities were strongly intensified during the Covid pandemic, as the development and application of the novel mRNA vaccines caused a high need for public information. BTN represented by Borgos, was interviewed and disseminated own contributions in several of the largest Norwegian newspapers and popular science channels, as well as radio and US television **[S6-1], [S6-2], [S6-3]**. The public dissemination and building of trust towards vaccines and other medical technology is of high priority, aligned with SINTEF’s vision of 'Technology for a better society', and has contributed to the very high acceptance and penetration of vaccines in Norway.

Influence on the attention to nanomedicine technology on a European level

The European Technology Platform on nanomedicine (ETPN) is a think tank created in 2005 and set up together with the European Commission. BTN contributed to the creation of ETPN, had the chair position 2019-2021 by Ruth Schmid, and the chairman of the working group Nanotherapeutics and Targeted Delivery by Research Scientist Dr Andreas Åslund (in SINTEF from 2019) from 2021 onwards. ETPN has through its influence created awareness in the European Commission and industry on the necessity for developing nanomedicines with a high potential impact, thereby securing the funding for nanomedicine innovation projects. To the framework programs FP7 and H2020, 95% of the nanomedicine related calls were formulated by the ETPN based on its Strategic Research and Innovation Agenda. ETPN acts also as a

driving force for industrialization of nanomedicine in Europe since 2014, detecting the best innovations of the field and facilitating their transfer from innovative design to clinical development through the nanomedicine Translation HUB, a global set of premium services, free-of-charge for the beneficiaries **[S7], [S8], [S9]**.

Education of students within nanomedicine

Many of the activities within formulation of nanomedicines have involved students, mostly in collaboration with NTNU. Together, the research units have educated bachelor, master and PhD candidates with relevant nanomedicine competence for industry, communities, and research organizations through the synergies of basic and applied research. Only within the PACA technology development more than 30 candidates (including 8 PhDs) have been educated. BTN personnel (Schmid) has also given lectures in international courses for PhD-students on nanoparticles for medical applications.

5. Sources to corroborate the impact

[S1] Industrial impact of PACA technology: Statements from NaDeNo, Biosergen, Algipharma. Appendix 2.

[S2] Patent EP3004183B1/US10,967,039 "Process for preparing stealth nanoparticles" (filed 2013), inventors **Schmid R., Stenstad P., Mørch Y., Johnsen H.** https://patents.google.com/patent/EP3004183B1/en

[S3] EUNCl website with assay descriptions for assays developed by BTN and partners: https://www.euncl.org/about-us/assay-cascade/
Assays cover pre-screening, physiochemical characterization, and *in vitro* assays. Corroborates the impact of BTN as a part of the EUNCL European resource and knowledge base, guiding researchers and industry and facilitating regulatory review of new nanomaterials, enabling the transition of innovative nanomaterials into medical applications.

[S4] The importance of the EUNCL results for innovation in nanomedicine and example of the intercontinental collaboration between EUNCL and National Cancer Institute - Nanotechnology Characterization Laboratory (NCI-NCL):
Caputo, F., Mehn, D., Clogston, J.D., Rösslein, M., Prina-Mello, A., **Borgos, S.E**., Gioria, S. and Calzolai, L., *Asymmetric-flow field-flow fractionation for measuring particle size, drug loading and (in) stability of nanopharmaceuticals. The joint view of European Union Nanomedicine Characterization Laboratory and National Cancer Institute-Nanotechnology Characterization Laboratory*. Journal of Chromatography A, 1635, p.461767 (2021). https://doi.org/10.1016/j.chroma.2020.461767 *(18 citations)*

[S5] Impact of the methods that are available from B-SMART and REFINE: European Commission's Innovation Radar pointing out two innovations in REFINE and one in B-SMART. https://www.innoradar.eu/ Appendix 3.

[S6] Development, function and safety of COVID19-vaccines, and impact on public opinion: **S6-1**: https://norwegianscitechnews.com/2020/11/this-is-how-the-new-covid-19-vaccine-works/ (Published 18.11.2020),**S6-2**:https://sciencenorway.no/covid19-epidemic-health/a-revolution-in-vaccine-development--but-will-we-all-benefit/1662994 (Published 30.03.2020), **S6-3**: https://www.abcactionnews.com/news/national/covid-19-vaccine-paves-the-way-for-new-types-ofmedication (Interview published 09.02.2021),

[S7] The power of nanomedicine: Germain M, **Caputo F**, Metcalfe S, Tosi G, Spring K, **Åslund AKO**, Pottier A, Schiffelers R, Ceccaldi A, **Schmid R**. *Delivering the power of nanomedicine to patients today.* J Control Release **326**, pp. 164-171 (2020). https://doi.org/10.1016/j.jconrel.2020.07.007 *(141 citations)*

[S8] The importance of the ETPN influence on policy and agenda: Precision NanoSystems Webinar on Youtube: *Nanomedicine Today and Tomorrow: How the European Tech Platform is Driving Nanomedicine Forward*. By Ruth Schmid. 964 views per august 2021. https://www.youtube.com/watch?v=6Xxp5Q6VU7o&t=24s.

[S9] Boosting the translation of medical innovations: https://etp-nanomedicine.eu/about-etpn/nanomedicine-translation-hub/

Appendix 1

Fernández, Y., Movellan, J., Foradada, L., Giménez, V., García-Aranda, N., Mancilla, S., Armiñán, A., **Borgos, S.E., Hyldbakk, A.,** Bogdanska, A. and Gobbo, O.L. *In Vivo Antitumor and Antimetastatic Efficacy of a Polyacetal-Based Paclitaxel Conjugate for Prostate Cancer Therapy*. Advanced Healthcare Materials, **11(7)**, p. 2101544 (2022). https://doi.org/10.1002/adhm.202101544

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In Vivo Antitumor and Antimetastatic Efficacy of a Polyacetal-Based Paclitaxel Conjugate for Prostate Cancer Therapy

Yolanda Fernández, Julie Movellan, Laia Foradada, Vanessa Giménez, Natalia García-Aranda, Sandra Mancilla, Ana Armiñán, Sven Even Borgos, Astrid Hyldbakk, Anna Bogdanska, Oliviero L. Gobbo, Adriele Prina-Mello, Jessica Ponti, Luigi Calzolai, Oleksandr Zagorodko, Elena Gallon, Amaya Niño-Pariente, Alison Paul, Simó Schwartz Jr, Ibane Abasolo, and María J. Vicent**

Prostate cancer (PCa), one of the leading causes of cancer-related deaths, currently lacks effective treatment for advanced-stage disease. Paclitaxel (PTX) is a highly active chemotherapeutic drug and the first-line treatment for PCa; however, conventional PTX formulation causes severe hypersensitivity reactions and limits PTX use at high concentrations. In the pursuit of high molecular weight, biodegradable, and pH-responsive polymeric carriers, one conjugates PTX to a polyacetal-based nanocarrier to yield a *tert*-Ser-PTX polyacetal conjugate. *tert*-Ser-PTX conjugate provides sustained release of PTX over 2 weeks in a pH-responsive manner while also obtaining a degree of epimerization of PTX to 7-epi-PTX. Serum proteins stabilize *tert*-Ser-PTX, with enhanced stability in human serum versus PBS (pH 7.4). In vitro efficacy assessments in PCa cells demonstrate IC_{50} values above those for the free form of PTX due to the differential cell trafficking modes; however, in vivo tolerability assays demonstrate that *tert*-Ser-PTX significantly reduces the systemic toxicities associated with free PTX treatment. *tert*-Ser-PTX also effectively inhibits primary tumor growth and hematologic, lymphatic, and coelomic dissemination, as confirmed by in vivo and ex vivo bioluminescence imaging and histopathological evaluations in mice carrying orthotopic LNCaP tumors. Overall, the results suggest the application of *tert*-Ser-PTX as a robust antitumor/antimetastatic treatment for PCa.

1. Introduction

Metastasis, the last stage of cancer progression, represents a sequential series of interrelated steps, including local invasion, intravasation, survival in the bloodstream and lymph, extravasation, and growth within a secondary organ and the cause of most cancer-related death.[1] Therefore, the formation of incurable metastases represents a significant problem in cancer treatment rather than the eradication of the primary tumor itself. While early-stage prostate cancer (PCa) is treatable, with a five-year survival rate exceeding 90%, nearly 30% of men treated by radical prostatectomy suffer from disease relapse, and their prognosis remains poor.[2–4] Androgen ablation represents a commonly used therapy for advanced metastatic PCa,[5] to which most patients initially respond; however, most patients eventually relapse and succumb to androgen-independent PCa and metastasis. The design of new therapeutic strategies to improve the anti-tumor and antimetastatic

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efficacy of PCa drug treatments represents an essential step to ensure adequate disease management.[6]

Nanomedicines have emerged as exciting new therapeutic modalities for unmet clinical needs, and in this study, we focused on the potential of polymer therapeutics, particularly polymer–drug conjugates (PDCs), for the treatment of advanced metastatic PCa.[7] PDCs are defined as macromolecular complexes in which a drug is covalently bound to a water-soluble polymeric carrier.[8] Compared to conventional small molecule-based therapies, PDCs have several advantages for cancer therapy, including i) enhanced aqueous solubility, ii) higher drug loading capacity, iii) prolonged blood circulation times and, therefore, improved bioavailability and biodistribution via the so-called enhanced permeability and retention (EPR) effect,[9] and consequently, iv) reduced toxicity for healthy tissues and v) increased anti-tumor efficacy.[10]

The design of improved biodegradable polymeric carriers that exploit EPR-mediated tumor targeting and/or controlled drug release at specific loci, represents an ongoing multidisciplinary challenge. Biodegradable polymers such as polyacetals[11,12,13] constitute promising candidates for the design of PDCs as they display pH-dependent degradation; while they remain stable at pH 7.4, polyacetals rapidly degrade in response to the acidic environments encountered in endosomes and lysosomes (e.g., pH5–5.5).[11,13] In vitro and in vivo studies using polyacetals have previously confirmed a lack of toxicity and low uptake by the liver and spleen combined with enhanced blood circulation times.[11,12]

The microtubule-interfering agent paclitaxel (PTX) is a clinically well-established and highly effective chemotherapeutic drug used to treat advanced tumors, including prostate, breast, ovarian, and non-small cell lung cancer. In addition to anti-neoplastic activity, PTX exhibits antiangiogenic and pro-apoptotic effects at low doses;[14–16] however, observed severe side effects limit the application of PTX. Given its hydrophobic nature, the clinical application of PTX requires solubilization in Cremophor EL or ethanol, which prompt hypersensitivity reactions.[17] PTX also displays inherent severe side effects, including neurotoxicity.[18] Additional limitations of PTX include a poor pharmacokinetic profile (short half-life, low selectivity), which leads to a negligible level of PTX reaching the tumor site and the development of drug resistance due to the nature of PTX as a substrate for efflux pumps.[19]

The development of an albumin-based PTX nanoparticle (Abraxane –ABI-007, Celgene Corporation) represents a successful approach for PTX delivery. The United States Food and Drug Administration (FDA) approved Abraxane in 2004 for the treatment of breast cancer after the failure of combination chemotherapy for metastatic disease or relapse within six months of adjuvant chemotherapy. In this formulation, PTX is physically complexed within the Abraxane nanoparticle, leading to the enhanced solubility of PTX and the avoidance of harmful solubilizing agents.[20] Even given the success of Abraxane, the conjugation of PTX to a polymeric carrier might offer further pharmacological advantages. Conjugation of PTX to N-(2-hydroxypropyl) methacrylamide (HPMA), a nonbiodegradable copolymer, led to improved pharmacokinetics and promising anti-tumor efficacy;[21] however, this strategy failed at the clinical stage due to the premature release of PTX in the circulation, producing a similar toxicity profile to free PTX. Cell Therapeutics Inc. (Seattle, USA) took a different approach and conjugated PTX to polyglutamic acid (a biodegradable polymer) to create OPAXIO, which displayed clinical benefits compared to free PTX when used alone or in combination with radiotherapy or other small drugs such as cisplatin.[22–24]

In this study, we synthesized and exhaustively characterized (demonstrating batch-to-batch reproducibility and endotoxin-free large-scale synthesis) a pH-responsive polyacetal-PTX conjugate (*tert*-Ser-PTX) to understand conjugate behavior at the cellular and whole-organism levels. Polyacetal conjugation inhibited the early release of PTX in the bloodstream but supported the pH-triggered release of PTX after the EPR-mediated accumulation within tumors. Encouragingly, *tert*-Ser-PTX demonstrated robust anti-tumor efficacy in primary tumors and significantly inhibited metastatic dissemination. Overall, we hypothesize that the conjugation of PTX to a pH-responsive polyacetal carrier will offer enhanced clinical benefits to PCa patients by enabling a low-dose clinical regime, controlled release of the drug, and a reduction in harmful side effects.

2. Experimental Section

2.1. Synthesis and Characterization of *tert*-Polyacetal-Paclitaxel (*tert*-Ser-PTX)

2.1.1. Synthesis of Polyacetal tert-FmocSerinol

PEG_{4000} (5 g, 1.25 mmol) and *p*-toluenesulfonic acid (pTSA) (8 mg, 0.0464 mmol) were first azeotropically distilled from toluene

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(40 mL) at 150 °C for 2 h. Then, Fmoc-serinol (472 mg, 1.53 mmol) in anhydrous tetrahydrofuran (THF) was added, and the mixture was dried under a high vacuum. The mixture was redissolved in anhydrous THF, and di(ethylene glycol) divinyl ether (DEGDVE) (456 µL, 2.78 mmol) was added dropwise. The mixture was allowed to stir for 3 h in the dark at room temperature. 1 mL of triethylamine was added to the reaction mixture under vigorous stirring. After 10 min, the mixture was precipitated into hexane (400 mL), decanted, washed with another 400 mL of hexane, and then collected by vacuum filtration.

Yield: 90%. ^{1}H-NMR (Acetone-d_6, 300 MHz) δ (ppm) 0.85–0.90 (t, J = 0.9 Hz, 2.5H), 1.22–1.27 (12H, m, PEG-acetal CH_3), 3.35–3.88 (204H, m, PEG CH_2, DEG CH_2, Serinol CH_2), 4.24 (1H, m, Fmoc Ar–CH–CH_2–), 4.36 (2H, m, Fmoc Ar–CH-CH_2-), 4.74-4.77 (3H, m, acetal CH), 7.33–7.41 (4H, m, ArHFmoc), 7.71 (2H, m, ArHFmoc), 7.85 (2H, m, ArHFmoc).

2.1.2. Fmoc Deprotection

tert-Fmoc Serinol was dissolved in a flask using 20% piperidine/acetonitrile (40 mL) as the deprotection reagent, and the reaction mixture was stirred for 1 h at room temperature. Then, the crude product was precipitated once in diethyl ether (400 mL) and twice in hexane (400 mL). The solid was redissolved in a minimum amount of acetone between each precipitation. Finally, *tert*-Serinol was dried under a high vacuum for 1 h. Yield: 90%.

tert-FmocSerinol: ^{1}H-NMR (Acetone-d_6, 300 MHz) δ (ppm) 1.22–1.33 (m, 3H, PEG-acetal –CH_3), 3.35–3.85 (m, 67H, PEG DEG –CH_2, Serinol–CH_2), 4.80 (m, 1H, PEG-acetal –CH).

2.1.3. Synthesis of 2'-Succinyl-Paclitaxel (PTX_{COOH})

PTX (300 mg, 0.35 mmol) and succinic anhydride (450 mg, 4.5 mmol) were dissolved in anhydrous pyridine (5 mL) and stirred under a nitrogen atmosphere at room temperature for 4 h. Then, pyridine was evaporated under high vacuum, and the crude product was washed with water and filtered. The white solid obtained was recrystallized in acetone/water and freeze-dried.

Yield: 64%. PTX_{COOH}: ^{1}H-NMR (Acetone-d_6, 300 MHz) δ (ppm) 1.20 (s, 3H, –CH_3), 1.22 (s, 3H, –CH_3), 1.67 (s, 3H, –CH_3), 1.96 (s, 3H, –CH_3), 2.17 (s, 3H, –CH_3), 2.47 (s, 3H, –CH_3), 2.64 (d, 2H, CH_2–suc), 2.71 (d, 2H, CH_2–suc), 3.89 (m, 2H, –CH_2), 4.20 (m, 2H, –CH_2), 4.45 (m, 1H, –CH), 4.98 (m, 1H, –CH), 5.57 (d, 1H, –CH–suc), 5.70 (d, 1H, –CH), 5.98 (m, 1H, –CH), 6.17 (t, 1H, –CH), 6.43 (s, 1H, –CH), 7.31-7.70 (m, 11H, ArH), 7.88 (d, 2H, ArH), 8.14 (d, 2H, ArH), 8.45 (d, 1H, NH).

2.1.4. Synthesis of tert-Ser-PTX

tert-Ser-PTX was obtained through a reaction between PTX_{COOH} (0.13 g, 0.134 mmol), *tert*-Serinol (2.13 g), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMTMM-BF4) (50 mg, 0.152 mmol) using 15–20 mL anhydrous *N,N*-dimethylformamide (DMF) as a solvent. PTX_{COOH} and DMTMM-BF_4 were first poured into a Schlenk flask and dried under a high vacuum for 1 h and were then dissolved in DMF (3 mL) while stirring under a nitrogen atmosphere protected from the light. *tert*-Serinol was added after 10 min using DMF (15 mL) to dissolve completely. The pH of the reaction mixture was adjusted to 8 using *N,N*-diisopropylethylamine (DIEA). After 16 h, the crude product was precipitated in diethylether (200 mL) and collected by vacuum filtration. The white solid obtained was redissolved using a minimum amount of acetone and dialyzed against acetone using a regenerated cellulose membrane (molecular weight cutoff [MWCO] 3.5 kDa).

Yield: 85%. ^{1}H-NMR (Acetone-d_6, 300 MHz) δ (ppm) 1.19–1.34 (m, 76H, –CH_3 PEG acetal, 2 –CH_3 PTX), 1.66 (s, 3H, –CH_3 PTX), 1.97 (s, –CH_3 PTX), 2.19 (s, 3H, –CH_3 PTX), 2.45 (s, 3H, –CH_3 PTX), 2.53 (m, 5H, PTX), 3.34–3.84 (m, 1627H, PEG DEG CH_2, Serinol-CH_2), 4.10–4.17 (m, 3H, PTX), 4.42 (m, 1H, –CH PTX), 4.73–4.79 (m, 21H, –CH PEG-acetal), 4.95–4.98 (d, 1H, 5.0 Hz, –CH PTX), 5.53 (d, 1H, 5.5 Hz, CH PTX), 5.70 (d, 1H, 5.7 Hz, –CH PTX), 5.93–5.98 (t, 1H, 5.9 Hz, –CH PTX), 6.11–6.18 (t, 1H, 6.1 Hz, –CH PTX), 7.07 (m, 1H, ArH PTX), 7.31 (m, 1H, ArH PTX), 7.45-7.70 (m, 11H, ArH PTX), 7.91 (m, 2H, ArH PTX), 8.13 (d, 2H, 8Hz, ArH PTX), 8.4 (m, 1H, NH PTX).

2.1.5. Physicochemical Characterization of tert-Ser-PTX

Physicochemical characterization of *tert*-Ser-PTX involved 1D and 2D NMR, dynamic light scattering (DLS), transmission electron microscopy (TEM), size exclusion chromatography (SEC), reversed-phase liquid chromatography with electrospray positive ionization tandem mass spectrometry (LC-MS/MS) of PTX and the epimer 7-epi-PTX, and small-angle neutron scattering (SANS) measurements. Please see the Supporting Information for further details.

2.1.6. Determination of Total Drug Loading and Free Drug Content by LC-MS/MS

Reconstituted *tert*-Ser-PTX was incubated at 37 °C in phosphate-buffered saline (PBS) adjusted to pH 7.4, 5.5, and 4.0 at a target concentration of 2 µg mL^{-1} conjugated PTX based on the PTX loading determination by NMR (8% w/w PTX). Sampling was performed at the following times: 0, 1, 4 h, 1 , 2, 4 d, one week, and two weeks. Organic solvent (acetonitrile) was added at 9 volumes (1+9) to solubilize the released PTX, which was subsequently quantified by LC-MS/MS. As a degradation control, free PTX at 2 µg mL^{-1} was incubated under identical conditions.

For the forced degradation study, 100 µg mL^{-1} of free PTX or PTX equivalent (*tert*-Ser-PTX, theoretical PTX loading 8% w/w) was incubated in 4 M H_2SO_4 at 90 °C overnight. After 100× dilution in water, the samples were analyzed by LC-MS/MS to quantify benzoic acid.

2.2. Drug Release in Simple and Complex Media

2.2.1. pH-Dependent Degradation

Polyacetals (3 mg mL^{-1}) were incubated at 37 °C in PBS at pH 5.5, 6.5, and 7.4 for 20 d. 100 µL samples for high-performance liquid

chromatography (HPLC) analysis were isolated at various time points (0, 8 h, and then every 24 h) until complete degradation. Before analysis, the pH of acidic samples was neutralized with ammonium formate buffer (0.1 M, 100 µL for pH 5.5 and 50 µL for 6.5) to stop any further degradation, and concentrations were normalized with PBS (100µL PBS were added to the samples of pH 7.4 and 50 µL to the sample of pH 6.5). Then, samples were directly analyzed by reversed-phase HPLC (RP-HPLC), using a C18 LiChroSpher 100 column (5 µm, 15 cm length), with the UV detector set at $\lambda = 280$ nm with a flow rate of 1 mL min^{-1}, 20 µL injection. Eluent A was H_2O and eluent B was acetonitrile. Estradiol was used as an HPLC internal reference standard; 100 µL of a 10 µg mL^{-1} stock solution was added to each sample. The elution was performed by the following gradient: from 35% B to 80% B over 20 min (PTX retention time: 7 min). A calibration curve of PTX was used to quantify the total PTX release from the conjugates by HPLC.

2.2.2. Serum Stability

Polyacetals (3 mg mL^{-1}) were incubated at 37 °C in freshly extracted serum from Wistar rats for up to 24 h. Samples of 100 µL were collected at regular intervals of time. 10 µL of 100 µg mL^{-1} solution of estradiol in methanol (internal standard) and 135 µL of acetonitrile were added to each sample to precipitate serum proteins. Following centrifugation (14 000 rpm, 5 min), supernatants were analyzed by HPLC (gradient 35% to 80% acetonitrile/water, 20 min, 20 µL injection).

Additional studies were performed by LC-MS/MS. Free PTX and *tert*-Ser-PTX were incubated at 37 °C for up to two weeks in the following matrices: i) PBS, pH 7.4; ii) HepG2 cell culture medium (unbuffered) with 10% fetal bovine serum (FBS); iii) RPMI cell culture medium (buffered at pH 7.4); and iv) human plasma, pH buffered at 7.4 with 50×10^{-3} M HEPES. 0.1% w/v sodium azide was added to all samples to prevent microbial growth. All incubations were performed in triplicates in 96-well plates where every plate constituted one sampling time. Sampling was performed at the following times: 0, 1, 4 h, 1, 2 d, 4 d, 1 week, and 2 weeks. Three PTX concentrations levels were studied; 0.5, 2, and 20 µg mL^{-1}, of which the 2 µg mL^{-1} concentration is closely based on PTX plasma concentrations used in the clinic. For the *tert*-Ser-PTX formulation, these target PTX concentrations were used to calculate the amount of prodrug added, based on the previously measured drug loading (6.8 w/w %). LC-MS/MS quantified PTX and 7-epi-PTX after adding nine volumes of acetonitrile, which solubilizes the PTX (from precipitation and protein binding) and simultaneously precipitates proteins. The free drug was separated by centrifugal ultrafiltration (regenerated cellulose membrane, MWCO 10 kDa) in well-plate format at 2000 g for 5 min.

2.3. In Vitro Efficacy Studies

2.3.1. Cells

PCa androgen-dependent (LNCaP.Fluc2) and -independent (PC3) cell lines[25] were used for cytotoxicity assays. LNCaP.Fluc2 PCa cells were obtained from PerkinElmer (Waltham, MA, USA) and PC3 cells from the American Type Culture Collection (Rockville, MD, USA). Briefly, LNCaP.Fluc2 cells were cultured in DMEM medium and PC3 cells in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA). All media were supplemented with 10% heat-inactivated FBS (Lonza, Verviers, Belgium), penicillin (100 U mL^{-1}), streptomycin (100 µg mL^{-1}), and fungizone (250 ng mL^{-1}) (Invitrogen). LNCaP.Fluc2 overexpressed firefly luciferase for in vivo bioluminescence imaging (BLI) monitoring, and reporter expression was maintained in cell culture with 500 µg mL^{-1} of geneticin (Invitrogen). Cells were maintained in a humid atmosphere at 37 °C with 5% CO_2.

2.3.2. In Vitro Cytotoxicity

The in vitro cytotoxicity of polyacetals was evaluated using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) method after 72 h incubation.[26–28] Cells were treated with *tert*-Ser-PTX or free PTX with final doses ranging from 0.000004 to 40 µg mL^{-1} (PTX equivalents).

2.4. In Vivo Efficacy Studies

All in vivo efficacy studies were performed using the NANBIOSIS Singular Scientific Technological Infrastructures at the In vivo Experimental Platform of the Functional Validation & Preclinical Research (FVPR) area of the Bioengineering, Biomaterials and Nanomedicine Networking Biomedical Research Centre (CIBER-BBN) in Barcelona, Spain. (http://www.nanbiosis.es/unit/u20-in-vivo-experimentalplatform/)

2.4.1. Animals

Biodistribution and repeated-maximum tolerated dose (r-MTD) assays were performed using C57BL/6 mice (sourced from Envigo Laboratories, UK and were bred on-site at Trinity College Dublin). Efficacy assays were performed with 8 week old male NOD-SCID mice (Charles River Laboratories, Inc., Barcelona, Spain). All animals were housed in individually ventilated cage units and maintained under pathogen-free conditions. Food and water were provided ad libitum. Specific pathogen-free conditions were employed during the surgery and the follow-up for the animals. The animals were euthanized for necropsy by cervical dislocation after sedation. The experimental protocols employed in this study were approved by the local Animal Research Ethics Committee and Health Products Regulatory Authority (HPRA, ref. AE19136 P073), in accordance with the guidelines of the Animal Ethics Committee Trinity College Dublin, Ireland and Vall d'Hebron's Animal Experimentation Ethical Committee (CEEA, 53/12), Spain and the European Council Directive 1986 (86/806/EEC). Throughout the study, the use and treatment of animals were performed within the Three R's guidelines for ethical animal testing.

Mice were housed in groups and kept under standard housing conditions at a constant temperature (20 ± 2 °C) and standard lighting conditions (cycles of 12 h light: 12 h dark). Food and water were available ad libitum.

2.4.2. Biodistribution, Repeated-Maximum Tolerated Dose of tert-Ser-PTX, and Tolerability Studies

For the in vivo safety investigation, the biodistribution of PTX after single intravenous (i.v.), subcutaneous (s.c.), and intraperitoneal (i.p.) administrations of *tert*-Ser-PTX (20 mg kg^{-1} in PTX equivalents) was analyzed in C57BL/6 mice ($n = 4/6$ per group). Quantification of PTX and 7-epi-PTX by LC-MS/MS was carried out in tissue samples collected 24 h postinjection, after enzymatic digestion of organs and precipitation of protein/extraction with acetone.

Repeated dose MTD (r-MTD) analysis was carried in C57BL/6 mice (male and female, $n = 4$ per group and sex) receiving ten daily s.c. injections at 40 mg kg^{-1} or the corresponding volume of the vehicle (PBS). Behavior, physical appearance, and weight were monitored and scored to evaluate any visible adverse effects (no observable effect level; NOEL) for 24 days (10 days of treatment plus 14-day follow-up). Different organs (e.g., liver, lung, kidney, spleen, and heart) were collected at the experimental endpoint, fixed in formalin, and processed for histological analysis as described in Section 3.5.

Tolerability studies in NOD-SCID mice with orthotopic LNCaP tumors (see below) were conducted before efficacy studies following the same treatment schedule. In detail, mice (7 to 9 mice per group) were treated with PBS, PTX, or *tert*-Ser-PTX at a dose of 15 PTX kg^{-1} by i.v. administration three times a week followed by a rest week followed by the reinitiation of the treatment for up to a total of 3 treatment-rest cycles. Weight loss, physical appearance, and response to stimuli were monitored during treatment, and animals were euthanized if they reached the humane endpoints defined by the Animal Experimentation Ethics Committee. At the experimental endpoint, blood samples were collected by cardiac puncture in EDTA-containing tubes (Sarstetd) to evaluate clinical biochemical parameters in the Servei de Veterinària Clínica of the Universitat Autònoma de Barcelona.

2.4.3. Orthotopic Prostate Cancer Model

LNCaP.Fluc2 (1×10^6) cells in 30 µL sterile PBS were inoculated into the prostate of NOD-SCID mice. The prostate of anesthetized mice was exteriorized through a laparotomy, and cells were injected into the dorsal prostate lobes using a 30-gauge needle attached to an insulin syringe. A well-localized bleb within the injected prostate lobe indicated a technically satisfactory injection. The prostate was washed with saline, returned to the abdominal cavity, and the abdominal wound closed by suturing. During pretreatment, body weight, physical condition, and tumor palpation were measured twice a week. Tumor growth was indirectly monitored through tumor BLI using the noninvasive IVIS Spectrum imaging system. Mice displaying successful prostatic injection of LNCaP.Fluc2 cells were imaged every two weeks with ventral and dorsal views for up to two months. The tumor bioluminescence was quantified over time to determine orthotopic prostate tumor growth in the abdominal cavity. Once the tumors reached a median tumor bioluminescent intensity of $\approx 1.60 \times 10^7$ photons per second (ph s^{-1}) (range 1.50×10^6 to 1.60×10^8 ph s^{-1}), mice were randomized into two groups according to their tumor bioluminescent signal. Randomized mice ($n = 9$ per group) were treated with the test conjugate at 15 mg PTX kg^{-1} by i.v. administration three times a week followed by a rest week followed by the reinitiation of the treatment. On administration days, the bodyweight profile of the experimental groups was monitored before dosing the animals. The animals were administered with treatments only if the mean weight loss of the group was > 5%. During treatment, supervision of the animals was performed every 24 h. Clinical observations recorded included changes in skin, eyes, mucous membranes, alterations in respiratory pattern, behavior, posture, response to handling, and the presence of abnormal movements. Bodyweight and tumor palpation were measured twice a week. Tumor bioluminescent intensity was visualized and quantified once a week for seven weeks. Moreover, the animals were imaged to monitor the metastatic development of other tissues from the dorsal and ventral mouse views. At termination (24 h after the last administration), animals were euthanized by cervical dislocation and subjected to gross necropsy comprising the macroscopic evaluation of all the external body orifices and the examination of the cranial, abdominal, thoracic cavities, and contents. Prostate tumors, lungs, lymph nodes (mesenteric, peripancreatic, and perirenal), and diaphragm were collected. Tumor and metastases were evaluated by ex vivo BLI monitoring before the histological analyses.

2.4.4. Bioluminescence Imaging

In vivo and ex vivo BLI was performed with the IVIS Spectrum Imaging System, and images and measurements of bioluminescent signals were acquired and analyzed using the Living Image 4.3.1 software (PerkinElmer). The in vivo and ex vivo BLI techniques were developed following procedures previously described in our group.[29]

For in vivo BLI, animals were administered 150 mg kg^{-1} of D-luciferin (Promega Biotech Ibérica S.L., Spain) in sterile PBS by i.p. injection and anesthetized using 1–3% isoflurane (Abbott Laboratories, IL, USA). Five mice were imaged simultaneously, and imaging settings were set depending on the bioluminescent signals of the orthotopic tumors or metastatic lesions. We imaged our model at a 5–25 min range after D-luciferin injection. The brightest abdominal signals were shielded to detect and quantify weaker signals in the thoracic region. Light emitted from the bioluminescent cells was detected in vivo by the IVIS Spectrum, digitalized, and electronically displayed as a pseudocolor overlay onto a grayscale animal image. Regions of interest from images were drawn automatically (threshold = 20%, lower limit = 1.0, and minimum size = 20) around the bioluminescent signals and quantified in ph s^{-1}.

For ex vivo BLI, mice were euthanized 5–10 min after D-luciferin administration, and tissues of interest were excised, incubated in 300 µg mL^{-1} D-luciferin solution, imaged, and quantified as described above. BLI images are set at the same pseudocolor scale in the associated figures to show relative bioluminescent changes between different treatment groups.

2.4.5. Histopathology

Immediately after euthanasia, organs and tissues were cleaned with PBS, preserved in 4% formaldehyde solution, and then

Table 1. Characterization of polyacetal-based conjugates.

	PTX loading [wt%]	Size [nm[a)]]	$Đ$[b)]	Z[c)] [mV]	M_w [kDa][c)]	M_n [kDa][c)]	$Đ4$[d)]	R_h [nm][e)]
tert-Ser	-	6 ± 1	0.4	1.2	27.0	15.7	1.7	5.0
tert-Ser-PTX	6.8	9 ± 2	0.3	1.5	23.6	34.4	1.48	6.0

[a)] Data obtained by DLS for 5 mg/ml solutions in PBS (mean ± SD). Size distribution by volume %; [b)] Polydispersity index determined by DLS; [c)] Z potential determined by electrophoresis light scattering in PBS; [d)] Data obtained by GPC in DMF/LiBr (1%) at 8 mg mL^{-1}; [e)] Radius of hydration (Rh) determined by ^{1}H-diffusion ordered spectroscopy-NMR.

processed for histological analyses. All tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). All sections were assessed by an experienced histopathologist at Trinity College Dublin.

2.5. Statistical Analysis

The mean or median BLI intensities and corresponding standard errors of the mean (SEM) were determined and plotted. Non-linear regression plots were used to describe the relationship between BLI intensity and time after treatment. A non-parametric Mann-Whitney test was applied for peer comparisons in prostate weight and ex vivo bioluminescent data. The significance threshold was established at $p < 0.05$, and significance levels were schematically assigned *($0.01 \leq p < 0.05$), **($0.001 \leq p < 0.01$), ***($0.0001 \leq p < 0.001$) or ****($p < 0.0001$). All the analyses and graphs were performed using GraphPad Prism 5 software (GraphPad, San Diego).

3. Results and Discussion

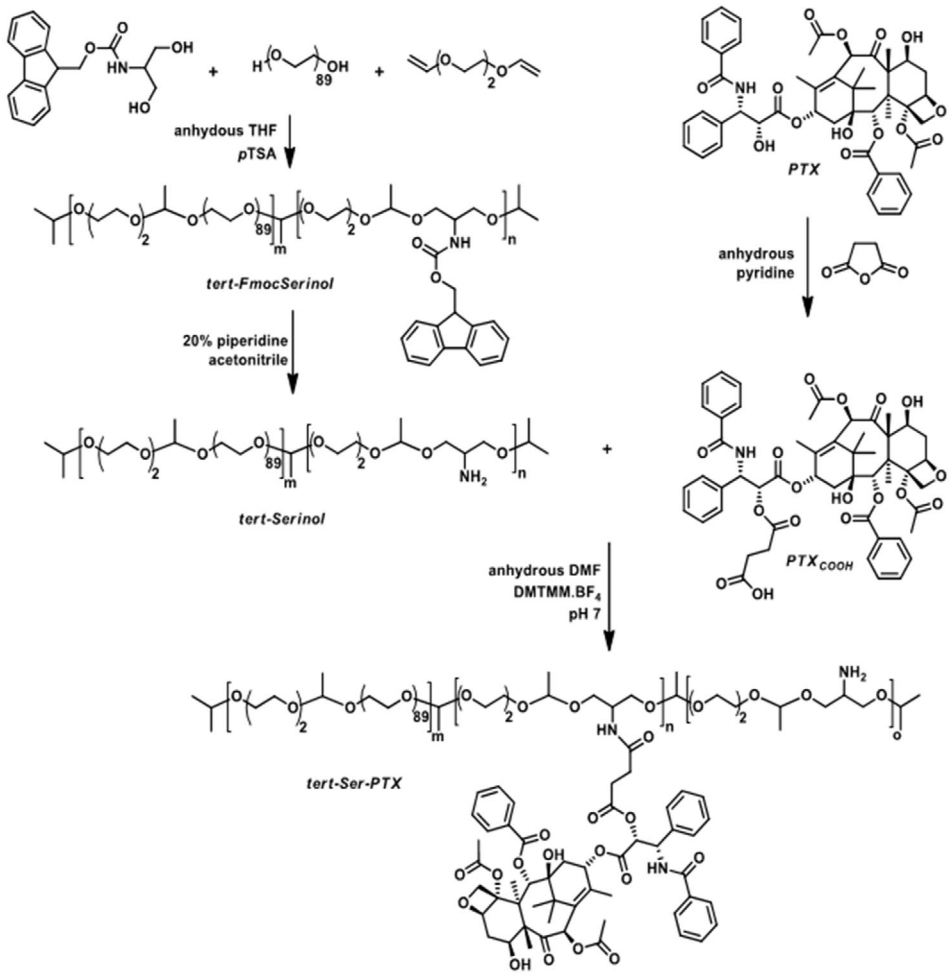
3.1. Synthesis and Characterization of *tert*-Ser-PTX

We incorporated PTX within the side-chains of a pH-susceptible biodegradable polymer to prepare a water-soluble polyacetal-PTX conjugate: *tert*-Ser-PTX (**Table 1**) and then compared the efficacy and potential toxicity to free PTX in an in vivo orthotopic LNCaP.Fluc2 PCa model. We prepared the *tert* structure using a simple one-pot synthetic procedure, as previously described.[11] In brief, this uses serinol moieties as co-monomers during the polymerization reaction, forming part of the polymer main-chain in a random manner. Incorporated serinol moieties offer new anchoring positions to incorporate PTX in a second step. Before conjugation, we modified the PTX molecule with succinic anhydride to generate 2'-succinyl-PTX. We established a synthetic protocol at the 10 g scale with high batch-to-batch reproducibility (**Scheme 1** and Table S1, Supporting Information).

We adopted complementary characterization techniques of incremental complexity to evaluate size distribution, Z potential, morphology, drug loading, drug release in complex media, and protein binding. Firstly, the characterization of polymers by ^{1}H-NMR ensured acetal bond formation and the correct insertion of Fmoc-serinol in the main polymer chain. The peaks corresponding to the –CH– of acetal groups appear at 4.77 ppm when the acetal bond lies next to an ethylene glycol moiety (**Figure 1**a). We found evidence for the esterification site of 2'-succinyl-PTX through the shift of the signal corresponding to C2' protons of PTX from 4.8 to 5.5 ppm for the succinoylated molecule. We also observed a new set of peaks due to the $-CH_2-CH_2-$ protons of the succinyl moiety at 2.64 and 2.76 ppm. The ^{1}H-NMR spectrum of the *tert*-Ser-PTX confirmed the formation of an amide bond between the *tert*-Ser conjugate and the 2´-succinyl-PTX by the presence of the peaks due to PTX protons after polymer purification.

We calculated the total PTX loading by integrating the ^{1}H-NMR of the characteristic peaks of PTX (7.46–8.49 ppm) corresponding to fifteen protons (Figure S1, Supporting Information), which we determined to be 8% w/w. A more exhaustive analytical assessment by LC-MS/MS (Figure 1b and Figure S1, Supporting Information) provided a figure of 6.8% w/w PTX total loading, with less than 1.5% free drug not bound to the polymer (Table 1). LC-MS/MS constitutes an orthogonal measurement principle to NMR, providing independent verification and ensuring confidence in the challenging analysis of polymeric prodrugs. LC-MS/MS is highly suitable for drug loading measurements, even in complex biological matrices, due to its sensitivity, selectivity, and specificity.

The formation of the *tert*-Ser-PTX conjugate requires the covalent bonding of PTX to the polyacetal carrier. Since LC-MS/MS is an analytical technique based on the drug's molecular properties, we adapted the standard operating procedures to measure drug loading, free vs. bound drug fractions, and drug release rates. These parameters can be measured in the same experimental setup by following the free drug concentration as a function of time and the buffer used. The free fraction can be measured at time $t = 0$. Subsequently, the total drug loading can be measured as the drug concentration at the time when equilibrium has been reached (corrected for drug degradation). Performing this experiment under different conditions also allows the investigation of drug release kinetics. Release of PTX in vivo occurs through the hydrolytic cleavage of the ester bond to the succinic acid moiety, with temperature and pH influencing hydrolysis in an aqueous solution. Additionally, endogenous esterases in plasma could contribute to hydrolysis. Notably, both PTX and the polymer/linker contain additional hydrolyzable bonds—four ester bonds within the PTX moiety, the amide bond attaching the succinic acid linker to the polymeric carrier, and the repeated acetal groups in the polymer (Figure S1, Supporting Information). One additional consideration for drug release from a covalent bond is the potential for concomitant drug degradation. PTX is known to epimerize in an aqueous solution to 7-epi-PTX. Additional main degradation products from PTX are baccatin and 10-deacetyl-PTX.[30,31] Although inconsistencies exist in the literature, 7-epi-PTX is assumed to possess similar bioactivity to PTX, whereas the latter two degradation products display lower bioactivity.



Scheme 1. Synthetic approach for the preparation of *tert*-Ser-PTX.

The initial analytical results obtained of drug loading after the spontaneous release of PTX (Figure 1b) suggested a possible incomplete release of PTX and/or concomitant degradation of PTX during analysis. Therefore, we devised a complementary drug quantification strategy that encompassed complete, harsh chemical degradation of both the polymer and the drug to accelerate the degradation of all hydrolyzable bonds. This strategy released two benzoic acid molecules per molecule of PTX. As no other sources of benzoic acid exist in the samples, the quantification of benzoic acid can function as a direct measure of released, degraded, and polymer-bound PTX. In the absence of biological conversion pathways (i.e., enzymes and cofactors), the conversion of benzoic acid should be minimal. Moreover, the compound is known to be stable both towards strongly acidic and strongly alkaline conditions that hydrolyze ester and amide bonds.

Free PTX measured as a function of incubation time in PBS with pH adjusted to 7.4, 5.5, and 4.0 showed as expected, the release rate increases markedly with pH, see Figure 1b. The initial experiments indicated a peak concentration at pH 7.4 after around 48 h at 636 ng mL^{-1} followed by an almost linear decrease in concentration up to the last sampling point at 336 h, suggesting PTX degradation. Sample incubation at lower pH prompted an upward trend in the concentration of PTX even at the last sampling point, suggesting the incomplete release of PTX. Here, the measured concentration of PTX of 592 ng mL^{-1} strongly indicates the underestimation of the value measured at 48 h (pH 7.4). Upon inspection of the LC-MS/MS data, we discovered a second chromatographic peak with a mass and fragmentation pattern of PTX. A comparison with analytical standards allowed the identification of the 7-epi-PTX impurity. The formation of this epimer seems to be accelerated at neutral and alkaline pH. Furthermore, an additional control experiment carried out by incubating free PTX in the same conditions described above, demonstrated loss of total PTX (the sum of PTX and 7-epi-PTX) over time, indicating the degradation of both species to other molecules. This degradation could occur through the hydrolysis of the ester to form baccatin.

Figure 1b shows the quantification of released PTX with 7-epi-PTX included and corrected for the degradation of free PTX. After correction, the peak concentration was 1692 ng/mL of PTX release, which corresponds to a concentration (w/w) of 1.69 μg PTX per 25 μg *tert*-Ser-PTX, resulting in a total PTX loading of 6.8% w/w. Using the samples at pH 4.0 and 5.5, we found a free PTX level in the formulation of less than 1.5% of total drug

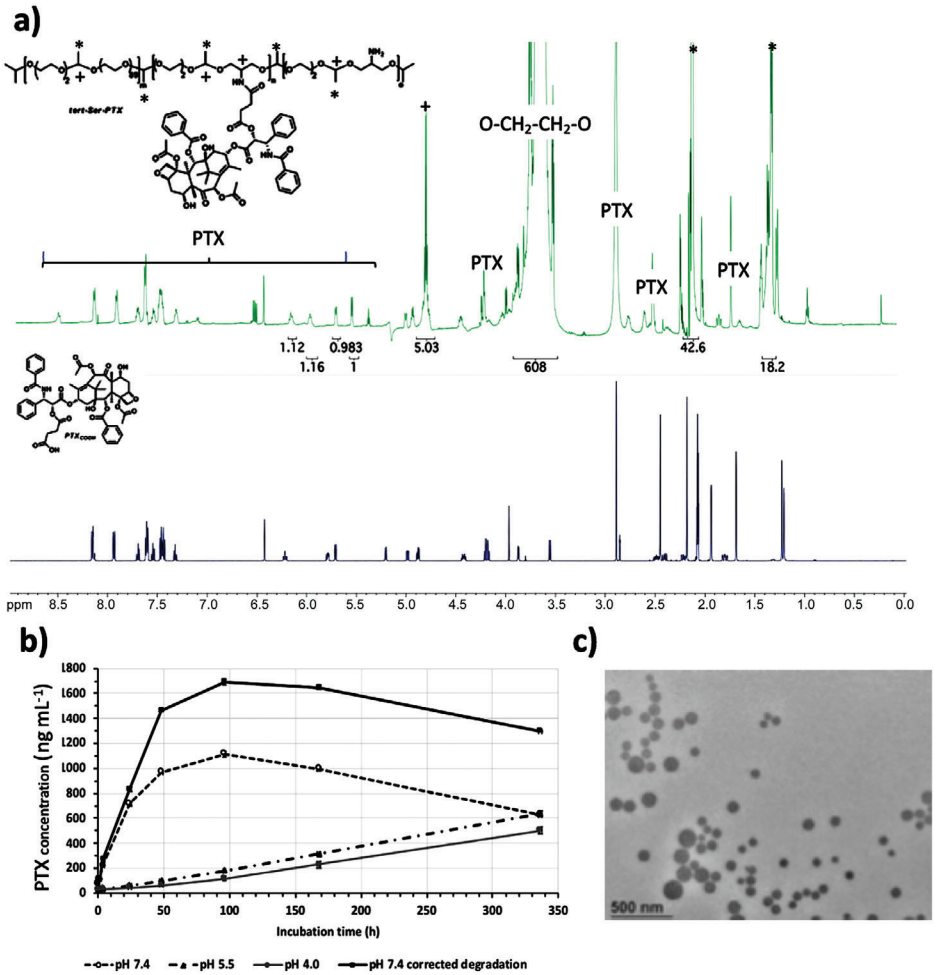


Figure 1. Characterization of *tert*-Ser-PTX a) High resolution 600 MHz ^{1}H NMR spectrum of *tert*-Ser-PTX (upper) with assigned signals and comparison with free PTX (lower). b) Cumulative PTX release from *tert*-Ser-PTX after incubation in PBS at 4 varying pH values (see legend) measured by LC-MS/MS. Graph shows total PTX release, including 7-epi-PTX epimer, without correction for PTX degradation (direct measurement for pHs 7.4, 5.5, and 4.0), and the concentration of total PTX at pH 7.4 with correction for degradation of free PTX (solid black line) as measured in a separate sample. Data as mean ± SD ($n \geq 3$). c) Representative image of *tert*-Ser-PTX show a smaller and more abundant population at around 10 nm, medium-sized particles ranging from 30 to 70 nm, and a few aggregates of 200 nm.

loading. As we determined a measured and calculated drug loading slightly lower than the value obtained by NMR, we designed a complementary drug loading measurement strategy by inducing complete chemical degradation (see Experimental Design Considerations) of PTX. This approach indicates an absolute lower limit for drug loading, as it constitutes a direct measurement of a fragment of the PTX molecule. After digestion of both free PTX and *tert*-Ser-PTX with 4 M H_2SO_4 at 90 °C overnight, we measured the concentration of benzoic acid. The theoretical concentration of benzoic acid in both samples was 234.2×10^{-6} M. For the free PTX sample, we found a concentration of 222.0×10^{-6} M benzoic acid, which constitutes a 94.6% recovery of the theoretical value. For the *tert*-Ser-PTX, we found a concentration of 161.0×10^{-6} M, which constitutes 1.46 mg PTX per 25 mg *tert*-Ser-PTX, 6% w/w. This experimental value of 73% of the value obtained by NMR constitutes a lower value for PTX loading. The detailed results obtained by LC-MS/MS align well with the initial results obtained by NMR; overall verification by orthogonal measurements techniques provides additional confidence.

DLS, diffusion ^{1}H-diffusion ordered spectroscopy (DOSY)-NMR (Figures S2–S3a, Supporting Information), and SEC analyses of *tert*-Ser-PTX combined to establish an average size distribution of around 9–12 nm (data expressed in volume) with the presence of a small percentage of larger aggregates as expected for neutral conjugates (Z potential = 1.5 mV) with hydrophobic moieties (Table 1). Of note, we observed *tert*-Ser-PTX stability for up to 24 h in PBS, with size not significantly affected during the incubation time (Figure S3b, Supporting Information). Importantly, transmission electronic microscopy (TEM) analysis also demonstrated the well-dispersed nature of the particles after preparation in dry conditions, but we observed particles of at least three families of size: the most prevalent of small particles around 10–15 nm, medium particles of 30 to 70 nm, and a few larger particles of around 200 nm (Figure 1d, Figure S3c, Supporting Information).

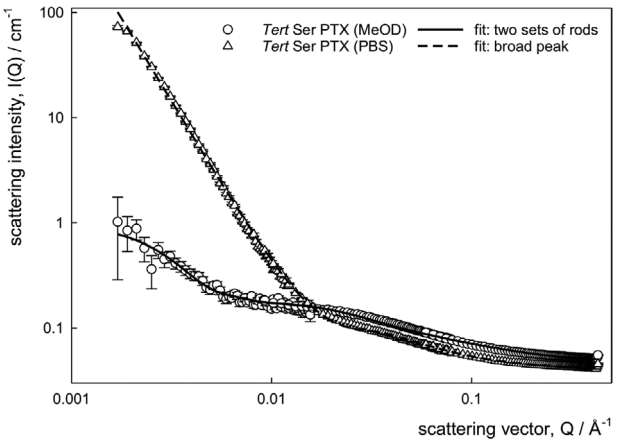


Figure 2. Characterization of *tert*-Ser-PTX by SANS. SANS data from 1 wt.% conjugate solutions in dPBS and MeOD. Representative error bars are shown.

We also investigated *tert*-Ser-PTX by SANS (methodology and data analysis are detailed in the Supporting Information). SANS provides detail regarding the size and morphology of structures in solution and can link different aspects of conjugate behavior to morphology. We studied *tert*-Ser-PTX in deuterated methanol solution (MeOD) and PBS (dPBS) at 10 mg mL^{-1} concentration, which allows the evaluation of the specific influence of PTX solvophobicity (significantly increased in PBS compared to methanol solution). Data revealed two regions in the scattering; at low Q, a steeper decline in $I(Q)$ versus Q was followed at intermediate and high Q by a shallower and nonlinear Q dependence (**Figure 2**).

In methanol, low Q data showed a Q^{-1} dependence at low Q and were fitted in FISH software. Data at low Q and intermediate/higher Q were fitted separately to a model for rods. The parameters were then refined using the whole data set to give a combined model for two sets of rods in solution, which was previously used for conjugates of this type.[13] The high Q region indicated short, thin rods with a radius of 0.7 ± 0.05 nm and a length of 17.0 ± 1.00 nm. We also observed larger, more globular structures with a radius of 78.0 ± 2.00 nm and a length of 50.0 ± 2.00 nm coexisting in solution.

In PBS solution, we observed a much higher intensity at low Q, which indicates larger structures present in solution at an equivalent concentration of the same material. The high Q data suggested the presence of broader rods in PBS compared to methanol. We also identified a fitted radius of 1.0 ± 0.05 nm (with the fitting insensitive to rod length), suggesting the existence of long thin structures indicative of fiber-like structures. Combined with the larger radius in PBS compared to methanol, the presence of PTX may drive polymer chain aggregation. Furthermore, considering the solvent contributions, the drug interactions would be much weaker in an ionic solvent.

As we could not fit the whole of the PBS data to a model for two separate populations of rods. Instead, we adopted a broad peak model, typically used for large structures caused by the aggregation of other rod-like structures (e.g., fiber bundles). This model provides information on the packing of structures within the aggregate, given by the scattering at low Q, and information on the individual chains within the aggregate, given by scattering at intermediate and high Q.

When low Q Porod scattering dominates, and the slope of the straight line (n) arises from scattering from either a mass ($n = 1$–3) or surface ($n = 3$–4) fractal. Indicative values are 1 for long rigid structures, 2 for Gaussian chains, and 3 for collapsed polymer systems. The individual chains within the aggregate are characterized by a correlation length ξ, peak position Q_0, and the fractal dimension m. The relation of these terms to $I(Q)$ is given in Equation 1 below, where two scaling factors determine the contributions of each term.[32] For the *tert*-Ser-PTX sample in PBS, the fitted parameters are in Table S2 (Supporting Information).

$$I(Q) = \frac{I_P(0)}{Q^n} + \frac{I_L(0)}{1 + (|Q - Q_0|\xi)^m} + bkg \quad (1)$$

To summarize, we found *tert*-Ser-PTX scattering patterns consistent with the formation of large globular structures of ≈100 nm in diameter coexisting with long thin rod-like structures in methanol; however, we observed more ordered structures in PBS (Figure 2). The modeling results suggest the aggregation of rods into densely packed bundles in PBS. The rods themselves in PBS are thicker (about 1.5×) than in methanol, consistent with the intramolecular association of PTX molecules, which increases the stiffness of the rod-like structures and drives intramolecular alignments into bundles (Figure 2 and Table S2, Supporting Information). Notably, the smaller structures observed by SANS are consistent with the sizes obtained from DLS. These findings could suggest that sample filtration before DLS measurements remove or disrupt any larger structures present, which leaves insufficient time for reformation. The scattering results from the *tert*-Ser-PTX sample fit best to a thin rod of radius 10 Å, length 300Å, with a Q^{-n} term with $n = 3.5$ (Figure 2 and Table S2, Supporting Information).

3.2. *tert*-Ser-PTX Stability and Drug Release Studies in Simple and Complex Media

Stability during circulation and the potential for controlled drug release from the polymeric carrier under selected physiological triggers represent two essential characteristics of PDCs.[13] While the released free drug fraction supports biological outcomes, the (reversibly) released fraction bound to plasma or cell medium proteins constitutes a 'reservoir' in equilibrium with free PTX. Therefore, we must understand the levels of both free and total released PTX.

In the absence of serum, the percentage release of PTX from *tert*-Ser-PTX displayed a pH-dependent profile, with an increase in PTX release upon increased pH (**Figure 3**a). We note that this is an unexpected result for polyacetal systems,[11–13] which generally degrade (and therefore release the conjugated active agent) more rapidly at a lower, acidic pH. Our previous studies involved incorporating drugs directly into the polymer backbone; however, the conjugate described in this study employs a polymer–drug linker susceptible to hydrolysis. This provides two possible mechanisms of PTX release from the polyacetal polymer involving hydrolysis: i) hydrolysis of acid pH-sensitive polymer backbone acetal bonds or ii) hydrolysis of the ester bonds of the polymer–drug

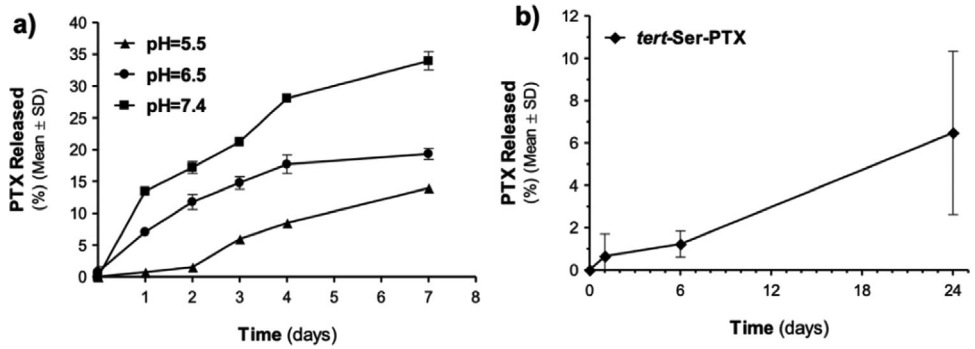


Figure 3. PTX release from *tert*-Ser-PTX in PBS and rat serum. a) pH-dependent release (3 mg mL^{-1}, 37 °C) in PBS at pH 5.5, 6.5, and 7.4 determined by HPLC-UV. b) Effect of rat serum on release (3 mg mL^{-1}, 37 °C). Data as mean ± SD ($n \geq 3$).

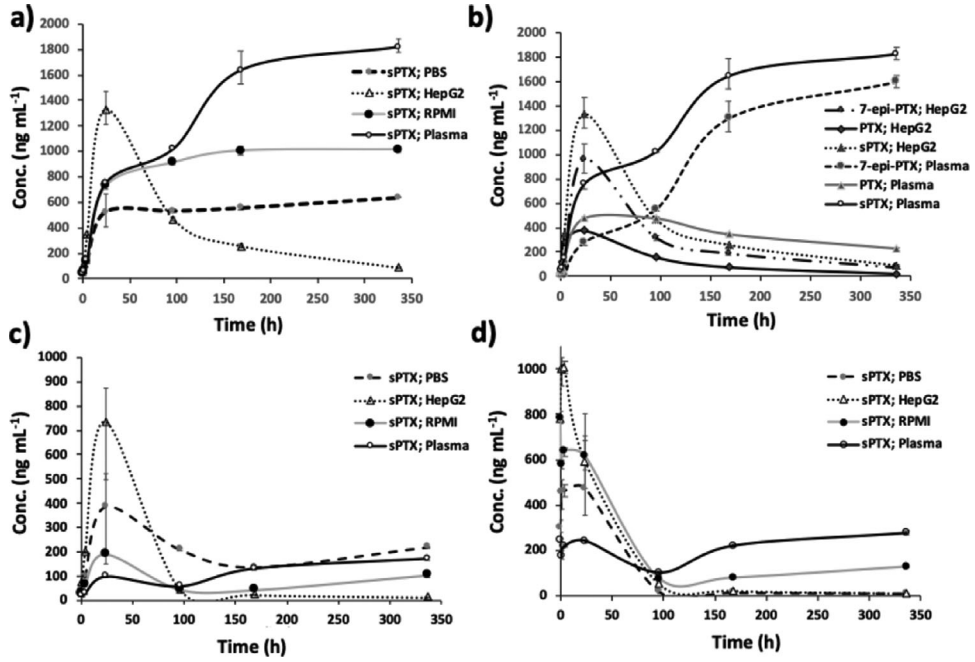


Figure 4. a) PTX released from *tert*-Ser-PTX after incubation in different media (PBS, RPMI cell medium, HepG2 cell medium, human plasma), measured by LC-MS/MS as the sum (sPTX) of PTX and 7-epi-PTX. b) Release into HepG2 cell medium and human plasma plotted as the single components PTX and 7-epi-PTX and the sum. RPMI and PBS showed similar ratio trends between PTX and 7-epi-PTX as human plasma but were omitted for visual clarity. c) PTX released from *tert*-Ser-PTX after incubation in different media (PBS, RPMI cell medium, HepG2 cell medium, human plasma), measured by LC-MS/MS as the sum (sPTX) of PTX and 7-epi-PTX, after centrifugal ultrafiltration (i.e., excluding protein-bound drug). d) Free PTX after incubation in different media (PBS, RPMI cell medium, HepG2 cell medium, human plasma), measured by LC-MS/MS as the sum (sPTX) of PTX and 7-epi-PTX, after centrifugal ultrafiltration (i.e., excluding protein-bound drug). Data as mean ± SD ($n \geq 3$).

linker (Figure S1, Supporting Information). Overall, our results suggest drug release driven by hydrolysis of the polymer–drug linker. PTX release at pH 7.4 is hydrolytically triggered by ester bond degradation; in buffer, this degradation is faster than the disruption of the polyacetal mainchain at pH 5.5, as we observed no significant metabolites containing Ser-PTX moieties (data not shown).

In the presence of rat serum, *tert*-Ser-PTX released low levels of PTX during a 24 h incubation at pH 7.4 (Figure 3b). Therefore, our data suggest that serum proteins protect the ester bond from hydrolytic degradation; however, the role of in vivo protein corona formation[33] in the protection of the *tert*-Ser-PTX ester bond requires further consideration. In any case, this is an encouraging finding as increased stability in serum favors systemic administration.[34] Overall, we found less than 2% of total PTX release after 6 h incubation at 37 °C, and less than 7% after 24 h incubation.

We performed further exhaustive analyses using LC-MS/MS to ratify our hypothesis—although we employed three different concentrations (see Experimental Section). The data provided similar trends; thus, we here present only the 2 μg mL^{-1} concentration point (the most clinically relevant concentration) in the following discussion for clarity (**Figure 4** and Figure S4, Supporting Information). Figure 4a,b demonstrates evident differences

in release and/or degradation kinetics in different media (in both panels, "sPTX" denotes the sum of PTX and 7-epi-PTX). We found low or negligible variability between replicates at most data points. Of note, we buffered the pH in PBS, RPMI, and plasma to 7.4, although HepG2 medium remained unbuffered (manual evaluation of several samples of HepG2 medium indicated a pH of around 8.0). In comparison with our drug loading studies, we observed the accelerated release of sPTX at higher pH (and we presumed this to cause the accelerated release of sPTX in HepG2 medium compared to RPMI and plasma). Furthermore, the increased degradation of sPTX in HepG2 medium may correlate to the increased susceptibility of the intramolecular ester bond to hydrolysis at elevated pH.

Of note, the measured concentrations of released sPTX in PBS, RPMI, and plasma converge on equilibrium values, albeit at distinct levels; for plasma, the concentration converges towards the theoretical loading of 2000 ng mL^{-1} after two weeks. We observed a similar initial shape for the sPTX release curves for PBS, RPMI, and plasma; however, the subsequent plateau for each condition occurs at distinct concentrations. We also note that similar total protein levels present in HepG2 and RPMI media (10% FBS in HepG2; 10% plasma in RPMI) remain much lower than that found in total human plasma. Therefore, one could speculate that the differences at later time points may correlate with solubility limits in the respective media, as observed by Abouelmagd et al.[35]

As PTX suffers from an extremely high degree of protein binding, the increased amount of protein in media (RPMI, plasma) may ensure an increased amount of solubilized sPTX. Interestingly, PTX release into plasma extends beyond two weeks with little or no loss of sPTX observed during this time, even as measured concentrations approach theoretical PTX loading. This may indicate that plasma proteins contribute to PTX stabilization and protection of PTX from degradation, thereby corroborating our hypothesis. Degradation may occur in PBS in a comparable manner to our observations in drug loading experiments (Figure 1b); however, this remains undetectable, as precipitated PTX would re-solubilize concomitantly up to the solubility limit. The complete evaluation of this hypothesis would require longer incubation times or the direct detection of degradation products.

Figure 4b depicts the conversion of PTX to 7-epi-PTX, which occurs to a significant degree in all media and reaches near completion in HepG2 medium. For PBS and RPMI, the conversion kinetics remain similar to those for plasma. These findings strongly advise the detailed investigation of the biological effect equivalence between PTX and 7-epi-PTX, as the latter compound could be the predominant epimer in plasma after around three days. Figure 4c and Figure S4a (Supporting Information) show the corresponding levels of free drug (sPTX) measured after spin filtration (i.e., excluding protein-bound drug) in the respective media. While the equilibrium concentrations measured in the supernatant from the PBS, RPMI, and plasma remain comparable after two weeks. Analysis in HepG2 medium revealed little detectable sPTX at longer incubation times, reinforcing the hypothesis that PTX degrades more rapidly in this medium. Even given the relatively large degree of variability for the HepG2 samples (and PBS) at 24 h, the notably high sPTX concentration found in the centrifuged supernatant could indicate that either pH or other compounds present in HepG2 medium effectively increase the aqueous solubility of PTX. We also note the low variability between replicates at the other sampling points.

Finally, a comparison, with the same amount of free PTX added as control, for the free drug after ultrafiltration presented in Figure 4d and Figure S4b (Supporting Information). We added free PTX solubilized in an organic solvent (acetonitrile); while this was necessary given the solubility profile of PTX, we appreciate that this could induce local precipitation effects and other potential inhomogeneities. Nevertheless, the availability of free sPTX after long incubation times correlates with the amount of protein in the medium, which conceivably acts as a depot to protect PTX from degradation, as demonstrated in Figure 4d and Figure S4b (Supporting Information). The loss of free PTX over time compared to *tert*-Ser-PTX is of interest (we detected free PTX during the whole incubation period), which may derive from continuous release from the conjugate. The almost complete loss of free sPTX over time for the HepG2 medium corresponds well with our observations regarding *tert*-Ser-PTX incubation, i.e., loss most likely reflects elevated levels of degradation.

In summary, *tert*-Ser-PTX conjugate provides for the sustained release of PTX over two weeks, which reaches the theoretical limit regarding PTX loading. PTX release markedly accelerates at alkaline pH (or in the presence of specific degradation factors in the HepG2 cell medium), as does PTX degradation. Furthermore, the significant degree of epimerization of PTX to 7-epi-PTX must be considered for any assessment of bioactivity.

3.3. In vitro Efficacy of *tert*-Ser-PTX

To further explore drug-release and efficacy, we incubated androgen-dependent and androgen-independent PCa cell lines with increasing concentrations of *tert*-Ser-PTX and compared results to free PTX. Free PTX and *tert*-Ser-PTX treatment reduced cell viability in both cell lines, and *tert*-Ser-PTX displayed a higher IC_{50} value than free PTX (**Figure 5** and **Table 2**). We obtained lower IC_{50} values for *tert*-Ser-PTX in LNCaP cells (0.152 ± 0.141 μg PTX mL^{-1}) compared to PC-3 cells (0.764 ± 0.381 μg PTX mL^{-1}) (Table 2), suggesting the increased sensitivity of the androgen-dependent LNCaP cells.

Interestingly, we found the greater efficacy of *tert*-Ser-PTX at high concentrations of PTX (> 0.4 μg mL^{-1}) when compared to the free drug in both cell lines, but especially in LNCaP cells (Figure 5c). We previously observed that free PTX does not entirely abolish cell viability and that increasing the drug concentration does not result in higher cytotoxic activity in colon and breast cancer cell lines.[36] This result could be driven by the existence of PTX drug resistance mechanisms such as the presence of P-glycoprotein 1 (P-gp) (also known as multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B member 1 (ABCB1)), which pumps the cytotoxic drug out of the cell.[19,37] Therefore, applying a drug delivery system that introduces drugs through the endocytic pathway might overcome this problem.[38]

3.4. In Vivo Biodistribution of *tert*-Ser-PTX

Next, we evaluated the biodistribution of *tert*-Ser-PTX as result of different routes of administrations (**Figure 6**a). This was carried out before the efficacy assessment.

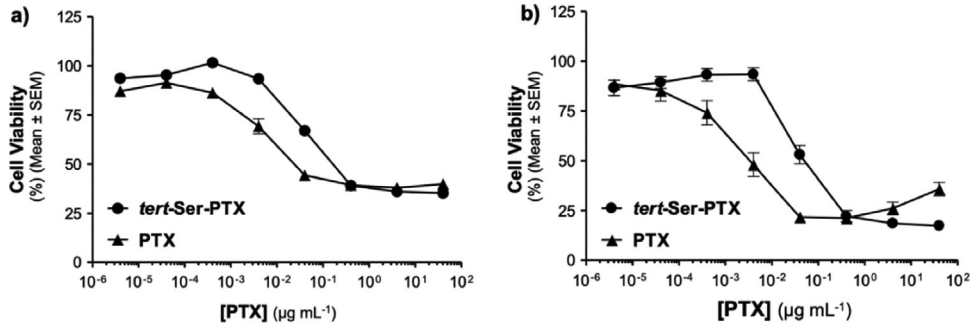


Figure 5. In vitro efficacy of *tert*-Ser-PTX in PCa cell lines. MTT assays were performed after 72 h incubation of a) androgen-independent PC-3 and b) androgen-dependent LNCaP PCa cells with increasing concentrations of *tert*-Ser-PTX or free PTX.

Table 2. IC_{50} values for free PTX and *tert*-Ser-PTX in different PCa cells. Each value is mean ± SEM ($n = 3$).

	IC50 [μg PTX mL^{-1}]	
PCa cell line	PTX	*tert*-Ser-PTX
PC-3	0.067 ± 0.027	0.764 ± 0.381
LNCaP	0.007 ± 0.003	0.152 ± 0.141

The results indicate that most of the administered PTX is located to the liver (36.1 ± 4.5% for i.v. injection at 24 h) and kidneys (34.3 ± 8.1% for i.v. injection at 24 h), regardless of the injection route. From the safety toxicity perspective, we observed no significant differences between the different administration routes. While PTX is i.v. administered in the clinical setting, we performed daily s.c. administrations of *tert*-Ser-PTX at high doses (40 mg kg^{-1}, 10 doses) during the r-MTD safety study. This was done with the intention of improving the consistency while reducing animal stress. Weight monitoring of the animals showed that repeated *tert*-Ser-PTX administrations induced mild weight loss in both sexes during the treatment period, which was fully recovered during the 14 d of follow-up. Figure 6b shows weights of male mice, sex where we observed the most significant differences between vehicle to *tert*-Ser-PTX treated mice). We also did not observe any behavioral changes during the treatment or observation periods. Histological assessment of different organs in these animals post-treatment did not detect any abnormalities in lung, kidney, spleen, and heart; only in liver tissue mitotic changes were detected, probably due to the high concentration of PTX. These results showed that *tert*-Ser-PTX was safe to use for repeated administrations up to a maximum dose of 40 mg kg^{-1} in PTX load. Upon the presence of moderate changes in liver histology, a lower PTX dose was adopted (15 mg kg^{-1} in PTX) for tolerability studies, as described next.

3.5. In Vivo PTX Tolerability of *tert*-Ser-PTX

We treated mice bearing orthotopic LNCaP.Fluc2 tumors with free PTX and *tert*-Ser-PTX and investigated toxicity by monitoring body weight and physical and clinical observations.

A summary of the data from the animal welfare monitoring is provided for this study in **Table 3**. Of note, two animals from the group treated with free PTX died 24 h after treatment, while the remaining animals displayed signs of toxicity and were euthanized a week after treatment initiation. After necropsy, we observed macroscopic lesions such as an enlarged urinary bladder (7/9 (78%)) and clear and enlarged kidneys (2/9 (22%)); however, we failed to observe similar lesions in the *tert*-Ser-PTX treated group (Table 3). Additionally, we failed to encounter significantly adverse effects on animal body weight, although we did detect fur alteration and black faces as minor adverse side effects after the first week of administration. By the end of the treatment period, we observed a treated to control (T/C) ratio of body weight change of -3% for the *tert*-Ser-PTX treated animals. Notably, the toxicities observed for free PTX at specific concentrations became significantly reduced following polymer conjugation (treating with the same concentration of PTX equivalents). Blood samples showed no differences in biochemical parameters between the *tert*-Ser-PTX and vehicle-treated mice (Table S3, Supporting Information), indicating the safety of repeated administrations of *tert*-Ser-PTX in animals. Overall, we found that *tert*-Ser-PTX was well tolerated and failed to cause adverse effects or animal deaths over the treatment period.

Our results indicate that polyacetals might constitute a promising drug delivery system that reduces systemic PTX toxicity by avoiding Cremophor and improvements to tissue biodistribution, as demonstrated for other PTX delivery systems.[39] Currently, two clinically approved nanoparticle taxane (i.e., PTX family of compounds) formulations exist—nab-PTX (Abraxane, Abraxis Bioscience, Los Angeles, USA) and Genexol-PM (Samyang Biopharm, Daejeon, South Korea). While both formulations increase the maximum tolerated dose of PTX,[40] dose-limiting toxicities such as neutropenia, myalgia, and neuropathy remain significant problems, and improvements to clinical outcomes remain modest.[39,40] Therefore, we still require further development/refinements of drug delivery systems to improve efficacy and safety profiles, including a direct comparison of *tert*-Ser-PTX polymers with already approved PTX formulations.

3.6. Inhibition of Orthotopic Tumor Growth by *tert*-Ser-PTX

We next orthotopically implanted LNCaP.Fluc2 cells into the mouse prostate and non-invasively monitored tumor growth through bioluminescent optical imaging to determine the antitumor efficacy of *tert*-Ser-PTX. We treated *tert*-Ser-PTX and free

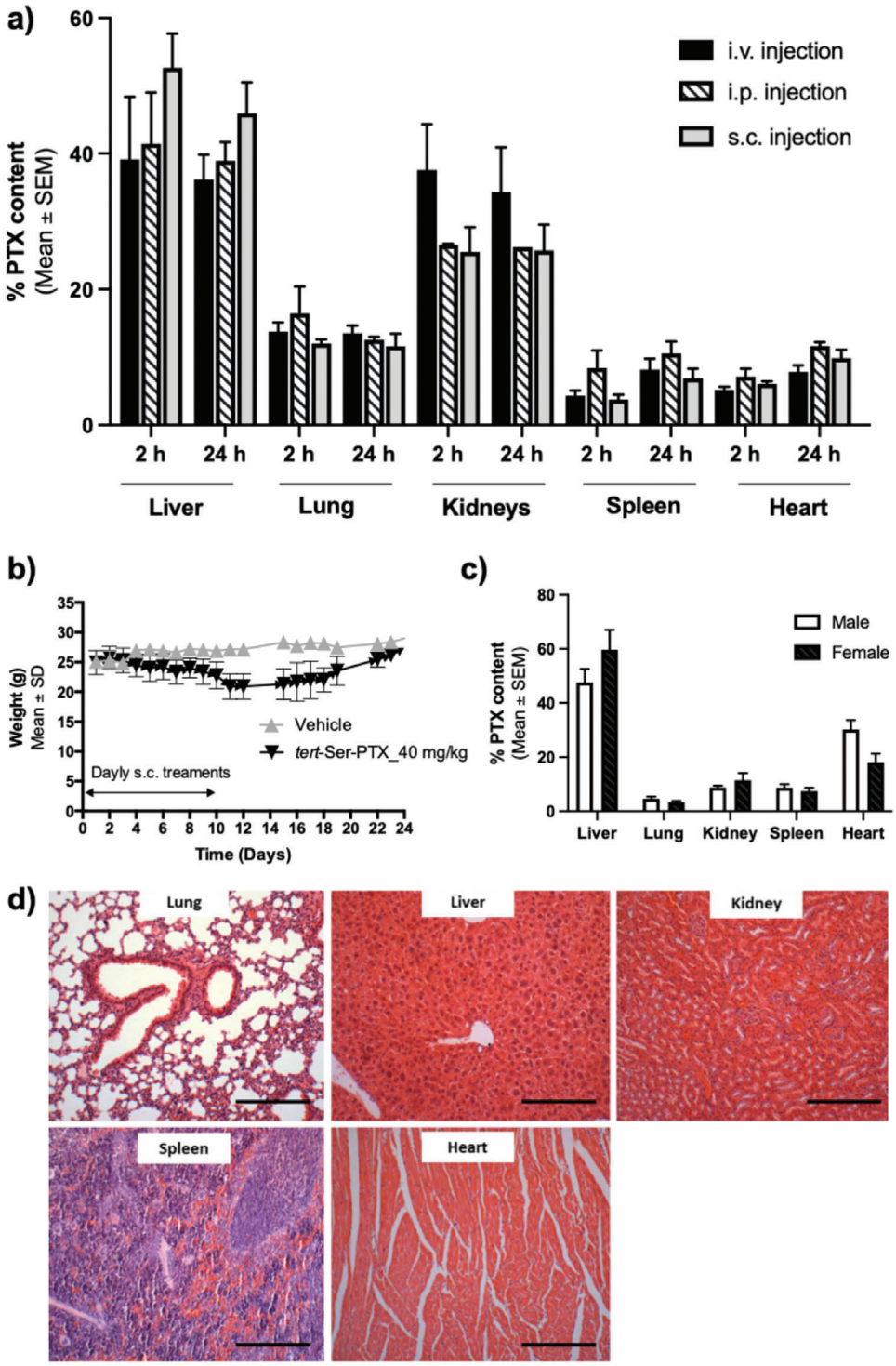


Figure 6. In vivo biodistribution and safety after single-dose and repeated administration of *tert*-Ser-PTX. C57BL/6 mice were administered with a single dose at 20 mg kg^{-1} or repeated administrations of 40 mg kg^{-1} *tert*-Ser-PTX at PTX-equivalent doses. Thereafter, PTX content was determined by LC-MS/MS analysis. a) PTX biodistribution among different organs 2 and 24 h after single i.v., i.p., or s.c. injection (n = 2–3 per time point). b) Weight monitoring in male animals receiving 10 s.c. doses of 40 mg kg^{-1} *tert*-Ser-PTX (n = 4). c) PTX content in organs of male and female mice after ten s.c. administrations of 40 mg kg^{-1} *tert*-Ser-PTX (n = 8, 4 males and 4 females). d) representative histological H&E images of lung, liver, kidney, spleen, and heart tissue slices collected from a representative male mouse treated with 10 doses of 40 mg kg^{-1} *tert*-Ser-PTX (Scale bar = 250 µm).

Table 3. Main parameters monitored in toxicity evaluation of PTX and *tert*-Ser-PTX after repeated administration of 15 mg kg-1 of PTX equivalents (The toxicity of PTX and *tert*-Ser-PTX and their respective vehicles (ethanol:cremophor EL:saline for PTX and PBS for *tert*-Ser-PTX). The number of animals and the corresponding percentage (in brackets) are given.

	PTX vehicle	PTX	*tert*-Ser-PTX vehicle	*tert*-Ser-PTX
Found dead or euthanized due to side effects	0/9 (0%)	9/9 (100%)	0/9 (0%)	0/9 (0%)
Enlarged urinary bladder	0/9 (0%)	7/9 (78%)	0/9 (0%)	0/9 (0%)
Enlarged kidneys	0/9 (0%)	2/9 (22%)	0/9 (0%)	0/9 (0%)

PTX (group ended a week after treatment initiation, as indicated above) groups via i.v. injection with the maximum tolerated dose of free PTX (15 mg kg^{-1} PTX-equivalent dose) three times a week every second week for four weeks. We quantified the growth rate of primary tumors from the ventral abdominal region over-time via bioluminescent intensity as the intra-abdominal location of the tumor precluded direct measurement of tumor volume. **Figure 7** demonstrates the bioluminescent signal in the vehicle control and *tert*-Ser-PTX treated-group over time. The BLI intensity measurements and direct images demonstrated that LNCaP.Fluc2 tumors treated with the *tert*-Ser-PTX conjugate at 15 mg PTX kg^{-1} did not significantly change in size compared to the vehicle control group, which displayed an exponential increase of bioluminescent signal within the prostate (Figure 7a). These differences were reflected when calculating bioluminescent absolute growth delay, relative tumor bioluminescence, and T/C ratio.

On day 43 (day 46 represents the endpoint of the study) of treatment, we found a delay in bioluminescent absolute growth delay of 21 days for the *tert*-Ser-PTX treated group, and relative tumor

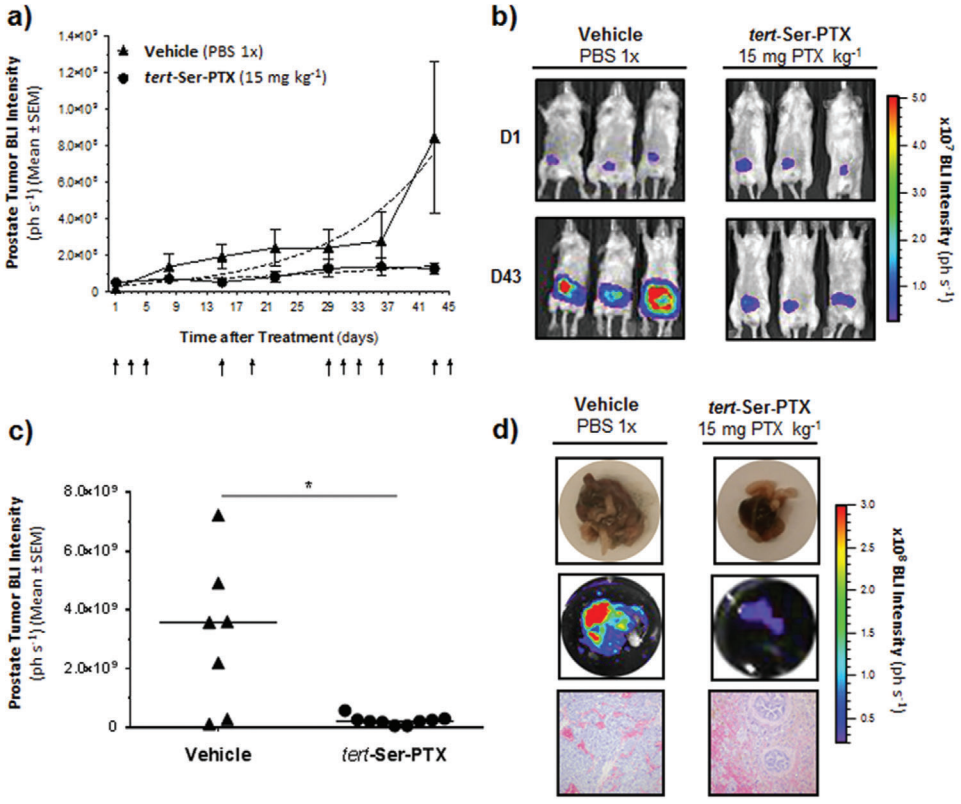


Figure 7. In vivo impact of *tert*-Ser-PTX treatment on orthotopic LNCaP.Fluc2 tumor growth. Comparative analysis of PCa growth longitudinally and at the endpoint following treatment with *tert*-Ser-PTX i.v. administered at 15 mg PTX kg^{-1} or vehicle as a control. a) Primary tumor growth rate from the ventral abdominal region was quantified weekly using bioluminescent intensity (ph s^{-1}). The dotted line indicates the non-linear regression fits of exponential tumor growth. Arrows on the X-axis indicate the administration schedule. b) Representative examples of mouse bioluminescence (ventral views) from *tert*-Ser-PTX and vehicle control groups are shown over the treatment timeline. c) Scatter dot plots of prostate tumor bioluminescence ex vivo at day 46 (study endpoint). d) *Ex vivo* comparisons of gross morphology, bioluminescence, and histopathology of LNCaP.Fluc2 tumors treated with vehicle or 15 mg kg^{-1} of *tert*-Ser-PTX. The mean or median BLI intensities and corresponding standard errors of the mean (SEM) were determined and plotted ($n \geq 7$). The significance threshold was established at $p < 0.05$, and significance levels were schematically assigned *($0.01 \leq p < 0.05$), **($0.001 \leq p < 0.01$), ***($0.0001 \leq p < 0.001$) or ****($p < 0.0001$).

bioluminescence values of 1821% and 481% for vehicle and *tert*-Ser-PTX, respectively, demonstrating the smaller nature of the tumors (and the lower levels of emitted bioluminescence) from *tert*-Ser-PTX treated mice. Finally, we found a T/C ratio of tumor bioluminescence of 26% for *tert*-Ser-PTX, indicating the robust inhibition of tumor growth in those animals treated with the *tert*-Ser-PTX.

At the endpoint of the experiment (day 46), ex vivo tumor bioluminescence and *ex vivo* tumor weight analyses further confirmed statistically significant tumor growth inhibition (Figure 7c—$p = 0.0164$ and Figure S2, Supporting Information, $p = 0.0464$, respectively). We also found T/C ratios for tumor weight and tumor bioluminescent growth of 17% and 8%, respectively, for the *tert*-Ser-PTX treated group.

Thus, *tert*-Ser-PTX at 15 mg PTX kg^{-1} administered at the above-noted schedule significantly inhibited tumor growth compared to the vehicle control group. Moreover, *ex vivo* analysis of prostates and histological images (Figure 7c) revealed that cancer cells occupied the whole prostatic gland in the vehicle control group, while *tert*-Ser-PTX treated mice displayed typical prostate structures and the maintenance of gland histology. Overall, these findings confirm the elevated antitumoral efficacy of PTX formulated as a polyacetal-based polymer therapeutic.

We also note that the obtained results improve on those found for the i.v. administration of a novel anti-microtubule agent (LG308),[41] an albumin-binding prodrug of PTX,[42] PTX microspheres,[43] and PEG-based PTX–polymers[44] in orthotopic PCa models.

3.7. Inhibition of Distant and Locoregional Metastasis by *tert*-Ser-PTX

The orthotopic implantation of tumor cells into the prostate provides a PCa model that includes all steps of metastatic progression that resembles the human clinical setting.[45] The LNCaP.Fluc2 orthotopic PCa model employed mimics the clinical pattern of spontaneous PCa metastases, including lymphatic, hematologic, and coelomic dissemination. At the experimental endpoint, ex vivo BLI and histopathological evaluation of excised tissues allowed the identification and localization of sites of spontaneous metastases (incidence rates) for *tert*-Ser-PTX and vehicle control-treated groups (see Table S4, Supporting Information). Significantly, *tert*-Ser-PTX reduced the incidence of metastases from orthotopic prostate LNCaP.Fluc2 tumors when administered at a concentration of 15 mg PTX kg^{-1} at the described schedule.

Furthermore, *tert*-Ser-PTX treatment reduced levels of distant hematological dissemination to lungs, locoregional lymphatic dissemination to perirenal, mesenteric, and peripancreatic lymph nodes, and coelomic dissemination to the diaphragm, as measured as incidence (Table S4, Supporting Information) or by BLI (**Figure 8**). We observed a statistically significant reduction ($p = 0.0143$) in distant hematological (lung metastases) dissemination and growth of LNCaP.Fluc2 PCa cells in *tert*-Ser-PTX polymers compared to vehicle control treated-group (Figure 8a, first two columns). We also found a T/C ratio of bioluminescent lung metastases growth of 8% for the *tert*-Ser-PTX treated group. Furthermore, ex vivo BLI and histopathological images of spontaneous orthotopic metastatic lesions from a representative mouse from each treatment schedule demonstrated reduced metastasis growth following *tert*-Ser-PTX treatment (Figure 8b).

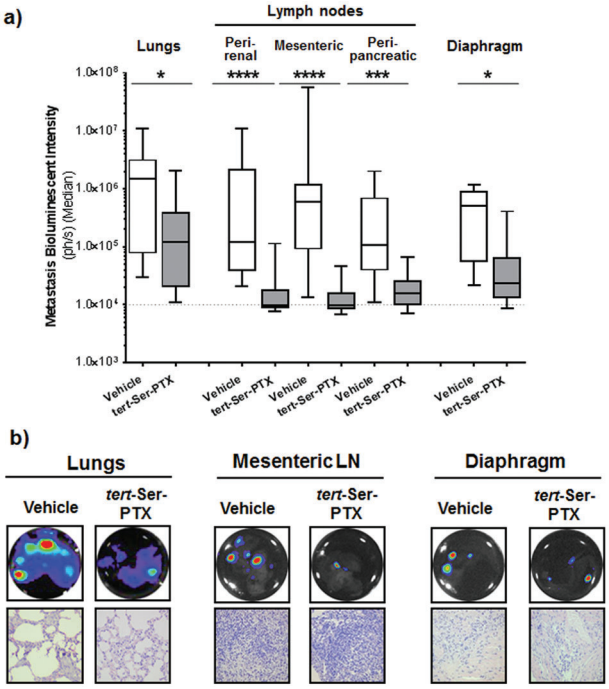


Figure 8. Effect of *tert*-Ser-PTX treatment on distant metastasis in orthotopic LNCaP.Fluc2 prostate tumor-bearing mice. At the study endpoint, ex vivo BLI of excised lungs, lymph nodes (perirenal, mesenteric, and peripancreatic) and diaphragm allowed the identification and localization of spontaneous hematologic (lung), lymphatic (lymph nodes), and coelomic (diaphragm) metastases. a) Ex vivo bioluminescent quantification of metastasis and b) comparisons of bioluminescence and histopathology of different tissues of LNCaP.Fluc2 bearing mouse treated with 15 mg kg^{-1} of *tert*-Ser-PTX and vehicle control. The significance threshold was established at $p < 0.05$, and significance levels were schematically assigned *($0.01 \leq p < 0.05$), **($0.001 \leq p < 0.01$), ***($0.0001 \leq p < 0.001$) or ****($p < 0.0001$).

As for locoregional metastasis to lymph nodes and diaphragm, ex vivo BLI analysis demonstrated a significant reduction in metastatic colonization of the lymph nodes ($p = 0.0004$ for peripancreatic lymph nodes and $p < 0.0001$ for perirenal and mesenteric lymph nodes) and the diaphragm ($p = 0.0001$), indicating that *tert*-Ser-PTX inhibits both lymphatic and coelomic dissemination (Figure 8a). Interestingly, we noted greater inhibition of lymphatic and coelomic metastasis than hematologic (lung) metastasis. The inhibition of dissemination and growth of lymphatic metastasis is highly relevant in PCa, as 14% of metastases locate in the locoregional lymph nodes.[46] Unfortunately, the orthotopic prostate model employed does not readily metastasize to the bone, which has an incidence of 65% in PCa,[46] and, therefore, we could not evaluate the impact of *tert*-Ser-PTX treatment on bone metastases.

We do note that we cannot exclude that the inhibition of primary tumor growth by *tert*-Ser-PTX indirectly limited the growth of the metastatic foci; however, studies have established that metastasis may develop in parallel with the development of the primary tumor, indicating early tumor cell dissemination.[47]

Therefore, significant inhibition in local and distant metastasis might suggest the direct impact of *tert*-Ser-PTX on metastatic foci. Nonetheless, the EPR effect may not occur in metastases as in the primary tumor due to the lack of the angiogenic switch and the lack of macromolecule accumulation at metastatic sites,[48] suggesting that EPR does not control *tert*-Ser-PTX efficacy on metastatic lesions.

With the EPR effect excluded, we hypothesize that the direct impact of *tert*-Ser-PTX could be driven by the inhibitory effect of PTX on the vascular system[14–16] and/or the potential effectiveness of drug delivery systems on cancer stem cells (CSCs).[48] CSCs may be responsible for metastasis formation,[50] and some drug delivery systems appear to overcome the inherent chemoresistance of CSCs.[49,51] This fact could justify the significant reduction in metastasis incidence and growth in *tert*-Ser-PTX treated mice. Moreover, microtubule-binding drugs such as PTX directly affect endothelial cells, and neoangiogenic tumor vessels by extension, at low PTX concentrations. Therefore, the slow release of PTX from *tert*-Ser-PTX polymers may increase anti-angiogenic activity.[15,16] Indeed, the delivery of PTX in endothelially-targeted nanosystems can increase PTX efficacy.[52]

Regardless of the precise mechanism of action, we highlight the effectiveness of *tert*-Ser-PTX in reducing distant and locoregional metastasis and inhibiting the growth of primary orthotopic LNCaP.Fluc2 prostate tumors. Overall, our results demonstrate the potential of *tert*-Ser-PTX in the treatment of PCa.

4. Conclusions

In this study, we report the development of a potentially effective PCa treatment via the conjugation of a known chemotherapeutic agent Paclitaxel (PTX) to a biodegradable polyacetal polymer. Polymer conjugation increases stability in circulation, inhibits off-target drug effects, and promotes tumor accumulation via the EPR effect. Once internalized within PCa cells, the alteration in pH mediates ester bond cleavage and PTX release together with polyacetal mainchain degradation. In vivo toxicity and efficacy profiles of *tert*-Ser-PTX suggest robust anti-tumor improvements compared to treatment with the standard formulations of PTX. Contrary to free PTX, we found that *tert*-Ser-PTX was well tolerated after 11 doses at 15 mg PTX kg^{-1} with no adverse effects observed during the treatment period. Significantly, *tert*-Ser-PTX inhibited orthotopic prostate LNCaP tumor growth and significantly reduced metastatic incidence. Overall, this study highlights the potential of polyacetal conjugation as an improved strategy for the delivery of chemotherapeutic drugs.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords

antitumor efficacy, nanomedicines, paclitaxel, pH-responsiveness, polyacetals, polymer–drug conjugates, prostate cancer

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Appendix 2

Industrial impact of PACA technology: Statements from NaDeNo, Biosergen, Algipharma

To whom it may concern

Support of the impact cases for the evaluation of SINTEF Dept. Biotechnology and Nanomedicine (2011-2021)

NaDeNo Nanoscience AS

NaDeNo is a SINTEF spin-off building on more than a decade of research at Dept. Biotechnology and Nanomedicine on a nanotechnology drug delivery platform designed for unleashing the potential of hard-to-deliver hydrophobic small molecule drugs. The decision to start NaDeNo was taken in 2021, and the company was established June 2022. NaDeNo aims at bringing new proprietary nanomedicines to patients and at offering its platform technology to pharma and biotech companies in need of overcoming drug delivery hurdles of promising drugs.

The lead candidate is a proprietary nanoformulation of cabazitaxel encapsulated in polymeric nanoparticles, supported by strong preclinical safety and efficacy data generated in collaboration with the Norwegian Radium Hospital, and is intended for local treatment of cancer metastasis in the peritoneum. The lead candidate is a result of extensive research on poly(alkylcyanoacrylate) nanoparticles at SINTEF through numerous national and international research projects with academia and industry. NaDeNo's technology is thus a mature technology where the whole discovery phase has been completed at SINTEF. NaDeNo is currently ready to enter the formal preclinical testing phase on its lead drug candidate and was in August 2022 accepted to enter a full preclinical testing program offered free of charge by the National Cancer Institute (NCI) in the US. The acceptance was based on the extensive physical/chemical, *in vitro* and *in vivo* data generated at SINTEF.

The target patient group for NaDeNo's lead candidate in Europe and US constitutes about 37 000 patients annually. These are patients where the metastasis originates from colorectal and ovarian cancer who are eligible for surgery and have the peritoneum as the sole site of metastasis. The NaDeNo patents are however broader, and long term also metastasis originating from other cancers such as gastric and appendiceal cancer can be targeted. The identified competitive landscape within this segment is overall modest, and there is currently no standardized treatment for these patients.

Future key milestones will encompass tech transfer and scale up of the manufacturing process to a GMP facility (2023) and completion of the formal preclinical phase (2025). SINTEF is a key secured partner to reach these milestones and is in direct dialogue both with NCI and NaDeNo's CDMO partner.

It is a common strategy for biopharmaceutical companies to develop candidates through phase II and then seek partnerships, acquisition, or IPO. This is also NaDeNo's strategy, and the main driver will be the development and progress of the lead-candidate PACAB-002. Additional value inflection points will be generated based on pipeline candidates and platform development. NaDeNo very recently received funding from RCN for pipeline development with SINTEF as key research partner.

NaDeNo and SINTEF have entered into a collaboration agreement defining SINTEF as NaDeNo's key R&D partner. NaDeNo's current team is Annbjørg Eide Falck, CEO and co-founder and Yrr Mørch, CTO and co-founder, formerly employed at SINTEF as one of the lead scientists on the poly(alkyl cyanoacrylate) nanoparticle technology. NaDeNo does not hold own laboratories or technical staff, and thus all experimental work related to nanoparticle design and pipeline development, as well as key physical/chemical, in vitro and in vivo characterization on NaDeNo's products is carried out at SINTEF. Yrr Mørch is in daily dialogue with SINTEF staff who in tight collaboration design the

experimental work. To this date no other institutions can match the deep knowledge on NaDeNo's technology, and the related infrastructure needed to advance on the technology.

Further, NaDeNo and SINTEF have entered into a technology licence agreement. Though this agreement NaDeNo has an exclusive license with a purchase option for four patents covering the core business of the company. In addition, NaDeNo will have exclusive license to all new discoveries related to the company's core technology.

NaDeNo has recently completed its first round of investments. **The technology licence agreement together with the collaboration agreement with SINTEF have been key elements in attracting external investors.**

Trondheim, 2023-01-30

_____

Ýrr Mørch
CTO
NadDeNo

Biosergen

22 January 2023.

Biosergen AS and Sintef AS (Department of Biotechnology and nanomedicine) R&D co-operation on BSG005 – a new antifungal drug for use in Invasive Fungal Infections (IFI).

Impact of Sintef work on Biosergen's development.

The co-operation between Biosergen and Sintef goes many years back. The company was originally started as a spin off by SINTEF TTO, based on technology established over years of basic research by SINTEF and Norwegian University of Science and Technology (NTNU).

During the last 4 – 5 years and until end of 2021 Sintef has been heavily involved in the development of BSG005. We have basically seen the Sintef lab as "Biosergen's lab". The co-operation for our "standard" BSG005 has mainly run in two ways. Either laboratory developments were done initially at Sintef on processes of manufacturing BSG005 or formulation and then moved out to our manufacturing partner for upscaling and GMP manufacture OR manufactured material or test results from other labs have been checked at Sintef to confirm data from external suppliers. Sintef has also performed improvements on the processes, participated in large upscaling for instance on the first step – the fermentation - at our supplier in Spain, where all the special knowledge at Sintef played a crucial role in achieving the right processes.

Sintef labs have built up an intimate understanding of the BSG005 molecule, have done important test on adherence to plastic surfaces of the infusion bags and lines – something very important before we initiated our "First in Man" clinical trial – so we could compensate in the important pharmacokinetic investigation in humans. The Sintef lab has performed many other important tests on BSG005 that has improved Biosergen's understanding of how to handle the final product, when going into the clinical phase – what external factors were important for drug stability as the BSG005 molecule in very sensitive to outside factors such as light, temperature, oxygen and pH.

For Biosergen the very close contact and co-operation with Sintef has been vital – and still is – for the development of the first generation BSG005.

In 2019 Biosergen approached Sintef with an idea of making BSG005 available as a "NANO-particle formulated version" with the aim to develop a version to specifically target the lung (typically the first organ attached in an IFI). There were several very important feedbacks from the Nano-group at Sintef. They had recently developed new Nanoparticles called PACA and they had a patented invention, that could make the idea of a lung targeted formulation possible much faster than anticipated.

For Biosergen that really was "a lucky punch", as we could see that the knowhow in the Nano-group was more, than we had hoped for. Together with the Sintef Nano-group the project has been moving on testing many types of Nanoparticles, the loading of BSG005 into the particles. The sensitivity of BSG005 created some issues and the testing of alternative Nanoparticles moved on through 2021.

For Biosergen it has also for the Nano development been very important that both the "standard BSG005 development" as well as the Nano-development are in the same overall Sintef-group (Department of Biotechnology and nanomedicine) and that knowhow and test data are coming from same lab with the same technicians, so all information is shared within the full group.

And we expect to get a breakthrough in 2023, which may have significant impact on Biosergen as a company.

Peder M Andersen, MD

CEO

AlgiPharma

27th Jan 2023

To whom it may concern,

I am writing on behalf of AlgiPharma in support of the impact cases (evaluation period 2011-2021) presented by the SINTEF Industry, Department of Biotechnology and nanomedicine.

AlgiPharma is a privately owned clinical stage biopharmaceutical company with a focus on developing its proprietary alginate technology for: Delivery platforms for nanoparticles, small and large molecule-based drugs; Drug conjugates, adding functionality and reducing toxicity of parent compound; and as a stand-alone active pharmaceutical ingredient in disease areas such as cystic fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD), and infectious diseases. AlgiPharma has pharmaceutical scale production capabilities for its technology and is protected by a broad family of patents. AlgiPharma's aim is to address unmet medical needs and fight diseases effectively through our innovative alginate technologies targeting those diseases where there is a clinical need to mitigate abnormal mucus accumulation, microbial infection, biofilm formation and antibiotic resistance. AlgiPharma has worked closely with the Biotechnology and Nanomedicine research teams at SINTEF Industry since 2006.

Through the coordination, expertise and leadership provided by Håvard Sletta at SINTEF Industry, their multi-disciplinary research teams have been a major contributor to AlgiPharma's ongoing success in the development of its alginate oligosaccharide technology. The broad range and depth of expertise at SINTEF Industry has been and continues to be a crucial resource in AlgiPharma's drug development and manufacturing programs, including microbial biotechnology, alginate polymer production and nanotechnology. This long-standing collaboration has been central in securing non-dilutive research funding (more than 85 MNOK) for AlgiPharma's research activities including the following Norwegian Research Council co-funded Innovation projects for industry (IPN) grants awarded during the 2011-2021 impact case evaluation period: BIA grant: Tailored OligoG (228542); BIA grant: OligoG Formulate (245598); NANO2021 grant: MucosALG (281920).

SINTEF Industry has played a key supporting role in the evaluation of AlgiPharma's primary drug candidate in four phase 2 clinical trials (NCT01465529; NCT03822455; NCT02157922; NCT02453789) evaluating a new drug treatment for Cystic Fibrosis (CF) which is a life-threatening lung disease. SINTEF Industry's research expertise have allowed us to gain a better understanding of the complex mechanisms of action and therapeutic potential of AlgiPharma's alginate oligomer technology for the treatment of respiratory, wound healing, drug delivery and infectious diseases. The teams at SINTEF have also played a key role in realizing commercial value for AlgiPharma, in both knowledge and intellectual property.

SINTEF has been an enabling force in AlgiPharma gaining investment for further research and commercial scale-up of OligoG production processes from the Norwegian Research Council and the UK Technology Strategy Board. SINTEF has played a central role in AlgiPharma's manufacturing program, securing significant non-dilutive funding in support of the development of commercially relevant manufacturing processes: Innovate UK: Algiferm (131142); ALGIPRO (102148); Microbialg (228570); Oligo-DSP (281907); Algi-SCALEUP (317799). AlgiPharma is also a partner in the SINTEF led national Centre of excellence, SFI-Industrial biotechnology.

If you have any further questions, please do not hesitate to contact me.

Yours sincerely,

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Appendix 3

European Commission's Innovation Radar pointing out two innovations in REFINE and one in B-SMART.

From: CNECT-INNOVATION-RADAR@ec.europa.eu <CNECT-INNOVATION-RADAR@ec.europa.eu>
Sent: fredag 19. juni 2020 14:46
To: Alexandra Bech Gjørv <alexandra.bech.gjorv@sintef.no>; Anu Schei <anu.schei@sintef.no>; Berit Broli <Berit.Broli@sintef.no>; Ruth Schmid <Ruth.B.Schmid@sintef.no>; Sven Even Borgos <SvenEven.Borgos@sintef.no>
Subject: Innovation Radar - highlighting excellent innovations

Dear EU-funded innovator(s),

We are writing to you as you are an official contact person for your organisation's participation in the **REFINE** project funded by the European Commission under Horizon 2020.

We are delighted to inform you that one of the innovations developed in the project has been analysed by the European Commission's Innovation Radar. Details of this innovation, and how it was categorised by the analysis, are as follows:

- **Innovation Title:** New methods to study/predict the physiological distribution and/or biological responses to Nano(bio)materials;

- **Market Maturity of the Innovation:** 'Exploring' (based on a method described in this paper);
- **Market Creation Potential of the innovation:** High.

In this analysis Innovation Radar also identified the following project partner(s) - including your organisation - in the project as a 'Key Innovator' in the development of this innovation:

- GESELLSCHAFT FUR BIOANALYTIK MUNSTER EV
- SINTEF AS
- THE UNIVERSITY OF LIVERPOOL

In the near future we will publish the above information about this innovation on the European Commission's Innovation Radar platform (accessed via a website or a smartphone app - iOS or Android). The name(s) of the beneficiary organisation(s) in the project identified by Innovation Radar as a 'key innovator' for this innovation will also be published. Details of the EU-funded project the innovation was developed within will also appear on the platform (project acronym, project title, project description and project end-date).

The information will be accessible to the public via this platform from 20 July 2020, joining the 3600+ EU-funded innovations already showcased on the platform. If your organisation is already featured in the Innovation Radar, the innovation listed above will be added to the EU-funded innovations of your organisation that are already accessible on the platform.

If, as a key innovator, you consider the above information about this innovation to be confidential - or its publication risks compromising certain legitimate interests - you can, within 30 days of receiving this email, request that the information is not made public. Such requests, with explanations, should be submitted to the Commission by **COMMISSARIAT A L ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES**, who is the Coordinator of Project REFINE. This should be done by the Coordinator via the 'Grant management Services' part of the 'Funding & Tender Opportunities' portal (the request should be sent as a 'new message' under the 'Communication Centre').

The Innovation Radar platform builds on the information and data gathered by independent experts involved in reviewing ongoing research and innovation projects funded by the European Commission. These experts also provided an independent view regarding the innovations in the projects and their market potential (more information about the Innovation Radar methodology is available here).

The aim is to make information about EU-funded innovations from high-quality projects visible and accessible to the public via the EU's Innovation Radar platform. This will show citizens the many excellent technological and scientific advances being delivered by researchers and innovators around Europe, funded on their behalf by the European Commission. This initiative has the support of EU Members States and, to date, Ministers from 23 countries have signed the Innovation Radar declaration confirming their support for this initiative.

We believe that your organisation's inclusion in this initiative could open up new opportunities for you to partner with business or academic organisations and trigger interest from potential customers or investors in your innovations. Above all it will demonstrate to a global audience the innovative work your organisation is active in delivering.

From the perspective of the protection of personal data, please note that your personal data is processed by the Commission in compliance with Regulation 2018/1725. According to the Regulation, you are entitled to access your personal data, to rectify and/or block it in case the data is inaccurate, to erase data concerning you (the 'right to be forgotten'), or to restrict processing of your data. You can exercise your rights by contacting the data Controller, or in case of conflict the European Commission Data Protection Officer, and if necessary the European Data Protection Supervisor by using the contact information: edps@edps.europa.eu.

Please share this information with other relevant people in your organisation.

Wishing you continued success for your research and innovation activities.

The European Commission's Innovation Radar team

From: CNECT-INNOVATION-RADAR@ec.europa.eu <CNECT-INNOVATION-RADAR@ec.europa.eu>
Sent: mandag 1. august 2022 10:31
To: Ruth Schmid <Ruth.B.Schmid@sintef.no>
Subject: Innovation - Highlighting excellent innovations

Dear EU-funded innovator(s),

We are writing to you as you are an official contact person for your organisation's participation in the **REFINE** project funded by the European Commission under Horizon 2020.

We are delighted to inform you that one of the innovations developed in the project has been analysed by the European Commission's Innovation Radar. Details of this innovation, and how it was categorised by the analysis, are as follows:

- **Innovation Title:** Decision Support System (DSS) software tool for the selection of medical products and medical devices based on nanomedicines and biomaterials;
- **Market Maturity of the Innovation:** Exploring (more details on this categorisation are provided here);
- **Market Creation Potential of the innovation:** Addresses needs of existing markets (more details on this categorisation are provided here);

In this analysis Innovation Radar also identified the following project partner(s) - including your organisation - in the project as a 'Key Innovator' in the development of this innovation:

- GREENDECISION SRL
- SINTEF AS

In the near future we will publish the above information about this innovation on the European Commission's Innovation Radar platform (accessed via a website or a smartphone app - iOS or Android). The name(s) of the beneficiary organisation(s) in the project identified by Innovation Radar as a 'key innovator' for this innovation will

also be published. Details of the EU-funded project the innovation was developed within will also appear on the platform (project acronym, project title, project description and project end-date). However, please note that no detailed description of the innovation or project deliverables will be published on the Innovation Radar platform.

The information will be accessible to the public via this platform between 30 and 45 days from the date that this email was sent, joining the 7600+ EU-funded innovations already showcased on the platform. If your organisation is already featured in the Innovation Radar, the innovation listed above will be added to the EU-funded innovations of your organisation that are already accessible on the platform.

If, as a key innovator, you consider the above information about this innovation to be confidential - or its publication risks compromising certain legitimate interests - you can, within 30 days of receiving this email, request that the information is not made public. Such requests, with explanations, should be submitted to the Commission by **GESELLSCHAFT FUR BIOANALYTIK MUNSTER EV**, who is the Coordinator of Project REFINE. This should be done by the Coordinator via the 'Grant management Services' part of the 'Funding and Tender Opportunities' portal (the request should be sent as a 'new message' under the 'Communication Centre').

We take this opportunity to highlight two opportunities for SINTEF AS:

- Innovator organisations featured on the Innovation Radar platform can now ‘claim their page’ and upload additional information about their organisation and innovations. We encourage all innovators to take advantage of this feature of the platform. Further information on the steps to ‘claim a page’ are provided here: https://www.innoradar.eu/innovatorslogin.
- Innovator organisations with the ambition of bringing their EU-funded innovation to the market can now apply for "go to market" training and support from Dealflow.eu, the support action of Innovation Radar financed by Horizon Europe. Applications for Dealflow.eu support need to be submitted via this page: https://dealflow.eu/registration.

The Innovation Radar platform builds on the information and data gathered by independent experts involved in reviewing ongoing research and innovation projects funded by the European Commission. These experts also provide an independent view regarding the innovations in the projects and their market potential (more information about the Innovation Radar methodology is available here).

The aim is to make information about EU-funded innovations from high-quality projects visible and accessible to the public via the EU's Innovation Radar platform. This will show citizens the many excellent technological and scientific advances being delivered by researchers and innovators around Europe, funded on their behalf by the European Commission. This initiative has the support of EU Members States and, to date, Ministers from 23 countries have signed the Innovation Radar declaration confirming their support for this initiative.

We believe that your organisation's inclusion in this initiative could open up new opportunities for you to partner with business or academic organisations and trigger interest from potential customers or investors in your innovations. Above all it will demonstrate to a global audience the innovative work your organisation is active in delivering.

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Please share this information with other relevant people in your organisation.

Wishing you continued success for your research and innovation activities.

The European Commission's Innovation Radar team

Discover what's new on Innovation Radar

From: CNECT-INNOVATION-RADAR@ec.europa.eu <CNECT-INNOVATION-RADAR@ec.europa.eu>
Sent: mandag 16. januar 2023 10:00
To: Ruth Schmid <Ruth.B.Schmid@sintef.no>
Subject: Innovation Radar - Highlighting excellent innovations

Dear EU-funded innovator(s),

We are writing to you as you are an official contact person for your organisation's participation in the **B-SMART** project funded by the European Commission under Horizon 2020.

We are delighted to inform you that one of the innovations developed in the project has been analysed by the European Commission's Innovation Radar. Details of this innovation, and how it was categorised by the analysis, are as follows:

- **Innovation Title:** New methods for Mass Spectroscopy of nanobodies and LNP carriers;
- **Market Maturity of the Innovation:** Exploring (more details on this categorisation are provided here);
- **Market Creation Potential of the innovation:** Addresses needs of existing markets (more details on this categorisation are provided here);

In this analysis Innovation Radar also identified the following project partner(s) - including your organisation - in the project as a 'Key Innovator' in the development of this innovation:

- MALVERN PANALYTICAL LIMITED
- SINTEF AS
- UNIVERSITAIR MEDISCH CENTRUM UTRECHT

In the near future we will publish the above information about this innovation on the European Commission's Innovation Radar platform (accessed via a website or a smartphone app - iOS or Android). The name(s) of the beneficiary organisation(s) in the project identified by Innovation Radar as a 'key innovator' for this innovation will

also be published. Details of the EU-funded project the innovation was developed within will also appear on the platform (project acronym, project title, project description and project end-date). However, please note that no detailed description of the innovation or project deliverables will be published on the Innovation Radar platform.

The information will be accessible to the public via this platform between 30 and 45 days from the date that this email was sent, joining the 9000+ EU-funded innovations already showcased on the platform. If your organisation is already featured in the Innovation Radar, the innovation listed above will be added to the EU-funded innovations of your organisation that are already accessible on the platform.

If, as a key innovator, you consider the above information about this innovation to be confidential - or its publication risks compromising certain legitimate interests - you can, within 30 days of receiving this email, request that the information is not made public. Such requests, with explanations, should be submitted to the Commission by **UNIVERSITAIR MEDISCH CENTRUM UTRECHT**, who is the Coordinator of Project B-SMART. This should be done by the Coordinator via the 'Grant management Services' part of the 'Funding and Tender Opportunities' portal (the request should be sent as a 'new message' under the 'Communication Centre').

We take this opportunity to highlight three opportunities for SINTEF AS:

- Innovator organisations featured on the Innovation Radar platform can now ‘claim their page’ and upload additional information about their organisation and innovations. We encourage all innovators to take advantage of this feature of the platform. Further information on the steps to ‘claim a page’ are provided here: https://www.innoradar.eu/innovatorslogin.
- Innovator organisations with the ambition of bringing their EU-funded innovation to the market can now apply for "go to market" training and support from Dealflow.eu, the support action of Innovation Radar financed by Horizon Europe. Applications for Dealflow.eu support need to be submitted via this page: https://dealflow.eu/registration.
- Innovator organisations can apply for Horizon Results Booster services provided to Horizon Europe and H2020 projects at no cost. The services on offer are: “Portfolio Dissemination and Exploitation Strategy”, “Business Plan Development” and “Go to Market” support. Applications can be sent by anyone in an eligible project by filling in the application form available at the following link: https://www.horizonresultsbooster.eu/.

The Innovation Radar platform builds on the information and data gathered by independent experts involved in reviewing ongoing research and innovation projects funded by the European Commission. These experts also provide an independent view regarding the innovations in the projects and their market potential (more information about the Innovation Radar methodology is available here).

The aim is to make information about EU-funded innovations from high-quality projects visible and accessible to the public via the EU's Innovation Radar platform. This will show citizens the many excellent technological and scientific advances being delivered by researchers and innovators around Europe, funded on their behalf by the European Commission. This initiative has the support of EU Members States and, to date, Ministers from 23 countries have signed the Innovation Radar declaration confirming their support for this initiative.

We believe that your organisation's inclusion in this initiative could open up new opportunities for you to partner with business or academic organisations and trigger interest from potential customers or investors in your innovations. Above all it will demonstrate to a global audience the innovative work your organisation is active in delivering.

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Commission in compliance with Regulation 2018/1725. According to the Regulation, you are entitled to access your personal data, to rectify and/or block it in case the data is inaccurate, to erase data concerning you (the 'right to be forgotten'), or to restrict processing of your data. You can exercise your rights by contacting the data Controller, or in case of conflict the European Commission Data Protection Officer, and if necessary the European Data Protection Supervisor by using the contact information: edps@edps.europa.eu.

Please share this information with other relevant people in your organisation.

Wishing you continued success for your research and innovation activities.

The European Commission's Innovation Radar team

SINTEF AS_BTN, case number 2 (CONFIDENTIAL)

Institution: SINTEF AS
Administrative unit: Biotechnology and Nanomedicine (BTN)
Title of case study: Biopolymers: From basic research to innovation in medicine, animal breeding, and marine industries
Period when the underpinning research was undertaken: 2010-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact

BTN has over the last decade had a large research activity on marine biopolymers, particularly alginates from brown algae and bacteria, which has had a large impact on innovation and value creation for industries working on biopolymer production and application development. The research has been conducted in close collaboration with NTNU among others and involves microbial production systems and down-stream processing of biopolymers and biopolymer-modifying enzymes, biomass processing, chemoenzymatic modification, structural characterization, and application testing of biopolymers. Key innovation impacts are development and pre-clinical/clinical testing of pharmaceuticals (AlgiPharma, MicroA), commercialized technology for cell delivery (SpermVital), and the growth of a national seaweed industry (Seaweed Solutions, Dupont Nutrition Norge).

2. Underpinning research

Underpinning research on biopolymers at BTN has been crucial for the growth and commercial activities of our industry partners. Due to their confidential nature, not all results developed in collaboration with industry are published, but the activities are described herein with selected published works, and in the statements from our collaborators (Appendix 1). The activities on biopolymers in BTN builds on over 60 years of research at NTNU on alginate extracted from seaweed. Alginates are linear polysaccharides (i.e. biopolymers) consisting of mannuronic acid (M) and guluronic acid (G) that form hydrogels in the presence of divalent cations and are used in hundreds of industrial applications. BTN's involvement in alginate and biopolymer research started in the 1990s with expression, production, and characterization of alginate-modifying enzymes. BTN's biopolymer research has grown substantially within the evaluation period to include additional biopolymers, microbial production processes, downstream processing, chemical and enzymatic modification, structural characterization, bioactivity testing, and product/application development. Here, cross-disciplinary international collaboration and state-of-the-art infrastructure platforms for screening (enzyme discovery, bioactivity), structural characterization (mass spectrometry) and microbial production processes have been important enabling factors.

A) Biopolymer engineering and production

Production processes for microbial alginates were established and extensively studied at BTN in the 2000s, whereas a large breakthrough was made in the recent decade with the increased understanding of alginate-modifying enzymes. The work of Tøndervik, A and Sletta, H in 2013-2021 has led to the synthesis and characterization of new alginate epimerases used for modification of alginates. It has shown that by using native or engineered epimerases, the structural properties of alginate can be tailored in a controlled manner and towards specific applications, e.g. the G-content of algal alginates can be increased to become suited for gelation purposes. Further, continuous basic and mechanistic studies of the epimerases have increased the understanding needed to develop the enzymes for commercial applications [1]. Combined with extensive research on microbial cell factories by Sletta, H., Maleki, S. and others (see also BTN impact case Microbial Biotechnology), this allowed development of industrial processes for microbial production of alginates toward pharmaceutical applications [2]. This included microbial production of poly-M alginate, a unique polysaccharide not found in nature. Industry-driven projects such as Microbialg with Dupont and AlgiPharma (2013-2017 RCN IPN 228570) and the recently started Algi-SCALEUP with AlgiPharma (2020-2025 RCN IPN 317799) have been pivotal in the establishment of these processes and developing unique and world-leading competence on microbial alginate production.

Research on alginate lyases in 2010-2012 also led to improved methods for characterization of algal alginates and a greater understanding of their structure-function relationships [3], which later has been translated to enzymatic engineering and tailoring of alginates with specific monosaccharide sequence patterns.

B) Pharmaceutical technology development
Controlled enzymatic and chemical depolymerization of alginates further led to the identification of G-rich oligomers (OligoG) which have been studied by BTN for use in biofilm disruption and antibiotic potentiation in e.g. treatment of respiratory disease. Main published works in 2012 and 2014 by Tøndervik A. and co-workers showed that OligoG can enhance the effectivity of antimicrobial agents towards multidrug resistance bacteria [4] and fungi [5] and provided important pre-clinical data for AlgiPharma (see details of impact below) through key projects Tailored OligoG (2013-2017 RCN IPN 228542), OligoG Formulate (2015-2018 RCN IPN 245598) and Mucosalg (2018-2023 RCN IPN 281920).
BTN also published extensive research by Arlov, Ø. and co-workers between 2017-2021 on chemically sulfated alginates. Key results from this work were methods for synthesis, characterization of structural properties, and demonstration of anti-inflammatory properties and cell stimulation in biomaterials, in collaboration with ETH Zürich. BTN's main contribution was the structural design and characterization of these biopolymers and associated biomaterials, and evaluation in immunological assays. The leading position of BTN and ETHZ was emphasized with an invited review for *Adv. Funct. Mater.* on engineered sulfated polymers for biomedical applications in 2021, which is the most comprehensive work on the subject to date [6]. The research highlighted the large application potential for sulfated polysaccharides and has had impact for associated industry partners such as MicroA described below.

C) Biopolymers as immobilization materials for animal breeding
Applied research on alginate hydrogels has also had a commercial impact for the cell immobilization industry, exemplified by BTN's collaboration with SpermVital AS. Immobilization of bovine semen in alginate promotes a slow release after insemination and increased fertilization rates. Here, extensive research on the effect of alginate structure and gel formulation has been critical for the success of the applications in multiple industry-driven projects from 2012 and later (RCN IPN 282025, 244408, 269144, 317871, 207799). Results published by Klinkenberg, G and co-workers in 2015 and 2018 showed that immobilized sperm maintain function and motility after cryopreservation [7]. These results have been important demonstrators for the competitiveness and wide applicability of the technology, and are directly linked to growth in value creation for the company within the evaluation period (see details in point 4 below)

D) Seaweed biorefineries
The biopolymer research at BTN is tied to the Norwegian alginate industry, which has been based on wild harvested biomass. Here, BTN has collaborated with Dupont, in the ERA-MBT Mar3Bio project (2016-2021 RCN 264065), ERA-BlueBio SNAP project (2020-2023 RCN 311958), and the ongoing Center of research-based innovation (SFI) Industrial Biotechnology (2020-2028 RCN 309558) to develop existing and novel business areas for Dupont. The Mar3Bio project coordinated by BTN focused on biorefinery of brown algal and crustacean biomass to produce biopolymers and resulted in more than 30 scientific publications and formed the basis for subsequent research projects on seaweed biorefineries. In the recent decade, there has been an increased research activity and output coinciding with the establishment of seaweed cultivation in Norway. Research at BTN by Aasen, I.M. and coworkers in 2014-2021 initially focused on using the biomass as a protein source or as a feedstock for fermentative processes [8]. and has in recent years expanded to include preservation and biorefinery of the biomass, and development of alginate-based applications in collaboration with industry (publications from 2022 and later). Here, the Norwegian Seaweed Biorefinery Platform (2019-2024 RCN HAVBRUK 294946) has been and is a highly important project, serving as a national knowledge base with the most central research units in the field and a catalyst for many successive projects in collaboration with industry and international research units. The seaweed research at BTN involving industry partners had a collected project volume of 6 MNOK in 2021.

Persons, period of work in the evaluation period, Position held in 2021, [Contribution to research in A) Biopolymer engineering and production, B) Pharmaceutical technology

development, C) Biopolymers as immobilization materials for animal breeding, D) Seaweed biorefineries]

- Trond Erling Ellingsen, 2011-2021: Research director [A, B, C, D]
- Håvard Sletta, 2011-2021: Research Manager [A, B, C, D]
- Anne Tøndervik, 2011-2021: Senior Researcher [A, B]
- Susan Maleki, 2015-2021: Researcher [A, B]
- Geir Klinkenberg, 2011-2021: Research Manager [A, B, C]
- Inga Marie Aasen, 2011-2021: Senior Researcher [D]
- Øystein Arlov, 2015-2021: Senior Researcher [B, D]

3. References to the research

A) Biopolymer engineering and production

1. Tøndervik, A., Klinkenberg, G., Aachmann, F.L., Svanem, B.I.G., Ertesvåg, H., Ellingsen, T., Valla, S., Skjåk-Bræk, G., Sletta, H. (2013) Mannuronan C-5 epimerases suited for tailoring of specific alginate structures obtained by high-throughput screening of an epimerase mutant library. Biomacromolecules 14(8), https://doi.org/10.1021/bm4005194 (attached in Appendix 2)

2. Maleki, S., Almaas, E., Zotchev, S., Valla, S., Ertesvåg, H (2016) Alginate biosynthesis factories in Pseudomonas fluorescens: localization and correlation with alginate production level. App. Env. Microbiol. 82(4), https://doi.org/10.1128/AEM.03114-15,

3. Aarstad, O., Tøndervik, A., Sletta, H., Skjåk-Bræk, G. (2012) Alginate Sequencing: An Analysis of Block Distribution in Alginates Using Specific Alginate Degrading Enzymes. Biomacromolecules 13(1), https://doi.org/10.1021/bm2013026

B) Pharmaceutical technology development

4. Khan, S., Tøndervik, A., Sletta, H., Klinkenberg, G., et al (2012) Overcoming Drug Resistance with Alginate Oligosaccharides Able To Potentiate the Action of Selected Antibiotics. Antimicrob Agents Chemother 56(10) doi: https://doi.org/10.1128/AAC.00525-12

5. Tøndervik, A., Sletta, H., Klinkenberg, G. et al (2014) Alginate Oligosaccharides Inhibit Fungal Cell Growth and Potentiate the Activity of Antifungals against Candida and Aspergillus spp PLoS One 19;9(11) doi: https://doi.org/10.1371/journal.pone.0112518

6. Arlov Ø, Rütsche D, Korayem MA, Öztürk E, Zenobi-Wong M (2021) Engineered sulfated polysaccharides for biomedical applications. Adv. Funct. Mater. 31: 2010732 https://doi.org/10.1002/adfm.202010732

C) Biopolymers as immobilization materials for animal breeding

7. Standerholen, F.B., Waterhouse, K.E., Larsgard, A.G., Garmo, R.T., Myromslien, F.D., Sunde, J., Ropstad, E., Klinkenberg, G., Kommisrud, E. (2015) Use of immobilized cryopreserved bovine semen in a blind artificial insemination trial. Theriogenology 84(3) https://doi.org/10.1016/j.theriogenology.2015.03.028

D) Seaweed biorefineries

8. Sandbakken, I., Sæther, M., Funderud, J., Aasen, I.M. (2018) Acid preservation of Saccharina latissima for application as a carbon source for fermentation to biofuels and chemicals. Journal of Applied Phycology 30, https://doi.org/10.1007/s10811-018-1489-z

4. Details of the impact

The biopolymer research at BTN has over the recent decade had a significant impact on the growth of the research unit's collaborating partners. This is specifically linked to value creation for the industry partners, in the form of contribution to funding acquisition from public and private sources, development of new processes and technology used by the industry partners, generation of knowledge to improve existing processes/products, and evaluation of new applications through proof-of-principle studies. This impact is detailed below for four selected industry partners who have been central in the development of the biopolymer research field at BTN. The research has also had a substantial impact on BTN's main research partner NTNU, Department of Biotechnology and Food

Science (IBT), through increase and diversification of its research portfolio, education of MSc and PhD students, increased public communication, and increased employment.

NTNU collaboration
Many of the activities and the impacts listed below have been developed in close collaboration between BTN and NTNU. Together, the research units have educated candidates with relevant competence for industry, communities, and research organizations through the synergies of basic and applied research. Many previous NTNU-IBT candidates are currently employed by SINTEF, in particular with PhD competence in molecular biology or biopolymer/biomaterials, while the collaborative projects have also contributed to funding and increased employment at NTNU. BTN has participated in more than 62 research projects with NTNU between 2011-2021, corresponding to a combined research funding of >600 MNOK and leading to co-publication with 30 PhD students. Approximately 160 MNOK of this has been for biopolymer research, with a near equal distribution of funds between NTNU and BTN. Together, we represent world-leading competence on marine biopolymers, biopolymer-based biomaterials, and seaweed biorefineries, which has had a large impact in value creation for associated industries, and a societal impact through the education of students and young researchers, public communication of research, and influence on policymaking.

AlgiPharma
AlgiPharma is a privately owned clinical stage biopharmaceutical company with a focus on developing its proprietary alginate technology for: Delivery platforms for nanoparticles, small and large molecule-based drugs; Drug conjugates, adding functionality and reducing toxicity of parent compound; and as a stand-alone active pharmaceutical ingredient in infectious diseases and respiratory disorders. AlgiPharma has over the last decade been granted more than 85 MNOK in public national funding in addition to private funding and international grants (e.g. from the Cystic Fibrosis Foundation) for the company and collaborators toward development of their pharmaceutical technology (see point 5 ii). More than 65 MNOK of this funding has been allocated to research activities at BTN. The work in these projects contributed key pre-clinical data on the safety and efficacy of AlgiPharma's pharmaceutical candidates, leading to the completion of four phase 2 clinical trials in the same period for the treatment of cystic fibrosis (see point 5 iii). BTN's research in projects granted between 2011-2021 has also contributed to commercial scale-up of microbial OligoG production processes (see also section 2A). AlgiPharma has stated that the expertise of BTN has been a major contributor to the ongoing success of the company (see point 5 i).

SpermVital
SpermVital AS is a biotechnology company founded by the Norwegian cattle breeding company Geno and the SINTEF Group and have developed a revolutionary insemination technology for artificial insemination of domestic animals based on immobilization of sperm in alginate gels. The technology was based on a patent by Klinkenberg, G. at BTN. Artificial insemination (AI) is a well-established method in cattle in industrialized countries, and cryopreserved semen is mostly used. Insemination at the right time relative to the time of ovulation is essential for successful fertilization, however, challenging in large commercial herds. The SpermVital technology makes timing of AI less critical, while achieving increased fertilization and production efficiency for the end users. The impact of the research thus extends further to animal farmers and breeders in addition to SpermVital. For SpermVital, the collaboration with BTN has within the last decade contributed to approximately 100 MNOK funding from national and regional grants (see point 5 iv), where almost 29 MNOK of this has been allocated to research at BTN. SpermVital further had four new patents granted for their technology, and multiple completed field trials in the evaluation period. They have significantly increased their market share in Norway and entered new commercial partnerships with multiple actors in 10 different countries (http://www.spermvital.com/). BTN has contributed with knowledge, ideas, research, and development within biopolymer science and controlled release, thereby contributing to implementation of a range of innovations in the product and production process in the period from 2011 to 2022 (see point 5 i).

MicroA
MicroA is a privately owned Norwegian Biotechnology company, focusing on sustainable manufacture of products from microalgae. One of these products is a bioactive sulfated polysaccharide, Prasinoguard® (see point 5 v). The collaboration with MicroA was based on the underpinning research on sulfated polysaccharides, where initial consultation with BTN in 2020 led

to a strategic development plan for MicroA's proprietary polysaccharide technology toward healthcare applications. Joint proposals to RCN led to two granted ongoing projects, generating key results for ongoing commercialization of the technology. Through the PrasiNOSE (RCN IPN 321594) project started in 2021, BTN established downstream processing methods for the modification and purification of the microalgae-produced polysaccharide and generated important pre-clinical data necessary for *in vivo* animal studies later in the project. These projects and proof-of-concept studies will contribute to bringing new investors for the continued growth of the company, and also form a basis for the development of applications and markets.

Norwegian seaweed industry

BTN's impact on the seaweed industry can be exemplified by a 10-year collaboration with Seaweed Solutions (SES), where the research efforts of BTN has contributed strongly to the company's growth through acquired research funding and development of expertise and technical solutions. After a transition in core business areas at SES from biofuels and added coproducts to food and feed ingredients, the biopolymer competence of BTN has been important for the understanding of the biomass, development of processing technology, and driving forward new application areas such as bioplastics (see point 5 i). BTN has coordinated research proposals granting over 20 MNOK funding to collaborations between BTN and SES over the last decade, including ERA-BlueBio projects SNAP (RCN 311958), PlastiSea and QualiSea, with a still growing portfolio of collaborative projects. Other collaborations from 2021 and onward include projects coordinated by other industry partners, such as Orkla (OptiAlgae, RCN IPN 317864) and B'ZEOS (AlgiPack, RCN IPN 332449) on developing food ingredients and bioplastics, respectively (see point 5 vi). For SES, this R&D funding has been necessary for the development of their processes and products and has further resulted in new customers and collaborators contributing to their production upscaling and substantial market growth in the recent few years.

BTN has also had a long-standing relationship with the alginate production industry (Dupont) in Norway, related to biomass characterization, biorefinery processes, and development of novel applications of biopolymers (alginate, fucoidan, laminarin, cellulose). Research in the evaluation period, particularly within the Mar3Bio project, has contributed novel methods and data on fucoidan characterization, characterization of side streams for potential valorization, and new data on seasonal and geographical variations in the biomass (presented by Dupont at Seaweed Applications conference 2019, Inderøy, Norway).

5. Sources to corroborate the impact

i. Appendix 1: Impact of collaboration with BTN for the companies AlgiPharma, SpermVital, MicroA and Seaweed Solutions
ii. Algipharma project grants announcements: https://algipharma.com/news/
iii. AlgiPharma clinical trials https://clinicaltrials.gov/ct2/results?cond=&term=&type=&rslt=&age_v=&gndr=&intr=&titles=&outc=&spons=&lead=algipharma&id=&cntry=&state=&city=&dist=&locn=&rsub=&strd_s=&strd_e=&prcd_s=&prcd_e=&sfpd_s=&sfpd_e=&rfpd_s=&rfpd_e=&lupd_s=&lupd_e=&sort=
iv. SpermVital AS announcements of grants and commercial partnerships: http://www.spermvital.com/Startpage/NewsAndPress/News/
v. MicroA's Prasinoguard® product for healthcare applications: https://microa.no/products/prasinoguard/#healtcare-application
vi. Seaweed Solutions project overview: https://seaweedsolutions.com/project-overview/
vii. Appendix 2: Tøndervik, A., Klinkenberg, G., Aachmann, F.L., Svanem, B.I.G., Ertesvåg, H., Ellingsen, T., Valla, S., Skjåk-Bræk, G., Sletta, H. (2013) Mannuronan C-5 epimerases suited for tailoring of specific alginate structures obtained by high-throughput screening of an epimerase mutant library. Biomacromolecules 14(8)

AlgiPharma

27th Jan 2023

To whom it may concern,

I am writing on behalf of AlgiPharma in support of the impact cases (evaluation period 2011-2021) presented by the SINTEF Industry, Department of Biotechnology and nanomedicine.

AlgiPharma is a privately owned clinical stage biopharmaceutical company with a focus on developing its proprietary alginate technology for: Delivery platforms for nanoparticles, small and large molecule-based drugs; Drug conjugates, adding functionality and reducing toxicity of parent compound; and as a stand-alone active pharmaceutical ingredient in disease areas such as cystic fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD), and infectious diseases. AlgiPharma has pharmaceutical scale production capabilities for its technology and is protected by a broad family of patents. AlgiPharma's aim is to address unmet medical needs and fight diseases effectively through our innovative alginate technologies targeting those diseases where there is a clinical need to mitigate abnormal mucus accumulation, microbial infection, biofilm formation and antibiotic resistance. AlgiPharma has worked closely with the Biotechnology and Nanomedicine research teams at SINTEF Industry since 2006.

Through the coordination, expertise and leadership provided by Håvard Sletta at SINTEF Industry, their multi-disciplinary research teams have been a major contributor to AlgiPharma's ongoing success in the development of its alginate oligosaccharide technology. The broad range and depth of expertise at SINTEF Industry has been and continues to be a crucial resource in AlgiPharma's drug development and manufacturing programs, including microbial biotechnology, alginate polymer production and nanotechnology. This long-standing collaboration has been central in securing non-dilutive research funding (more than 85 MNOK) for AlgiPharma's research activities including the following Norwegian Research Council co-funded Innovation projects for industry (IPN) grants awarded during the 2011-2021 impact case evaluation period: BIA grant: Tailored OligoG (228542); BIA grant: OligoG Formulate (245598); NANO2021 grant: MucosALG (281920).

SINTEF Industry has played a key supporting role in the evaluation of AlgiPharma's primary drug candidate in four phase 2 clinical trials (NCT01465529; NCT03822455; NCT02157922; NCT02453789) evaluating a new drug treatment for Cystic Fibrosis (CF) which is a life-threatening lung disease. SINTEF Industry's research expertise have allowed us to gain a better understanding of the complex mechanisms of action and therapeutic potential of AlgiPharma's alginate oligomer technology for the treatment of respiratory, wound healing, drug delivery and infectious diseases. The teams at SINTEF have also played a key role in realizing commercial value for AlgiPharma, in both knowledge and intellectual property.

SINTEF has been an enabling force in AlgiPharma gaining investment for further research and commercial scale-up of OligoG production processes from the Norwegian Research Council and the UK Technology Strategy Board. SINTEF has played a central role in AlgiPharma's manufacturing program, securing significant non-dilutive funding in support of the development of commercially relevant manufacturing processes: Innovate UK: Algiferm (131142); ALGIPRO (102148); Microbialg (228570); Oligo-DSP (281907); Algi-SCALEUP (317799). AlgiPharma is also a partner in the SINTEF led national Centre of excellence, SFI-Industrial biotechnology.

If you have any further questions, please do not hesitate to contact me.

Yours sincerely,

Dr Phil D. Rye
Chief Scientific Officer
AlgiPharma AS
Email: phil.rye@algipharma.com
Tel: +47 97503033

30 January 2023

To whom it might concern,

Our partnership with SINTEF started in 2020 as part of our needs to demonstrate the properties of our marine sulfated polysaccharide technology. Our initial conversations with SINTEF's helped us identify a development plan and stablish a mutual interest in this novel biopolymer. Here, SINTEF's published research on sulfated biopolymers was an important starting point to explore bioactivities and application areas for our proprietary polysaccharide technology. This partnership has allowed us to bring an interesting compound with tested bioactivity in personal care applications to proof of concept drug substance for different healthcare applications. SINTEF's unique expertise in marine biopolymers has expedited our development process helping in the optimization of handling, downstream processing, purification, modification to enhance bioactivity, and structural and biological characterization of this biomolecule. Last but not least, SINTEF's technological and commercial awareness has been instrumental to the positioning of the technology that has resulted in the award of two IPN Research Council grants. These grants are allowing us to stablish a proof of concept for the technology and ultimately bring new investors to help in the further development and commercialization of the technology.

Please do not hesitate to contact me if further clarification or information is required.

Best regards

Enrique Tabares Rodriguez

CEO at MicroA Bioactives – MicroA

enrique@microa.no

To whom it may concern

spermvital

SpermVital AS
Holsetgata 22
N-2317 Hamar

www.spermvital.com

30.01.2023

SpermVital was founded in 2008 with Geno, Norsvin and SINTEF as owners, later being owned solely by Geno. The basic concept of the SpermVital technology, is that sperm are immobilized in an alginate gel, which is gradually dissolved within the female after artificial insemination (AI), resulting in presence of fertile sperm over an extended period. The technology makes timing of AI less critical, achieving increased fertilization and production efficiency. Extended shelf life of sperm cells gives flexibility and increased fertility for the end users and SpermVital is established as a new industry brand. The SpermVital technology is patented in more than 40 countries and in commercial use in cattle breeding in more than 10 countries.

In livestock production, good fertility is a critical success factor for the financial result. Today's food production in developed countries relies intricately on breeding programs, selecting individuals with preferable phenotypes harbouring advantageous alleles of genes for extensive propagation in the population. Artificial insemination is a well-established method in cattle in industrialized countries, and cryopreserved semen is mostly used. Insemination at the right time relative to the time of ovulation is essential for successful fertilization, however, challenging in large commercial herds. The reproductive capacity of dairy cows is declining across production systems worldwide, an alarming trend that is explained, among other things, by increased milk production that is genetically negatively correlated to fertility, increased herd size and physiological stress. The SpermVital technology was developed to contribute overcome these challenges. This revolutionary semen processing technology is designed to provide greater flexibility for the timing of AI and thereby change the negative trend of fertility. Contrary to standard AI where all sperm are immediately deposited, the gel with immobilized sperm will release distinct sperm populations for at least 24 hours. SpermVital has since market launch established a position as a strong brand in several European markets and has been implemented by some of the business's major players.

SpermVital has collaborated closely with SINTEF on development of the SpermVital technology since the start-up of the company.

SINTEF has contributed with knowledge, ideas, research, and development within biopolymer science and controlled release, thereby contributing to implementation of a range of innovations in the product and production process in the period from 2011 to 2022. Four patents are granted and four scientific papers have been published together with SINTEF in this period.

Managing Director
SpermVital AS

Seaweed Solutions

Our date	Our reference
31.01.2023	Ole Christian Norvik
Your date	Your reference
	Øystein Arlov

To whom it may concern,

I am writing on behalf of Seaweed Solutions AS in support of the impact cases (evaluation period 2011-2021) presented by SINTEF Industry, Department of Biotechnology and Nanomedicine.

SINTEF Industry, Department of Biotechnology and Nanomedicine (BTN) has over the last 10 years been one of the most important R&D partners for Seaweed Solutions (SES) and contributed strongly to the company's knowledge and technical solutions within biomass processing and application/market development. SES focused on fermentation of seaweed for bioethanol and co-products in the early phase of the company, but have more recently transitioned to work on innovative processing for food applications (including fermentation, acid preservation and blanching), while pursuing other markets such as feed, bioplastics and pharmaceuticals through our R&D collaborations.

SES has through our collaboration with BTN been included in multiple international R&D projects such as ERA-net projects ProSeaFood, PlastiSea, SNAP and QualiSea, as well as national industry-driven projects (IPNs). This has provided important funding for SES as well as establishing collaboration with other industry actors including customers within existing and novel markets. As a small company it is challenging to develop new R&D projects and partnerships, and here we have benefitted greatly from the network and competence provided by BTN.

BTN's unique competence builds on a long tradition of basic research and innovation development, where the research environments NTNU and BTN are and have been world-leading in alginates and other biopolymers from seaweed. Much of the value creation potential from seaweed lies within these components, and we consider this competence to be a large competitive advantage for Norway in the global seaweed industry.

Jan 31st 2023

Ole Christian Norvik

CEO

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Article

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Mannuronan C-5 Epimerases Suited for Tailoring of Specific Alginate Structures Obtained by High-Throughput Screening of an Epimerase Mutant Library

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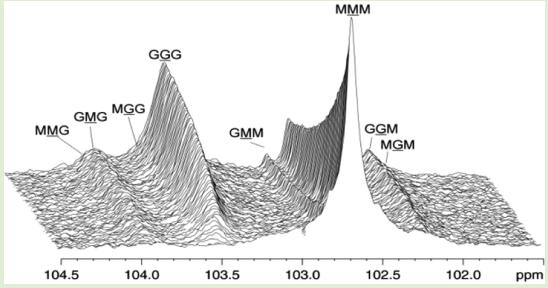
Supporting Information

ABSTRACT: The polysaccharide alginate is produced by brown algae and some bacteria and is composed of the two monomers, β-D-mannuronic acid (M) and α-L-guluronic acid (G). The distribution and composition of M/G are important for the chemical-physical properties of alginate and result from the activity of a family of mannuronan C-5 epimerases that converts M to G in the initially synthesized polyM. Traditionally, G-rich alginates are commercially most interesting due to gelling and viscosifying properties. From a library of mutant epimerases we have isolated enzymes that introduce a high level of G-blocks in polyM more efficiently than the wild-type enzymes from *Azotobacter vinelandii* when employed for in vitro epimerization reactions. This was achieved by developing a high-throughput screening method to discriminate between different alginate structures. Furthermore, genetic and biochemical analyses of the mutant enzymes have revealed structural features that are important for the differences in epimerization pattern found for the various epimerases.



INTRODUCTION

Alginate is a family of linear polysaccharides with numerous present and potential future application areas ranging from food, textile, and printing industry to biomedical and biopharmaceutical as well as electrochemical products.[1−5] The polymer is synthesized by brown algae and by *Azotobacter* and *Pseudomonas* species,[6,7] and currently all commercial production is based on extraction from algal resources. The alginate monomers β-D-mannuronic acid (M) and α-L-guluronic acid (G) are arranged in M-, G-, and MG-blocks along the polymer chain, and the length and distribution of these blocks determine the physicochemical properties of the polysaccharide.[8] Alginates containing G-blocks are dominating in commercial use due to their ability to form hydrogels. The polymer is first produced as mannuronan (polyM); subsequently, the block structure results from the activity of a family of mannuronan C-5 epimerases catalyzing nonrandom epimerization of β-D-mannuronic acid to α-L-guluronic acid at the polymer level.[9] *Azotobacter vinelandii* encodes a family of seven secreted mannuronan C-5 epimerases, AlgE1-AlgE7, involved in the cellular differentiation of the bacterium.[10−13] These enzymes display a modular structure being composed of one or two catalytic A-modules and from one to seven regulatory R-modules. Although highly homologous, these enzymes each create characteristic M/G patterns producing alginates with different properties. AlgE4, which is the smallest epimerase containing one A-module and one R-module, makes predominantly alternating M/G structure acting on polyM by a processive mode of action.[14−16] AlgE1, AlgE2, AlgE3, AlgE5, and AlgE6 make G-blocks of varying lengths, and AlgE6 is the epimerase able to make the longest G-block structures when acting on polyM.[17] AlgE7 encompasses dual functionalities in also having alginate lyase activity.[18] The 3D structure of the A- and R-module of AlgE4 has been reported, and the catalytic residues in the active site have been determined.[19,20] Given the similarity in amino acid sequence of the A-modules of the epimerases, it is clear that very minor and to a large extent unpredictable changes in the primary structure can lead to alterations in the epimerization pattern.

The microscopic structure, that is, the monomeric sequence distribution of biopolymers like alginate, determines the chemical and physical properties of the molecules and thereby the spectrum of possible applications.[21,22] Being able to structurally design biopolymers is therefore highly desirable for obtaining biomaterials with controllable and targeted properties.[2] For alginates this can be approached by utilizing mannuronan C-5 epimerases with defined properties in in vitro epimerization processes of, that is, polyM.[23,24] As opposed to current manufacturing strategies from algal resources, this enzymatic route would reduce batch-to-batch variations as well

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as introduce possibilities for obtaining reproducible alginate structures that are not readily obtainable from algae. Additionally, in vitro epimerization with specific enzymes could also offer a route for so-called upgrading of algal alginate, that is, increasing the level of G-blocks.[17] This strategy could represent a valuable supplement to the global alginate market, which is facing a shortage of G-rich alginates due to the lack of algal raw material.

In the present study, our main goal has been to develop mannuronan C-5 epimerases that can be used for efficient in vitro epimerization of either bacterially produced mannuronan or alginate substrates of algal origin. On the basis of the *algE1-algE6* genes from *A. vinelandii*, a mutant library was constructed by gene shuffling and subsequent error-prone PCR. A high-throughput screening protocol was developed that enabled discrimination of epimerised alginate based on the M/G content in the samples, that is, the microscopic structure of the polymers created by the mutant epimerases. To our knowledge, high-throughput screening studies based on biopolymer structure have not been previously performed. By this approach, we have obtained novel mannuronan C-5 epimerases that are more efficient in epimerizing polyM to high levels of G-blocks than any of the wild-type enzymes. Time-resolved NMR spectra indicate that at least one of these enzymes has altered enzyme kinetics compared with wild-type AlgE6. Furthermore, results obtained in this work indicate that the R-modules of the mannuronan C-5 epimerases play a role also in determining the epimerization pattern, a property that has previously been attributed only to the catalytic A-modules.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and DNA Manipulations. *Escherichia coli* strains DH5α (Bethesda Research Laboratories), JM109 (New England BioLabs), and XL1-Blue (Stratagene) were used as general cloning hosts, whereas XL10-Gold (Stratagene) was used for establishing the mutant library. *E. coli* strains were routinely grown at 37 °C in LB medium (yeast extract, 5 g/L; tryptone, 10 g/L; and NaCl, 10 g/L) or on LB agar (LB medium supplemented with 20 g/L agar). For protein expression, strains were grown in triple-strength LB medium (3 × LB; yeast extract, 15 g/L; tryptone, 30 g/L; and NaCl, 10 g/L). For growth in 96-well plates, a reduced Hi-Ye medium with the following composition was used: $Na_2HPO_4{\cdot}2H_2O$, 12.3 g/L; KH_2PO_4, 4.29 g/L; NH_4Cl, 0.43 g/L; NaCl, 0.71 g/L; glucose, 2.86 g/L; yeast extract, 2.86 g/L; citric acid, 1.43 g/L; $MgSO_4$, 1.86 mM; Fe(III)-citrate, 118 μM; H_3BO_3, 21.0 μM; $MnCl_2$, 37.6 μM; EDTA, 9.86 μM; $CuCl_2$, 3.86 μM; Na_2MoO_4, 4.29 μM; $CoCl_2$, 4.71 μM; and Zn-acetate, 17.3 μM. Cultures were induced for protein expression using an induction solution containing: glycerol (99%), 25.8 g/L; yeast extract, 24 g/L; and isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.25 mM. When appropriate, ampicillin (200 μg/mL) was added to the growth media. Standard recombinant DNA techniques were performed, as described elsewhere.[25] Plasmids were purified by the WizardPlus SV Minipreps DNA purification system (Promega) or the QIAGEN Plasmid Plus Midi Kit (QIAGEN). Transformation of XL10-Gold ultracompetent cells was performed according to instructions given by the manufacturer and for other *E. coli* strains according to RbCl transformation protocol (New England BioLabs). DNA sequencing was performed by Eurofins MWG Operon. Construction of vectors expressing epimerases with site-specific mutations and mutant A-modules combined with the R modules of AlgE6 is described in Table S1 in the Supporting Information.

Construction of an Epimerase Gene Library by Staggered Extension Process (StEP) and Error-Prone PCR. Vectors used and constructed are listed in Table S1 in Supporting Information, whereas sequences of primers utilized are given in Table S2 in Supporting Information. The sequences encoding the A-modules of AlgE1−6 (eight in total) were cloned as *Nco*I-*Xma*I fragments into *pTrc99A*, and the resulting vectors were used as templates in the StEP reaction. Primers StEP fwd (located 257 bp upstream of *Nco*I) and StEP rev (loacated 145 bp downstream of *Xma*I) were used for amplification of PCR fragments, and the reaction mixtures used were as follows: 12 ng of each template vector, 30 pmol of each primer, 0.2 mM dNTP, 1 × Taq 2000 buffer, and 3.75 U Taq 2000 DNA polymerase. PCR conditions used were as follows: 2 min at 96 °C, 80 cycles of 30 s at 95 °C, and 3 to 4 s at 40−45 °C. The StEP procedure was repeated several times, and because DNA sequencing of the PCR products revealed a predominance of the gene AlgE5A in the recombined sequences for the A-modules, the vector encoding this A-module was omitted from some of the StEP reactions. PCR reactions were treated with *Dpn*I to degrade template DNA, and fragments with correct size (1.1 kb) were isloated from agarose gels. The recombined fragments were digested with *Nco*I-*Xma*I and ligated into the same sites of pBL5 creating libraries of hybrid epimerase genes consisting of recombined A-modules (376 amino acid residues) and the R module (177 amino acid residues) from AlgE4. The ligation mixtures were transformed into XL-10 gold cells, and the resulting transformants were pooled together, grown for a few generations in LB medium, and used for plasmid isolation. Plasmids from each StEP reaction were mixed together and used as template for error-prone PCR. Random mutations were introduced into the recombined library either by using the GeneMorph PCR mutagenesis Kit from Stratagene (method 1) or by decreasing the fidelity of the *Taq* polymerase by manipulating the M_n/M_g ratio and the nucleotide concentration in the reaction mixture (method 2). Method 1: Conditions were chosen to give a mutation frequency of 3−7 mutations/kb: 8.5 ng template DNA (corresponding to 1 ng target DNA), 30 pmol each of primers StEP fwd and EU20 (located 52 bp downstream of *Xma*I), 0.8 mM dNTP, 1× mutazyme buffer, and 2.5 U mutazyme in a final volume of 50 μL. Method 2: 78 ng template DNA, 30 pmol each of primers StEP fwd and EU20, 0.2 mM dNTP, 5 μL 5 × PCR-buffer (300 mM Tris-HCl, pH 8.5, 75 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$), 1.25, 2.0, 2.5, or 3.0 μL of 10× mutagenic buffer (8 mM dTTP, 8 mM dCTP, 48 mM $MgCl_2$, 5 mM $MnCl_2$) and 5 U *Taq* polymerase (Promega) in a final volume of 50 μL. PCR conditions used for both methods were as follows; 1 min at 96 °C, 4 cycles of 30 s at 96 °C, 30 s at 55 °C, and 2 min at 72 °C and then 27 cycles of 30 s at 96 °C, 30 s at 60 °C, and 2 min at 72 °C. The five PCR reactions mixtures (one by method 1 and four by method 2) were treated with *Dpn*I, and fragments of correct size (1.1 kb) were isolated from agarose gels, digested with *Nco*I-*Xma*I, and ligated into the corresponding sites of pBL5 to creating recombined, mutated libraries of epimerase genes. The ligations mixtures were transformed into XL10-Gold cells, and the transformants were pooled together before the addition of glycerol and storage (−80 °C) of the library.

Production of Alginate Substrates and Alginate Lyases. High-molecular-weight mannuronan (polyM) was isolated from a mutant strain of *Pseudomonas fluorescens* NCIMB 10525.[26] ^{13}C-1 labeled mannuronan was produced by growing *P. fluorescens* on a minimal media with 99% D-^{13}C-1 fructose as carbon source. The obtained mannuronan was selectively enriched to 59% with ^{13}C at carbon position C-1.[15,27] Alginate with a strictly alternating structure (polyMG; F_G = 0.47 and F_{GG} = 0) was prepared by epimerization of polyM with recombinant mannuronan C-5 epimerase AlgE4 and characterized by NMR as described previously.[27] G-blocks (polyG; F_G = 0.94 and DP = 18.5) were prepared from *Laminaria hyperborea* stipes, as described elsewhere.[28,29] Production of M-M-specific M-lyase (AlxM), G-M, and G-G-specific G-lyase (AlyA) and G-G-specific GG-lyase (AlyA5) by fermentation of recombinant *E. coli* strains was performed as described elsewhere.[30]

Robotic Screening of the Mutant Recombined Epimerase Library. The *E. coli* library was plated on LB agar in 25 × 25 cm Petri dishes (Corning CLS431301) and incubated overnight at 37 °C. Colonies were picked using a Genetix Q-Pix2 robotic colony picker and transferred to 96-well microplates (Greiner M3186) containing 80 μL of reduced Hi-Ye medium. The microplates were incubated at 30

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°C, 900 rpm (3 mm amplitude) and 80% relative humidity. After 24 h, 40 μL of induction solution was added to the microplates using an Asys Hi-Tech Flexispence microplate dispenser. The microplates were incubated at 37 °C, 900 rpm, and 80% relative humidity for 7 h after induction and were frozen at −40 °C prior to analysis.

After thawing, to the microplates was added 30 μL of B-per II solution (Pierce) (with $CaCl_2$ to a concentration of 25 mM) per well; they were shaken for 30 s (900 rpm, 3 mm amplitude) and incubated at room temperature for 1 h. After incubation, the microplates were shaken (850 rpm, 3 mm amplitude) for 10 min and then centrifuged for 30 min at 3500*g*. For epimerization of alginate, 10 μL of enzyme extracts was added to 190 μL of assay buffer (40 mM MOPS, 20 mM NaCl, 2 mM $CaCl_2$, pH 6.8) containing polyM alginate (0.1 mg/mL). The plates were sealed after the addition of enzyme extract using sterile sealing film (Nunc 236366) and incubated at 37 °C for 48 h. Microplates with epimerized alginate were frozen at −40 °C prior to analysis.

For analysis of G-content in epimerized alginate samples, we transferred 30 μL samples of alginate in assay buffer to wells in 384-well microplates (Corning CLS3675). To the wells was then added 10 μL of assay buffer containing the AlyA enzyme (0.14 U/mL on MG alginate), and it was shaken at 1700 rpm for 1 min. The microplates were then incubated at 25 °C for 5 h. The absorbance at 230 nm (A230) was read in a Beckman Coulter DTX880 microplate reader prior to the addition of alginate shortly after mixing and after incubation. The increase in absorption during incubation was calculated, and ΔA230 ($A230_{t=5} - A230_{t=0}$) was used for estimation of the total G content of the epimerized alginates.

To be able to discriminate between alginate samples containing MG- and GG-blocks, we developed a two-step protocol using an M-lyase and two alginate lyases with different specificity toward G-block and polyMG alginates. We transferred 30 μL of epimerized alginate in assay buffer to wells in 384-well microplates (Corning CLS3675). To each of the wells was added 5 μL of assay buffer containing M-lyase, and the microplates were shaken at 1700 rpm for 1 min and incubated at 25 °C for 12 h. To each of the wells was then added 5 μL of assay buffer containing either the AlyA enzyme or the AlyA5 enzyme (0.2 U/mL on polyG for both enzymes), and the microplate was shaken at 1700 rpm for 1 min. After mixing, the microplates were further incubated at 25 °C for 12 h. The absorbance at 230 nm (A230) was read in a Beckman Coulter Paradigm microplate prior to the addition of enzymes, shortly after mixing, and then each hour after addition enzymes. The difference in absorbance between the two first time points (ΔA230 = $A230_{t=1} - A230_{t=0}$) was used for evaluation of the G content and structure of the epimerized alginates. All liquid and microplate handling was performed by a Beckman Coulter Core system robotic liquid handling workstation.

Protein Expression and Purification. Epimerase expressing strains were grown in 100 mL of 3× LB medium in 500 mL baffled shake flasks at 37 °C for 3 h before induction with 0.5 mM IPTG. Growth was continued for 4 h at the same temperature before harvesting the cells by centrifugation. For preparation of protein extracts, the cells were sonicated in 10 mL of 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), 5 mM $CaCl_2$, pH 6.9, and then centrifuged for 30 min at 20 000*g*. The supernatant was filtered (0.2 μm) and applied on a 5 mL HiTrap Q HP column, and proteins were eluted using a stepwise NaCl gradient (0 to 1 M) in the same buffer as above. Protein-containing fractions were tested for epimerase activity by NMR (see below), and the total protein content was measured by the Bio-Rad microassay procedure using bovine serum albumin as standard. Purity of protein fractions was determined by SDS-PAGE.

End-Point and Time-Resolved NMR Analysis of Epimerised Alginate Samples. All experiments were recorded on a BRUKER Avance 600 or DPX 400 spectrometer equipped with a 5 mm cryogenic CP-TCI z-gradient probe and 5 mm z-gradient DUL (C/H) probe, respectively. End-point analysis of epimerised samples was recorded at 90 °C, while time-resolved NMR recording of the epimerization reaction was performed at 40 °C. To reduce the viscosity of the alginate samples prior to NMR measurements, the samples were depolymerized by mild acid hydrolysis to a final average DP_n ∼30 residues.[31] 3-(Trimethylsilyl)-propionic-2,2,3,3-*d*4 acid sodium salt (Aldrich, Milwaukee, WI) was used as an internal standard for the chemical shift and triethylenetetra-amine hexa-acetate (Sigma-Aldrich) was added to chelate residual calcium ions in end-point epimerised samples. For the time-resolved NMR analysis of epimerization reactions, a stock solution of 22 mg/mL ^{13}C-1-enriched polyM (average DP_n ∼70) in 5 mM MOPS, pH 6.9 with 75 mM NaCl in 99.9% D_2O was prepared. Purified enzyme fractions from ion exchange chromatography were subject to buffer exchange and upconcentrated (final concentration of 1.1 to 2.3 mg/mL) by spin columns (VivaSpin, Sartorius Stedim Biotech) with molecular cutoff 10 kDa. Samples were washed with 5 mM MOPS, pH 6.9 with 75 mM NaCl and 27.5 mM $CaCl_2$ in 99.9% D_2O. Protein concentrations were determined with a Nanodrop ND-1000 to ensure similar enzyme concentration in the epimerization reaction. 500 μL of ^{13}C-1-enriched polyM stock solution was preheated in the NMR instrument and 1D proton and carbon spectra were recorded to ensure that the sample has not undergone any degradation or contamination prior to the time-resolved NMR experiment. 50 μL of enzyme solution was added to preheated substrate and mixed by inverting the sample two to three times. The sample was then immediately inserted into the preheated NMR instrument and the experiment was started. The recorded spectrum is a pseudo-2D type experiment recording a 1D carbon NMR spectrum every 15 min. The recorded 1D carbon spectrum (using inverse gated proton decoupling) contains 8K data points and has a spectral width of 80 ppm, 64 scans with a 30° flip angle, and relaxation delay of 1 s (total recording time of 91s). The NMR data were processed and analyzed with Bruker XwinNMR 3.5, TopSpin 2.1, and TopSpin 3.0 software.

Bioinformatics Analysis of Epimerase Mutants. The experimental 3D structure of the A-module from AlgE4 with and without mannuronan trisaccharide bound (Protein Data Bank code 2PYH and 2PYG, respectively) in the substrate binding groove were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank.[32] The structures were used as template input for the SWISS-MODEL platform[33−35] modeling the mutant A-modules identified from the library. The structures were visualized and analyzed with PyMol.[36] The alignment of mutant and wild-type protein sequences was done with ClustalX[37] and visualized with TreeView.[38]

RESULTS AND DISCUSSION

Construction and Characterization of a Mannuronan C-5 Epimerase Mutant Library. To obtain mannuronan C-5 epimerases with improved properties for in vitro epimerization, we used the genes encoding the secreted epimerases from *A. vinelandii* as the basis for construction of a mutant library. The catalytic site of the epimerases is located in the A-modules of the enzymes, and the gene sequences encoding these modules of AlgE1-AlgE6 (E1A1, E1A2, E2A, E3A1, E3A2, E4A, E5A and E6A) were therefore used as templates in the staggered extension process (StEP). DNA encoding AlgE7 was not included in the recombination reaction due to the combined epimerase and lyase activity of this enzyme.[39] The recombined A-module sequences were ligated into expression vectors containing the R-module sequence of AlgE4 creating complete epimerase genes. The resulting plasmids were transformed into XL10-Gold cells creating a library of about 120 000 clones. To test the diversity in the library, we sequenced the plasmids from 48 random clones to analyze the degree of recombination between the different A-module sequences, and the epimerases encoded by the same plasmids were also tested for epimerase activity. Sequence alignment showed that 33 plasmids (69%) encoded shuffled A-modules, and of these, 26 (78%) encoded epimerases displayed activity. To further increase the diversity of the DNA sequences, we performed error-prone PCR on the recombined A-module genes from the first library. Conditions

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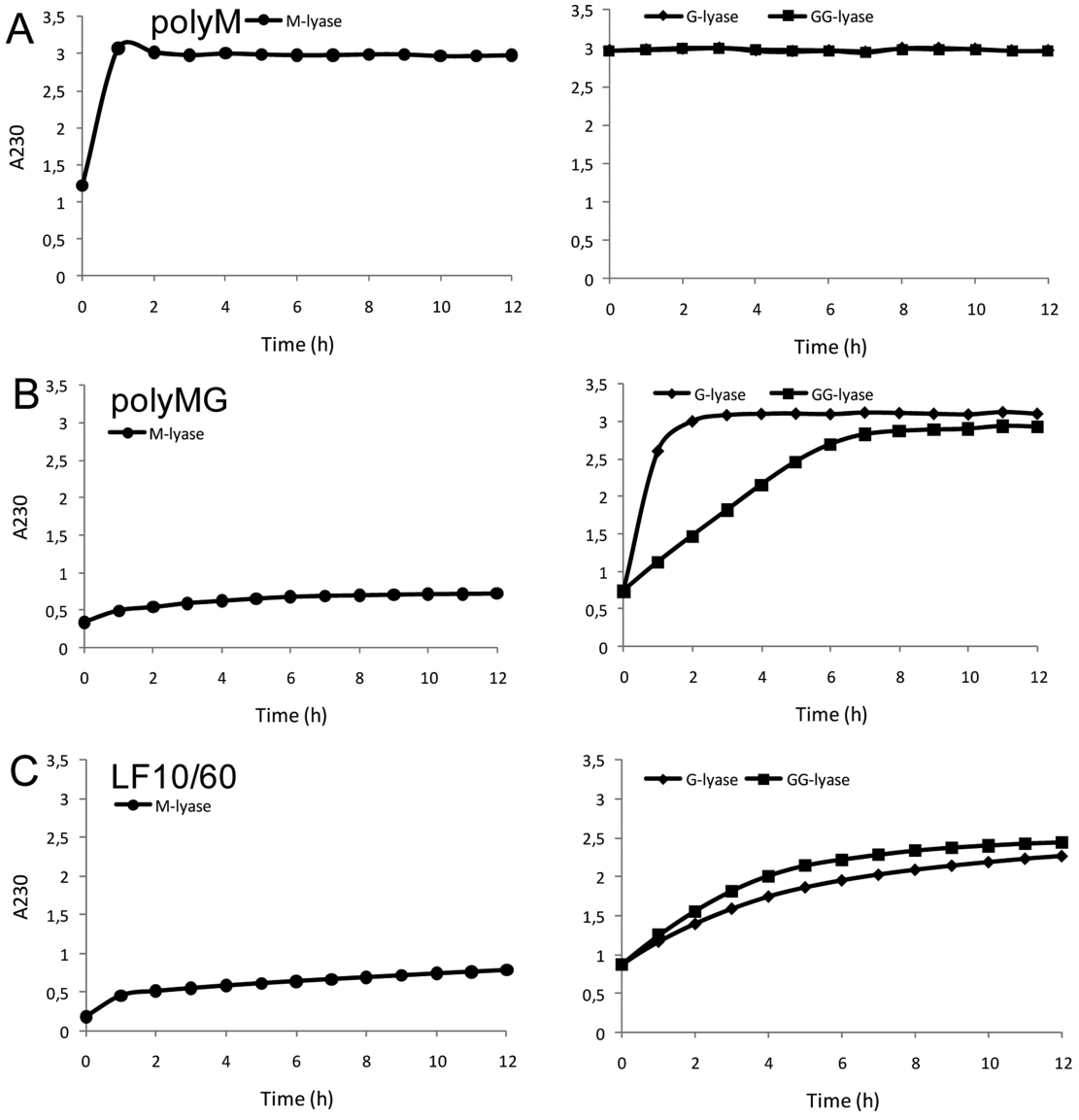

Figure 1. Degradation of polyM (A), polyMG (B), and LF10/60 (C) with M-lyase (left) and G- or GG-lyase (right) measured by monitoring A_{230}. To two parallel samples of the alginate substrate (1 mg/mL in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$) was added M-lyase (0.5 U/mL on polyM), and they were incubated for 12 h with A_{230} readings every hour. Then, G- and GG-lyase (0.2 U/mL on polyG) were added to parallel samples and incubation and A_{230} reading continued for an additional 12h. Degradation reactions for each sample were performed in 384-well plates in 96 parallels, and the curves shown are representatives of each kind. The standard deviation of the mean for the measurements is below 15%.

were adjusted to achieve a mutation frequency of about 3−8 nucleotide changes per gene. The mutagenized A-modules were ligated into expression vectors containing the R-module sequence from AlgE4, and the resulting plasmids transformed into XL10-Gold cells, creating a final library of about 100 000 clones. To verify the mutation frequency, we sequenced plasmid DNA from 56 random clones, and 18 of the mutant enzymes encoded by these plasmids (32%) were found to display epimerase activity. This final library containing epimerases with recombined and mutated A-modules was used for screening for enzymes with high G-block-forming activity.

Screening of the Mutant Library and Isolation of Enzymes That Are Able to Epimerise Mannuronan to High Levels of G-Blocks. An initial screening was performed by randomly picking 11 000 colonies from the final library, followed by cultivation and induction for epimerase expression with IPTG in 96-well microtiter plates. Cell-free extract from each culture was prepared and used for epimerization of polyM. To evaluate the G content in each epimerised sample, we degraded the resulting alginates using a G-lyase, which cleaves G-M and G-G linkages almost equally well (polyMG/polyG activity ratio of 1.2).[40−42] End-point measurements of A_{230} detecting the unsaturated uronic acid residues resulting from lyase degradation made it possible to identify samples containing epimerised alginate among the ones that were not epimerised, that is, containing polyM. However, by this method, it turned out to be difficult to distinguish between samples of alginates with medium G content (~45%) in alternating MG structure and high G content (~80%) in block structure. This is due to the similar amounts of linkages available for lyase degradation in the two structurally very different alginates. A two-step degradation protocol utilizing three alginate lyases with different specificities was therefore developed. The strategy was to first use an M-lyase to specifically degrade all of the M-M linkages in the alginate

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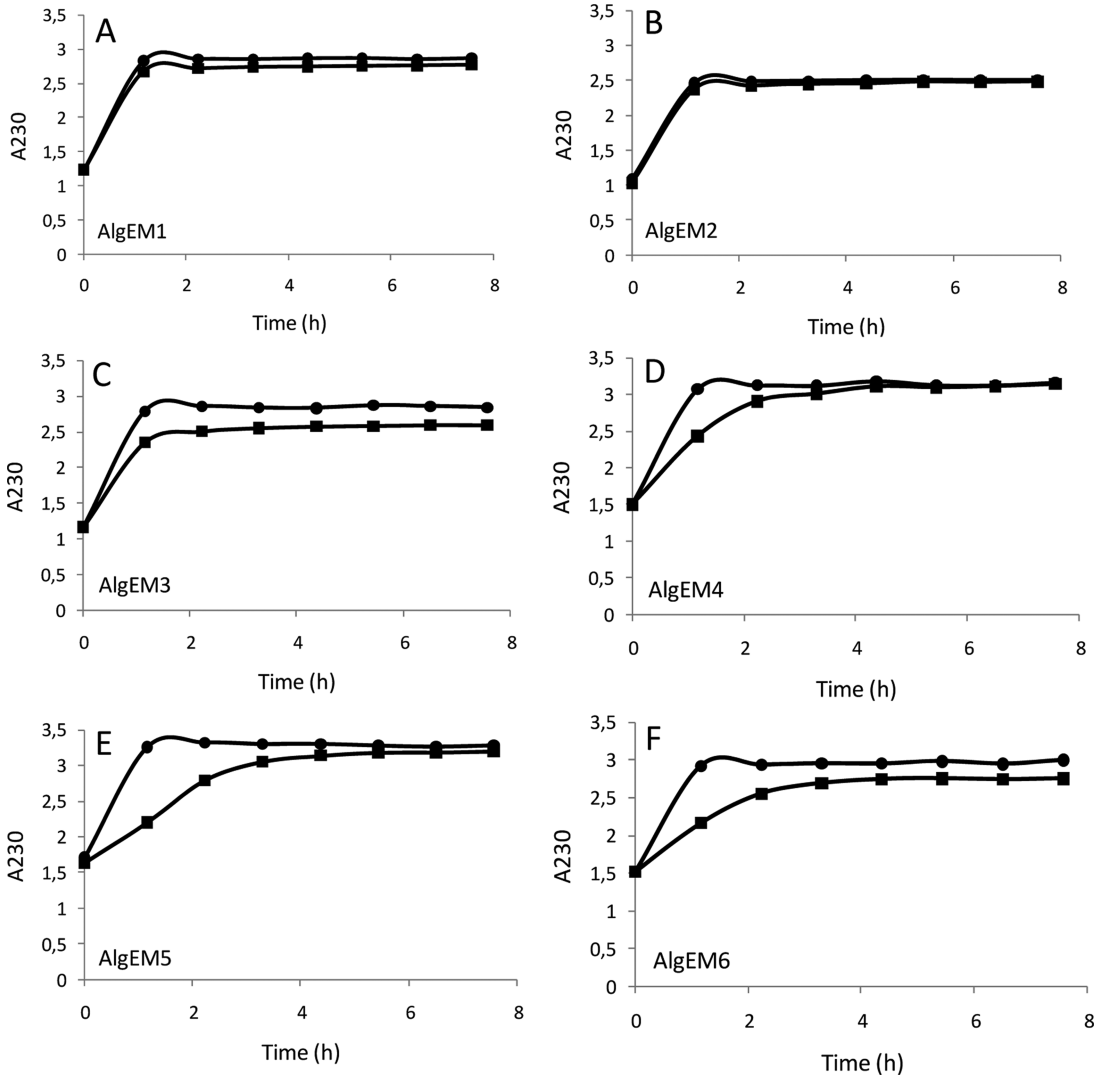

Figure 2. Degradation of polyM epimerised with mutant epimerases AlgEM1−6. Two parallel samples of each were first degraded with M-lyase for 12 h (not shown in the Figure); then, to samples were added G- (circles) and GG-lyase (squares), and incubation and A_{230} reading continued for additional 12h. The Figures show the A_{230} measurements only up to 8 h because after that there was no change in absorbance. The reaction conditions and enzyme concentrations used and the experimental uncertainty for the measurements were as described in the legend to Figure 1.

samples.[43] Samples containing high or low M would then be subject to extensive or limited degradation by this enzyme, respectively. The next step was to further degrade two parallels of the same samples with G-lyase and GG-lyase, with the latter enzyme displaying increased specificity toward G-G linkages (polyMG/polyG activity ratio of 0.1).[42] The GG-lyase was expected to display lower activity toward a sample containing alginate molecules with alternating MG structure than the G-lyase, whereas the two enzymes were expected to have similar activity toward samples rich in G-G linkages. To take full advantage of the different specificities of these two enzymes, it was necessary to follow the kinetics of alginate degradation. End-point measurements would give nearly the same A_{230} for both enzymes because the GG-lyase has some residual activity toward G-M linkages. The protocol was first tested on alginates with known composition: polyM (F_M = 1, F_G = 0), polyMG (F_G = 0.47, F_{GG} = 0), and LF10/60 (F_G = 0.66, F_M = 0.34, F_{GG} = 0.55, F_{MG}/F_{GM} = 0.12, F_{MM} = 0.22). First, M-lyase was added to the samples and degradation was followed by measuring A_{230} every hour for 12 h. Then, two parallel samples were added, G- or GG-lyase, and incubation continued with A_{230} monitoring for another 12 h. As expected, polyM is completely degraded in the M-lyase step; that is, there is no further increase in A_{230} by the addition of G- or GG-lyase (Figure 1A). Furthermore, the kinetics of degradation of polyMG and LF10/60 was clearly different in the second step (Figure 1B,C), and the largest difference in activity between the G- and GG-lyase was as expected obtained on the alternating substrate (Figure 1B). This showed that it was possible to perform a screening of the library based on discrimination between differences in the resulting polymer microstructures created by the mutant enzymes. To our knowledge, this kind of high-throughput screening study has not been previously performed. Because of the increasing applications and need for high-G alginates, we targeted the current screening approach to isolate mutant enzymes giving high content of G-blocks when epimerising polyM.

Alginate epimerised by 960 randomly selected mutants was evaluated using the two-step degradation protocol described above (9% of the amount in the initial screen). PolyM was epimerised with protein extracts from the randomly selected mutants and subjected to lyase degradation, as described for the

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test samples. From data analysis of the degradation kinetics of each sample, we identified three for which the degradation with G- and GG-lyase was almost identical (Figure 2A,B) or very similar (Figure 2C), indicating a high level of G-blocks in the epimerised alginate. Furthermore, around 25 samples displayed degradation kinetics similar to polyMG, indicating an F_G of ∼0.45 (three examples shown in Figure 2D−F). To test whether the observed degradation kinetics corresponded to the expected sample properties, we performed a preliminary characterization of crude protein extracts obtained from a total of 11 strains (including those shown in Figure 2). Strains were grown in shake flasks, and cell-free protein extracts were used to epimerise polyM for NMR analysis. These analyses showed that alginates with F_G in the range of 0.65 to 0.80 were obtained for the samples shown in Figure 2A−C and 0.40−0.45 for the remaining samples. Taken together, this confirmed the validity of the screening method for identification of samples with a specific composition. Furthermore, it also indicated that ∼0.2% of the mutants in the library encoded epimerases that were introducing such a high level of G-blocks into polyM.

It should be pointed out that the kinetic measurements performed to obtain the data underlying the isolation of mutant enzymes are time- and resource-demanding. If the aim was to isolate as many mutants as possible, then an alternative strategy allowing for screening of a larger portion of the library could be considered. As a first step, the entire collection of alginate samples would be degraded with M-lyase and end-point A_{230} measurements used to sort out the ones containing polyM (inactive enzymes) and low G (enzymes with low activity). The remaining samples, that is, with A_{230} below 1 like in Figure 1B,C, would then be chosen for further characterization following the kinetics during degradation with G- and GG-lyase. From the original library of about 100 000 clones, one would then expect to obtain around 200 mutants resulting in high G when starting with polyM.

Mutant Epimerases AlgEM1 and AlgEM2 Epimerise polyM to Higher G-Content than Wild-Type Epimerase AlgE6. To characterize enzyme properties in detail, we show the mutant epimerases corresponding to samples in Figure 2 (designated AlgEM1−AlgEM6), which were partially purified by ion exchange chromatography and used for epimerization of polyM. As a control, polyM was also incubated with AlgE6, which is the wild-type epimerase known to give the highest F_G.[17] The monomer composition and the diad and triad frequencies in the different alginate samples are shown in Table S3 in the Supporting Information. Two of the mutants, AlgEM1 and AlgEM2, epimerised polyM to F_G = 0.85 and 0.83, respectively, which is higher than what was obtained with AlgE6 (F_G = 0.77) under the conditions utilized. AlgEM1- and AlgEM2-epimerised alginate also display high F_{GG} (0.78 and 0.74) and low F_{MGM} (0.030 and 0.027), indicating that the majority of guluronic acid residues are present as blocks and that there is very little alternating structure present. Compared with AlgE6, these two enzymes produce alginates in which a larger fraction of the total guluronic acid residues are present as blocks. AlgEM3−5 resulted in alginates with F_G in the range of 0.54 to 0.67 and where the guluronic acid residues introduced are present both in blocks (F_{GG} = 0.22−0.44) and in alternating structures (F_{MGM} = 0.15−0.25). AlgEM6 epimerised polyM to F_G = 0.4, and most of the guluronic acid residues are present in alternating structures (F_{GG} = 0.039 and F_{MGM} = 0.33), which is very similar to wild-type AlgE4.

The availability of effective enzymes that can epimerise polyM in a single reproducible reaction step is valuable in in vitro design of alginates. Another approach for enzymatic preparation of specific alginates is to perform epimerization of algal alginates with the aim of increasing the G content, and we therefore wanted to test mutants AlgEM1 and AlgEM2 on a predefined complex alginate isolated from leaves of *L. hyperborea* to elucidate the efficacy on this kind of substrate. This natural substrate has F_G = 0.50, with G-residues present as both blocks and alternating structures. NMR analysis showed that AlgE6 and AlgEM1 acted very similar on this substrate, yielding alginates with F_G = 0.76 and 0.75, respectively, whereas AlgEM2 reached F_G = 0.69. This indicates that the enzymes act quite differentially on various substrates and that in screening for epimerases with targeted properties, the choice of substrate is very important.

AlgEM1 and AlgE6 Display Different Epimerization Kinetics on polyM. The results shown in Table S3 in the Supporting Information are end-point measurements of the final composition of polyM epimerised with the different enzymes. To reveal possible differences in the kinetics of epimerization for the mutant enzymes, we compared AlgEM1 and AlgEM2 with AlgE6; continuous NMR-spectra were recorded using ^{13}C-1-enriched polyM. AlgEM1 (Figure 3B) and displayed significantly different epimerization kinetics than AlgE6 (Figure 3A), whereas AlgEM2 displayed a spectrum very similar to AlgE6 (spectra not shown). AlgEM1 showed an almost immediate and fast introduction of G-blocks (evident as increase in peak marked G<u>G</u>G) into the substrate. This is accompanied by a simultaneous rapid decline in the content of M blocks (M<u>M</u>M) as well as a slow accumulation of alternating blocks (G<u>M</u>G and M<u>G</u>M). Moreover the G<u>G</u>M peak that signifies the number of G-blocks remains constant after the initial phase, indicating that the G residues are introduced predominantly as elongation of existing G-blocks. For AlgE6, the formation of G-blocks lagged behind the introduction of G residues in alternating sequences, indicating that AlgEM1 has higher affinity for the alternating polyMG structure than polyM compared with AlgE6. There are, in principle, two modes of action that could account for a predominant G-block formation; a processive mode where the enzyme slides along polyM, carrying out repetitive epimerization reactions without dissociating from the substrate, or a preferred attack mode where the enzymes affinity is higher for M-G than for M-M. In both cases, subsite −1 (by definition, epimerization takes place at subsite +1) must preferentially accommodate a G residue. A processive mode where consecutive residues are epimerized appears to be unlikely because the uronic acid residues are rotated 180° with respect to each other and the enzyme would then have to rotate while sliding around the polymer chain. We have previously demonstrated processivity for AlgE4 acting on polyM generating long alternating MG stretches or for AlgE6 when acting on polyMG. In both of these cases the enzymes act in processive modes, where every second M is converted while the enzymes slide along the polymer; however, this does not require the enzyme to rotate. Whether the properties of AlgEM1 are due to an increase in processivity or a result of an enhanced affinity for pre-existing G residues is not possible to conclude from the present experiments.

Alignment of Mutant and Wild-Type A-Modules Elucidates Sequence Properties Mediating Changes in Enzymatic Activity. To reveal possible structural features in the mutants underlying the enzymatic properties described

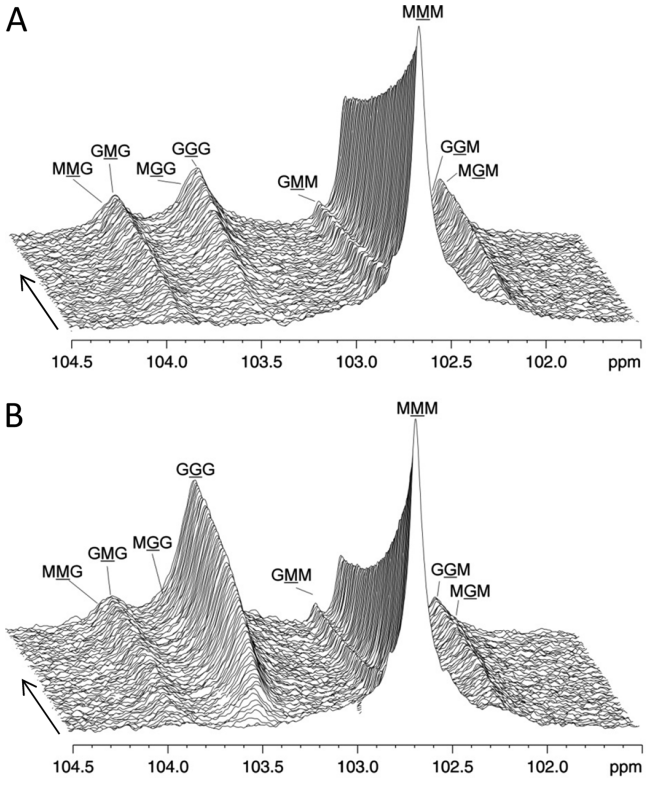

Figure 3. Continuously recorded NMR spectra showing epimerization of ^{13}C-labeled polyM with AlgE6 (A) and mutant enzyme AlgEM1 (B). Substrate (20 mg/mL) and enzyme were mixed and immediately inserted into the NMR instrument before recording of spectra every 15 min. Reactions were performed in MOPS, pH 6.9 with 75 mM NaCl and 2 mM $CaCl_2$. The position of each of the eight possible triads in the spectra is indicated, and the M or G moiety giving rise to the signal is underlined. Arrows indicate increasing reaction times. It should be noted that the enzyme reactions were not run to complete epimerization of the substrate, so the end composition of the resulting alginates in this experiment is not directly comparable to results given in Table S3 of the Supporting Information.

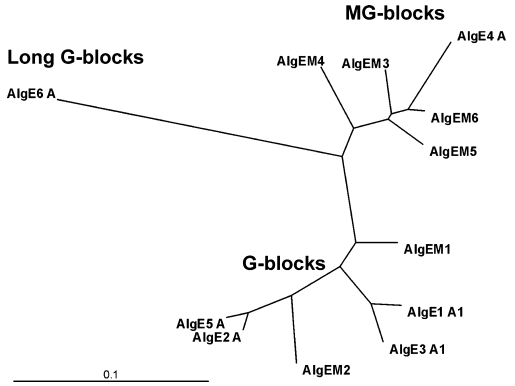

Figure 4. Phylogenetic tree displaying the relationship between the wild-type and mutate A-modules in relation to their function on alginates. Alignment of mutant and wild-type protein sequences was done with ClustalX[37] and visualized with TreeView.[38]

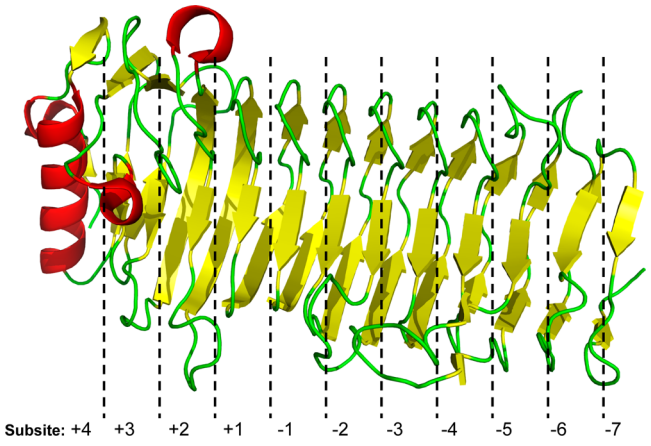

Figure 5. Ribbon structure of the A-module from AlgE4 with subsites for the substrate indicated. The division of the subsites is based on prolongation of the mannuronan trisaccharide bound to A-module from AlgE4 (Protein Data Bank code 2PYH) in the substrate binding groove. Subsite +4: Met1-Glu51, +3:Pro52-Ser91, +2: Ala92-Asn123, +1: Gly124−156+Asp178, −1: Thr157-Asp178+Leu228, −2: Tyr179-Leu228, −3: Glu229-Lys255, −4: Met256-Tyr278, −5: Gly279-Tyr318, −6: Thr319-Arg343, and −7: Asn344. The structures were visualized and analyzed with PyMol.[36]

above, we sequenced the genes encoding the A-modules of mutants AlgEM1−6, and the resulting protein sequences were compared with wild-type A-modules. Sequence properties, that is, similarity to wild-type A-modules and introduction of new amino acid residues due to error-prone PCR, are shown in Table S4 in the Supporting Information. The relationship between the A-modules is visualized in a phylogenetic tree (Figure 4), and alignment of sequences is given as Supporting Information (Figure S1A−C in the Supporting Information). The phylogenetic tree displays three groups, which can be characterized as formers of MG-blocks, G-blocks, and long G-blocks, the latter group comprising only AlgE6. A general tendency found for AlgE6 is that more amino acid residues promoting hydrogen bonding and hydrophobic interactions are identified along the alginate binding groove in the A-module compared with the other G-block-forming enzymes (Figure S1 in the Supporting Information).

Sequence alignment analysis shows that the A module of AlgEM1 is most similar to the G-block-forming A1 modules of AlgE1 and AlgE3 before the position of the active site, whereas it is most similar to AlgE6 after the active site. Furthermore, in AlgEM1 the alginate binding subsite at −3, −4, and −6 seems to have more residues supporting alginate binding through hydrogen bonds and hydrophobic interactions (e.g., Arg, Leu, Ser, Asn) than the other G-block formers (Figure 5, Figure S1 in the Supporting Information). The AlgEM2 A-module is most similar to AlgE2 and AlgE5, which are also both capable of producing G-blocks. Moreover, AlgEM2 has a substitution in subsite −2 (Ala220Asn) that putatively supports stronger alginate binding through hydrogen bonding. Furthermore, some amino acid residues change the N-terminal α-helix interfacing the rest of the protein and lead to minor rearrangement in packing of the hydrophobic core under subsite +1 (Val136Ala) and −1 (Ile200Val). This might result in a deeper substrate binding groove, hereby enlarging the contact surface to the alginate polymer. Because both AlgEM1 and AlgEM2 possibly have improved alginate binding properties compared with AlgE6, this can partially explain their ability to form long G-blocks. The mode of action for the G-block forming A-modules except for AlgE6 acting on polyMG has been characterized as a preferred attack mechanism,[44] and accordingly the N-terminal part before the catalytic site of AlgEM1 and AlgEM2 seems to originate from these A-modules.

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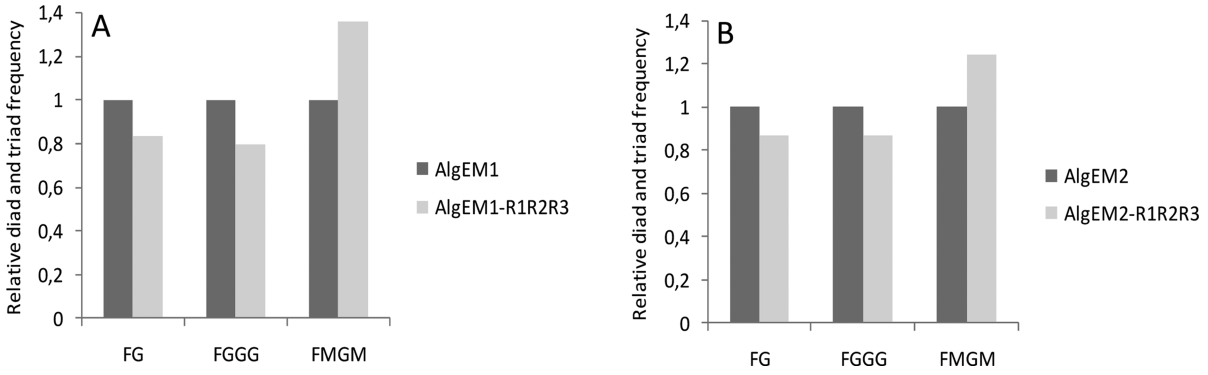

Figure 6. Relative frequencies of F_G, F_{GGG}, and F_{MGM} obtained by epimerization of polyM with mutant enzyme AlgEM1 and AlgEM1-$R_1R_2R_3$, that is, A-module from AlgEM1 and R-modules from AlgE6 (A). Corresponding results for AlgEM2 and AlgEM2-$R_1R_2R_3$ (B). Epimerization was performed with polyM (10 mg) and enzyme (0.2 mg) in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$.

Combining the preferred attack mode of action with better alginate-binding properties, that is, improved processivity may then have resulted in the high G-block-forming epimerases AlgEM1 and AlgEM2. The mutants AlgEM3−6 (Table S4 in the Supporting Information) have almost all mutations at the N-terminal part before the catalytic site, probably resulting in the ability to form G-blocks. After the active site they are almost identical to the AlgE4 A-module, presumably mediating the ability to create alternating structures (Figure S1 in the Supporting Information).

Introduction of R-Modules from AlgE6 ($R_1R_2R_3$) Behind Mutant A-Modules from AlgEM1 and AlgEM2 Modulates the Epimerization Pattern of the Enzymes. As a consequence of the construction strategy (see Materials and Methods), all mutants should contain the same C-terminal end, that is, 177 residues constituting the AlgE4 R-module. However, for AlgEM2 and AlgEM6, a deletion in the *Xma*I restriction site used for cloning leads to a frame shift and translation into a 52 residue long C-terminal end following the A-module. It has been previously shown that the A-modules alone are sufficient for epimerization,[45] and apparently the addition of 52 random residues C-terminally does not affect the enzymes detrimentally. Although it has been shown that the R-modules are Ca^{2+}-binding and stimulate the activity of the A-modules when present,[45] the function of the R-modules is not fully understood. AlgE6 is an efficient G-block forming epimerase with three R-modules ($R_1R_2R_3$), and we wanted to explore the effect of combining these R-modules with the mutant A-modules from AlgEM1 and AlgEM2. DNA sequences encoding $R_1R_2R_3$ were synthesized and cloned into the vectors encoding the two mutant A-modules, resulting in the expression of hybrid enzymes, AlgEM1-$R_1R_2R_3$ and AlgEM2-$R_1R_2R_3$. As previously described, polyM was epimerised, and the resulting alginate structures were analyzed by NMR. For both enzymes, the F_G obtained was lower for the hybrids containing $R_1R_2R_3$ than for the original mutants isolated from the screen (Figure 6). It also appeared that the level of alternating MG structures increased with a concomitant decrease in G-blocks. Furthermore, time-resolved NMR spectra recorded from the epimerization of polyM with AlgEM1-$R_1R_2R_3$ (Figure S2 in the Supporting Information) showed that this enzyme behaved kinetically like AlgE6 and not like AlgEM1. These results show that the R-modules modulate not only the epimerization rate as previously thought but also the epimerization pattern of the individual enzymes. This might be connected to individual differences between the R-modules in binding affinity for the epimerized substrate, which will influence the number of residues that are epimerized before the substrate is released from the enzyme.

Residue Asp119 in the A-Module of the Epimerases Is Important for the Epimerization Pattern. AlgEM3−5 displayed similar properties in introducing both MG- and G-blocks, although being up to 92% identical to AlgE4, which does not make G-blocks at all. Of these three mutants, AlgEM3 was found to have one substitution that was considered to be of particular interest (Table S4 in the Supporting Information). The tyrosine substituting the aspartic acid residue found at position 119 in all wild-type enzymes might influence the catalytic activity due to being in the vicinity of catalytic site (Figure 7A). To elucidate whether this residue is somehow determinative for the epimerization pattern, we made targeted substitutions at position 119 in both AlgE4 and AlgEM3. Site-specific mutations were introduced in the corresponding genes,

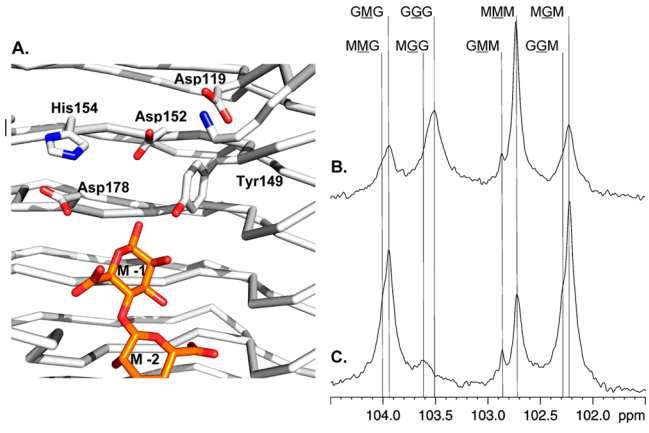

Figure 7. View of the active site with substrate-bound (PDB code: 2PYH) and ^{13}C NMR spectra for end-point products from the epimerization reaction. (A) Bound mannuronan trisaccharide (only two (M-1 and M-2) of the sugar units are shown on the figure), the catalytic residues (Tyr149, Asp152, His154, and Asp 178), and ionic pair (Lys117, Asp119) are shown in stick representation. The ionic pair is involved in the coordinate of the carboxyl group on the mannuronan at subsite +1. The structure was visualized with PyMol.[36] (B) End-point products as a result of epimerization with the GG-block forming epimerase AlgE6. (C) End-point products as a result of epimerization use mutant epimerase AlgEM3. This clearly shows that the mutant epimerase AlgEM3 is not able to form more than only GG-block and not polyG and fill in a G in a GMG sequence.

dx.doi.org/10.1021/bm4005194 | *Biomacromolecules* XXXX, XXX, XXX−XXX

and epimerization of polyM was performed with purified protein samples. Results from NMR analysis of the epimerized polyM are given in Table S5 in the Supporting Information. AlgE4 is forming strictly alternating MG-blocks, but by substitution of Asp119 to Tyr, Phe, or Ala, the resulting enzymes introduce a low level of two sequential G residues (F_{GG} = 0.044 to 0.089). Furthermore, a closer inspection of the end-point ^{13}C spectrum from the time-resolved NMR experiments shows that AlgEM3 (Figure 7C) is not able to produce GGG triads. This might indicate that GG-formation takes place because the enzyme moves only one residue forward instead of two before making the next epimerization reaction, and hereafter AlgEM3 dissociates from the alginate polymer. This points to residue 119 as one of probably many that are directly involved in determining epimerization pattern and also indicates that a negative charge on the side chain might be essential for obtaining the strictly alternating MG structure, as is the case for AlgE4. Effects on the epimerization pattern are also found for AlgEM3 when substituting Tyr119 to Asp or Arg, which in both cases leads to an increase in the frequency of MG. Again, this points to charged residues as being determinative for epimerization pattern.

CONCLUSIONS

In the present study, we have constructed a library of mutant mannuronan C-5 epimerases by gene shuffling and error-prone PCR. Furthermore, a screening method was developed that enabled the identification of specific alginate sequences created by the mutant enzymes. By screening nearly 1000 mutant strains we were able to isolate two epimerases that are more efficient in introducing G-blocks in polyM than the naturally occurring enzymes, and one of these apparently acts kinetically different than the G-block former AlgE6. Such mutant epimerases with new or improved functionalities can be valuable tools in future in vitro design of alginate structures and especially in manufacturing G-rich alginates, of which there is inadequate supply in the global alginate market. The results obtained also emphasize the need for careful design of the screening protocol, in that the AlgEM1 and AlgEM2 did not display superior properties to AlgE6 in epimerizing an alginate substrate with a complex composition. For isolation of robust enzymes with an industrial potential for upgrading of algal alginates the current method can be expanded to screen for enzymes efficient in epimerizing algal alginates under conditions of, for example, defined pH, temperature, ionic strength, and salinity that are relevant for the actual process.

ASSOCIATED CONTENT

Supporting Information

Vectors used and constructed and primers utilized are listed in Tables S1 and S2. Results from NMR analysis of polyM epimerized with AlgE4, AlgE6, and the epimerase mutants are shown in Table S3 and S5. Sequence properties of the A-modules from epimerase mutants AlgEM1−6 are shown in Table S4. Multiple sequence alignment of epimerase A-modules from *A. vinelandii* and the mutant A-modules for AlgEM1−6 is shown in Figure S1. Continuously recorded NMR spectra for epimerization of polyM with AlgEM1-$R_1R_2R_3$ are shown in Figure S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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[BTN] [Case 3] - Confidential

Institution: SINTEF AS
Administrative unit: Department of Biotechnology and nanomedicine (BTN)
Title of case study: Microbial biotechnology
Period when the underpinning research was undertaken: 2011-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021
Period when the impact occurred: 2011-2021

1. Summary of the impact: Microbial biotechnology has been a core activity for BTN historically and in the evaluation period (2011-2021). This activity, that has more than tripled in annual volume in the evaluation period (71 mill NOK for BTN in 2021), includes molecular biology, synthetic- and systems biology, high throughput screening, 'omics and other advanced analyses, fermentation and bioprocess technology. BTN's generic competence and state of the art infrastructure platforms have been developed and used to secure value and impact for our collaborators in academia and industry and for the society. **Impacts**: i) contribution to national and international competence building and cooperation, ii) access to state-of-the-art infrastructure iii) examples of innovations with impact for our collaborators and customers, iv) societal impact and dissemination of BTN's research.

2. Underpinning research: BTN has developed the branch of knowledge on microbial biotechnology across three different interlinked key fields (see below) with impact at industrial and fundamental levels both nationally and internationally. BTN's research in this field is performed in national fundamental projects, in huge European projects, where several are coordinated by BTN, and in numerous projects with industry. BTN collaborates with excellent research groups and innovative industry customers and is a trusted collaborator both for academia and industry (see Appendix 1 for documentation). BTN is the leader of the National Centre for Research-Based Innovation on Industrial Biotechnology (https://sfi-ib.com/). The SFI-IB encompasses BTN's expertise on microbial biotechnology and is based on decades of output and excellence in fundamental and industry-oriented research operated by BTN and its closest national co-operators and industry customers. 4 research institutions and 16 companies are partners in the centre.

A. Bioprospecting (Ref 1-3): BTN is one of the key players in the development of the Norwegian bioprospecting field with the first activities started in 2003. Throughout the years BTN has established strain collections and/or metagenome libraries from multiple environmental niches (from Svalbard terrain, oil reservoirs, moose's gastrointestinal tract and sediment-, neustonic- and sponge associated biomes from the Trondheim fjord). From 2011 to 2021 the key activities have been on establishing and use of technology for utilizing all these resources (strain collection, environmental samples, strain and metagenome sequence data sets, cloned metagenome (e.g. fosmid) libraries, as well as bioinformatics workflows, HTS and microbial production) for finding and producing novel enzymes and bioactive molecules. Examples of bioprospecting based projects where BTN is or have been in a key position are; European: Metafluidics (2016-2020), Marbles (2021-2026), MarBioTech (2018-2020), Oxypol (2015-2018), AtlantECO (2020-2025), EnZylaScope (2021-2025); Bluetools (2022-2026); National: Genome-based bioprospecting (2011-2014), NorZymeD (2013-2018), MarPol (2013-2017), MetaBiopOR (2011-2014), SFI-IB (2020-2028), Oxymod (2017-2022).

B. Strain engineering, enzyme evolution, synthetic- and systems biology (Ref 4-6): One of BTN's strongest assets is the development of advanced cell factories for efficient and sustainable production of enzymes, biopharmaceuticals, platform chemicals, biopolymers, food and feed ingredients using a range of substrates. These activities are rooted in the earlier research (2000-2010) at the department, where BTN started developing industrial genetics as a strategic area of research. This was, and is still done, in close collaboration with strong national and international research groups, supported by strategic recruitment and continuous investment in new infrastructure (bioinformatics, 'omics, screening and fermentation). In the evaluation period BTN has established a strong knowledge base on numerous relevant strains producing a variety of industrially relevant products accompanied by establishment of a vast toolbox for the engineering of large sets of strain (bacteria, yeast and fungi) that includes targeting metabolic engineering (i.e. CRISPR/Cas); system

biology with implementation of 'omics driven genome scale metabolic modelling; random mutagenesis for strain improvement and high-throughput characterization of the cell factories.

Nationally and internationally BTN has been at the technological and scientific forefront in the development, understanding and optimization of cell factories for production of important products in partnership with Norwegian universities (NTNU, NMBU, UiO) and prominent research groups internationally. Notable project examples on fundamental research on establishment (and use) of cell factories are: i) Bioactive secondary metabolites: RCN: INBioPharm (2016-2019), European: Tacrodrugs (2016-2020), SYSTERACT (2015-2018), Syntheroids (2018-2021) ii) Amino acids from C-1 feedstocks: RCN-GassMaks Cadaverine (2011-2014), European (ERA)-Systems biology of methylotrophy (2014-2017) and C1-Pro (2018-2021); iii) Microbially produced lipids: RCN-Mira (2014-2019) and AurOmega (2017-2021); iv) Biopolymers, biochemicals and biofuels: RCN: BioZEment (2019-2021), Bio4fuels (2017-2025), European: Bester (2018-2021), DAFIA (2017-2021), Perfecoat (2021-2024); v) Industrial enzymes: Polysaccharide Modifying enzymes (2016-2021), AlgmodE (2021-2025), SFI-IB (2020-2028).

The examples given above are from fundamental researcher driven projects, but the majority of them (especially the European) have had an applied focus and include collaboration with industry. Development of high producing cell factories is in addition a key activity in numerous projects where BTN is partner in industry owned projects. These projects normally includes both strain and bioprocess optimisation and are described in the next section.

C. Fermentation process development (Ref 7-8): BTN is the leading national group on bioprocesses and microbial fermentation technologies which have been key activities in the department since its establishment in 1979. This platform has been steadily expanding to cover nationally leading laboratories for high throughput screening, advanced analyses and fermentation. The fermentation infrastructure includes microreactor systems (ml-scale), 56 laboratory scale fermentors (0.2-L to 3-L) and pilot scale reactors (60-L and 200-L). Most of the projects mentioned under Bioprospecting and the cell factory sections above have included activity on fermentation. Furthermore, the development of industrial fermentation processes has been the focus in numerous of industry projects (RCN co-funded Innovation projects for companies (IPNs), and projects 100% financed by industry). Several of these activities have led to processes now under industrialisation or already established by our customers. The largest industry customers the last years have been **Leo-Pharma** (antibiotics), **Xellia Pharmaceuticals** AS (antibiotics), **AlgiPharma** (biopolymer-based drugs, industrial enzymes), **Biosergen** (antifungals), **Nykode** (DNA-based vaccines), **PHARMAQ** (fish vaccines), **Vectron Biosolutions AS** (enzymes), **ArcticZymes** (enzymes), **NattoPharma** (vitamins). For more information see attached statements from companies regarding impact of the collaboration with BTN (Appendix 1). The activity for the industry has included advanced strain engineering, classical mutagenesis and high throughput screening, fermentation and downstream processing, demonstration and technology transfer to our customers directly or to our customer's sub-contractors. For more details and links to industry projects see also form 11a in the Self-assessment for the administrative unit BTN.

As mentioned, several of the European projects involves fermentation activity. Another relevant project example is the recently initiated European Green Deal project PyroCO2 https://www.pyroco2.eu/ (2021-2026). The project has a total funding of 43 mEUR. The project will demonstrate the scalability and economic viability of carbon capture and utilization (CCU) to make climate-positive acetone out of industrial CO_2 and renewable electricity derived hydrogen by fermentation. Core of the technology is an energy-efficient thermophilic microbial bioprocess that is going to be demonstrated in small production scale (100 m^3). PyroCO2 has an industry-driven consortium of 20 partners (11 being SME/LE) and is coordinated by BTN.

For references to the research and details of the impact, see section 3, 4 and 5.

Persons, period of work in the evaluation period, position held in 2021, [Contribution to research in A) Bioprospecting, B) Strain engineering, enzyme evolution, synthetic- and systems biology, C. Fermentation process development]

- Trond Erling Ellingsen, 2011-2021, Research director [A, B, C]
- Håvard Sletta, 2011-2021, Research manager [A, B, C]
- Francesca Di Bartolomeo, 2019-2021, Research manager [B, C]
- Geir Klinkenberg, 2011-2021, Research manager [A, B, C]
- Trygve Brautaset, 2011-2015, Professor (synthetic biology) NTNU [B]

- Alexander Wentzel, 2011-2021, Senior scientist [A, B, C]
- Anne Tøndervik, 2011-2021, Senior scientist [B, C]
- Inga Marie Aasen, 2011-2021, Senior scientist [B, C]
- Kjell Domaas Josefsen, 2011-2021, Senior scientist [A, B, C]
- Kristin Fløgstad Degnes, 2011-2021, Research scientist [A, B]
- Susan Maleki, 2015-2021, Research scientist [B, C]
- Tonje Marita Bjerkan Heggeset, 2011-2021, Research scientist [A, B]
- Snorre Sulheim, 2016-2021, Research scientist [B]
- Simone Balzer Le, 2013-2021, Research scientist [B]
- Ingemar Nærdal, 2013-2021, Research scientist [B, C]
- Giang-Son Nguyen, 2015-2021, Research scientist [A, B]
- Tonje Husby Haukaas, 2018-2021, Research scientist [B, C]
- Anna Nordborg, 2013-2021, Research scientist [B, C]

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Bioprospecting:

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Fermentation processes development

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4. Details of the impact

The details of the impact of BTN's research within microbial biotechnology can be divided in 4 main outputs.

Contribution to national and international competence building and cooperation.

BTN's research is almost exclusively performed in collaboration with national and international research institutions and/or industry in national or international projects. The activity within microbial biotechnology includes both national and European basic research projects, and confidential research for our industry customers. Using 2021 as an example the activity on Microbial biotechnology represented approximately 40% (71 mill NOK for SINTEF) of BTNs total activity, with

split 50:50 between confidential industry research and other projects. In average the BTN share of the projects represent approximately 15-20% of the total project volume, thus the impact for our collaborators in joint projects with BTN is huge. This includes grants for PhDs and postdoctoral researchers and scientists in the collaboration research institutions and secures access to non-dilutive research funding (e.g in IPN-projects and European projects) for our industry customers. Our collaborative model of operation, combined with our scientific competence and state of the art laboratories have positioned us as a trusted partner for industry and academia. The internal training and strategic recruitment of excellent scientist in BTN, gives us the opportunity to take leadership in huge national and international projects (e.g INBioPharm, SFI-Industrial Biotechnology, PyroCO2, Perfecoat). NTNU has historically and in the evaluation period been BTN's most important academic collaborator, both in basic projects and in collaboration projects with industry. Projects active in the evaluation period (2011-2021) within microbial biotechnology represents a total project volume for BTN and NTNU of approx. 550 mill NOK (approximately 50:50 distributed between BTN and NTNU). From BTN this includes direct collaboration of 23 PhDs (finished or under education) and numerous master students with competence highly relevant for national biotech-based industries. For BTN value creation for industry is the main target, but fundamental projects are necessary to establish the competence and technology relevant and required to serve the industry. Furthermore, IP generated in fundamental projects is of potential great value for industry who want to licence/utilize this IP, furthermore this IP is basis for spin-out of start-up companies. Involvement of national industry in European projects where SINTEF has a key role, is also highly valuable for the industry.

State-of-the-art infrastructure. BTN has over the years been developing infrastructure platforms pinpointed for cost efficient microbial bioprocess optimisation (equipment value >150 mill NOK). BTN has co-located, nationally-leading laboratories in high throughput screening, mass spectrometry and fermentation, and also excellent facilities for microbiology molecular biology (including several BSL2 laboratories). We have established a huge array of methods and models that are important for BTNs activities. These facilities and established methods are highly beneficial for our collaborators. We work with both start-ups and established industry companies and are able to offer research infrastructure not possible to acquire or implement at the industry companies' site without huge investments in instruments and competence. For several companies we serve as their main laboratory (see e.g. letters from AlgiPharma and Biosergen, in Appendix 1). Our infrastructure has been very important for establishment and maintenance of long-term collaborations, e.g. we have had continuous collaboration with Xellia Pharmaceuticals (previously Alpharma) since 1991 (see attached letter and below).

Examples of innovations with impact for our collaborators and customers. In the evaluation period BTN has collaborated with numerous companies within microbial biotechnology, the majority of the industry collaboration is confidential research, and thereby not public information. In the period from 2017-2021, a total of 92 confidential reports were delivered from BTN. Selected examples of impact of collaboration connected to industrial research are given below. More details are given by collaborating companies in Appendix 1 and in RCNs Prosjektbanken (https://prosjektbanken.forskningsradet.no/) for description of RCN co-funded IPN projects (references limited to 2011-2021).

Development of novel drugs for treatment of invasive fungal infections – ***Biosergen AS.***

Biosergen was established by SINTEF Technology Transfer Office (TTO) based on results from basic research and patented technology generated from activities at NTNU and SINTEF BTN (Ref 9). The bacterial strain producing their lead drug candidate antifungal drug BSG005 (now in clinical stage) was established based on genetic engineering of the nystatin biosynthetic cluster in the soil bacterium *Streptomyces noursei.* BTN was involved from start and in addition to contributing to the strain development work, BTN has established the fermentation process and in the last years also been involved in establishment of formulation and testing of the drug products (see Appendix 1 for additional details) and technology transfer of the manufacture process to GMP-CDMOs. Key RCN co-funded IPN projects; RCN 168352, RCN 256886, and RCN 309865.

Alginate Oligomer based therapeutics and drug delivery systems – ***AlgiPharma AS***

BTN has been cooperating with AlgiPharma since 2006, including development of several manufacturing processes for different alginate oligomer based products, and formulation thereof. This has included development of genetically engineered strain and fermentation process for production of microbially produced alginate oligomers and establishment of production technology for industrial novel enzymes used in the manufacturing. These novel processes are now being scaled up to full industrial scale (see Appendix 1 for details on the collaboration). Key RCN co-funded

IPN projects RCN 221576, RCN 281920, RCN317799, RCN 228570, RCN 245598. AlgiPharma is partner in SFI-industrial biotechnology.

Production technology for heterologous enzyme production – ***Vectron Biosolutions AS***

BTN has been collaborating with Vectron since 2008. All projects have been focusing on development and use of Vectron's microbial protein expression technology platform. Some of the Vectron projects have been in cooperation with another national enzyme company, ArcticZymes. Vectron and ArcticZymes are both partners in SFI-Industrial biotechnology. For details regarding impact of the collaboration between Vectron and SINTEF BTN, see Appendix 1. BTN's work has been financed directly from Vectron and in RCN co-funded IPN projects (RCN 193193, RCN 296624 and RCN 256877).

Development of microbial bioprocesses for antibiotics – ***Xellia Pharmaceuticals AS***

BTN has been collaborating with Xellia Pharmaceuticals since 1991, in numerous of strain and development projects for establishment of cost-efficient manufacturing processes for important antibiotics. Several of the processes that has been developed are now established manufacturing processes at Xellia. This long-term collaboration has had impact for Xellia and for SINTEF and furthermore increased the national competence in development of industrial biopharmaceutical processes. This competence has been beneficial for many of BTNs other industry customers. See Appendix 1 for details regarding recent collaborations. The activity at SINTEF has been financed both from the company directly, and through RCN co-funded IPN projects (recent IPNs: RCN 219652, RCN 219652, RCN 296372, RCN 187999).

Development of manufacturing processes for DNA-based vaccines – ***Nykode Therapeutics AS***

BTN has for the last years collaborated with Nykode with evaluation of vaccine candidates and development of efficient microbial manufacturing processes for production of candidate products. Furthermore, this collaboration has included technology transfer from BTN to GMP-CROs. See Appendix 1 for details, RCN-IPN projects: RCN 321622 and RCN 310070 in addition to financing directly from Nykode.

Development of a new manufacturing process for Fusidic acid – ***Leo Pharma.*** Leo Pharma (Denmark) has the last years collaborated with BTN on development of a new production strain and suitable process for production of the antibiotic Fusidic acid. The new process will be implemented in production scale in Leo Pharma's new production plant in Ballerup, Denmark. The activity (see Appendix 1 for details) has been financed by Leo Pharma.

Societal impact and dissemination of BTNs research: BTNs Microbial biotechnology activities are directly addressing SINTEF's vision: Technology for a better society. We work with science addressing huge societal needs and challenges, including unmet medical needs, green technology and project and innovations needed for a transition to a future sustainable bioeconomy. BTN contributes actively to the long-term competence building needed for a growing biotech-based industry. BTN takes responsibility for leading huge national and international projects. Furthermore, we publish our open research in open access journals, and present scientific findings for the public and in popular science channels. One example of dissemination from the SINTEF coordinated Centre of Digital Life projects INBioPharm is given in Ref. 10.

5. Sources to corroborate the impact (for references 1-8, se section 3)

9. The extensive research behind BSG005 – Biosergen https://biosergen.net/bsg005/publications
10. Dissemination from the RCN Digital life project InBioPharm (72 registrations in Cristin) https://wo.cristin.no/as/WebObjects/cristin.woa/wa/fres?action=sok&prosjektfinansieringskilde=NFR&prosjektEksternkode=248885

Appendix 1: Impact of collaboration with BTN for the companies AlgiPharma AS, Biosergen AS, Xellia Pharmaceuticals AS, Nykode Therapeutics AS, Vectron Biosolutions AS and Leo Pharma

Appendix 2: PDFs of article and book chapter not available on-line (Ref 3 and Ref 4 in section 3

Appendix 1: BTN -Impact case 3 – Microbial biotechnology

Impact of collaboration with SINTEF BTN, letters from key collaborating companies:

- **AlgiPharma AS**
- **Biosergen AS**
- **Xellia Pharmaceuticals AS**
- **Nykode Therapautics AS**
- **Vectron Biosolutions AS**
- **Leo Pharma**

AlgiPharma

27th Jan 2023

To whom it may concern,

I am writing on behalf of AlgiPharma in support of the impact cases (evaluation period 2011-2021) presented by the SINTEF Industry, Department of Biotechnology and nanomedicine.

AlgiPharma is a privately owned clinical stage biopharmaceutical company with a focus on developing its proprietary alginate technology for: Delivery platforms for nanoparticles, small and large molecule-based drugs; Drug conjugates, adding functionality and reducing toxicity of parent compound; and as a stand-alone active pharmaceutical ingredient in disease areas such as cystic fibrosis (CF), Chronic Obstructive Pulmonary Disease (COPD), and infectious diseases. AlgiPharma has pharmaceutical scale production capabilities for its technology and is protected by a broad family of patents. AlgiPharma's aim is to address unmet medical needs and fight diseases effectively through our innovative alginate technologies targeting those diseases where there is a clinical need to mitigate abnormal mucus accumulation, microbial infection, biofilm formation and antibiotic resistance. AlgiPharma has worked closely with the Biotechnology and Nanomedicine research teams at SINTEF Industry since 2006.

Through the coordination, expertise and leadership provided by Håvard Sletta at SINTEF Industry, their multi-disciplinary research teams have been a major contributor to AlgiPharma's ongoing success in the development of its alginate oligosaccharide technology. The broad range and depth of expertise at SINTEF Industry has been and continues to be a crucial resource in AlgiPharma's drug development and manufacturing programs, including microbial biotechnology, alginate polymer production and nanotechnology. This long-standing collaboration has been central in securing non-dilutive research funding (more than 85 MNOK) for AlgiPharma's research activities including the following Norwegian Research Council co-funded Innovation projects for industry (IPN) grants awarded during the 2011-2021 impact case evaluation period: BIA grant: Tailored OligoG (228542); BIA grant: OligoG Formulate (245598); NANO2021 grant: MucosALG (281920).

SINTEF Industry has played a key supporting role in the evaluation of AlgiPharma's primary drug candidate in four phase 2 clinical trials (NCT01465529; NCT03822455; NCT02157922; NCT02453789) evaluating a new drug treatment for Cystic Fibrosis (CF) which is a life-threatening lung disease. SINTEF Industry's research expertise have allowed us to gain a better understanding of the complex mechanisms of action and therapeutic potential of AlgiPharma's alginate oligomer technology for the treatment of respiratory, wound healing, drug delivery and infectious diseases. The teams at SINTEF have also played a key role in realizing commercial value for AlgiPharma, in both knowledge and intellectual property.

SINTEF has been an enabling force in AlgiPharma gaining investment for further research and commercial scale-up of OligoG production processes from the Norwegian Research Council and the UK Technology Strategy Board. SINTEF has played a central role in AlgiPharma's manufacturing program, securing significant non-dilutive funding in support of the development of commercially relevant manufacturing processes: Innovate UK: Algiferm (131142); ALGIPRO (102148); Microbialg (228570); Oligo-DSP (281907); Algi-SCALEUP (317799). AlgiPharma is also a partner in the SINTEF led national Centre of excellence, SFI-Industrial biotechnology.

If you have any further questions, please do not hesitate to contact me.

Yours sincerely,

Dr Phil D. Rye
Chief Scientific Officer
AlgiPharma AS
Email: phil.rye@algipharma.com
Tel: +47 97503033

Biosergen

22 January 2023.

Biosergen AS and Sintef AS (Department of Biotechnology and nanomedicine) R&D co-operation on BSG005 – a new antifungal drug for use in Invasive Fungal Infections (IFI).

Impact of Sintef work on Biosergen's development.

The co-operation between Biosergen and Sintef goes many years back. The company was originally started as a spin off by SINTEF TTO, based on technology established over years of basic research by SINTEF and Norwegian University of Science and Technology (NTNU).

During the last 4 – 5 years and until end of 2021 Sintef has been heavily involved in the development of BSG005. We have basically seen the Sintef lab as "Biosergen's lab". The co-operation for our "standard" BSG005 has mainly run in two ways. Either laboratory developments were done initially at Sintef on processes of manufacturing BSG005 or formulation and then moved out to our manufacturing partner for upscaling and GMP manufacture OR manufactured material or test results from other labs have been checked at Sintef to confirm data from external suppliers. Sintef has also performed improvements on the processes, participated in large upscaling for instance on the first step – the fermentation - at our supplier in Spain, where all the special knowledge at Sintef played a crucial role in achieving the right processes.

Sintef labs have built up an intimate understanding of the BSG005 molecule, have done important test on adherence to plastic surfaces of the infusion bags and lines – something very important before we initiated our "First in Man" clinical trial – so we could compensate in the important pharmacokinetic investigation in humans. The Sintef lab has performed many other important tests on BSG005 that has improved Biosergen's understanding of how to handle the final product, when going into the clinical phase – what external factors were important for drug stability as the BSG005 molecule in very sensitive to outside factors such as light, temperature, oxygen and pH.

For Biosergen the very close contact and co-operation with Sintef has been vital – and still is – for the development of the first generation BSG005.

In 2019 Biosergen approached Sintef with an idea of making BSG005 available as a "NANO-particle formulated version" with the aim to develop a version to specifically target the lung (typically the first organ attached in an IFI). There were several very important feedbacks from the Nano-group at Sintef. They had recently developed new Nanoparticles called PACA and they had a patented invention, that could make the idea of a lung targeted formulation possible much faster than anticipated.

For Biosergen that really was "a lucky punch", as we could see that the knowhow in the Nano-group was more, than we had hoped for. Together with the Sintef Nano-group the project has been moving on testing many types of Nanoparticles, the loading of BSG005 into the particles. The sensitivity of BSG005 created some issues and the testing of alternative Nanoparticles moved on through 2021.

For Biosergen it has also for the Nano development been very important that both the "standard BSG005 development" as well as the Nano-development are in the same overall Sintef-group (Department of Biotechnology and nanomedicine) and that knowhow and test data are coming from same lab with the same technicians, so all information is shared within the full group.

And we expect to get a breakthrough in 2023, which may have significant impact on Biosergen as a company.

Peder M Andersen, MD

CEO

xellia
PHARMACEUTICALS

To whom it may consern

27 January 2023

Impact of the collaboration between SINTEF Industry, biotechnology, and Nanomedicine and Xellia Pharmaceuticals AS'

Xellia is a leading global industry supplier of several established anti-infective products including vancomycin, colistimethate sodium (CMS), bacitracin and daptomycin. Our B2B business in the global anti-infective market, serves customers from over 500 branded, specialty and generic pharmaceutical companies across 70 countries, while Xellia continues to develop a fast-growing US injectables business delivering critical care medicines, with direct presence in the US institutional market.
Xellia's purpose is to save lives by leading the fight against infections. We achieve our goal by prioritizing the patient and providing a strong and resilient supply of critical care medicines.
Xellia's products are used for treatment of serious bacterial and fungal infections globally.

SINTEF Industry, biotechnology, and Nanomedicine, (SINTEF-BTN) and Xellia have had an on-going cooperation for more than 30 years concerning research with the purpose of developing new or improving existing antibiotic production processes. The work has focused on the development of better production strains for antibiotics such as colistin, daptomycin, anidulafungin, vancomycin, amphotericin and dalbavancin. Furthermore SINTEF-BTN has been an invaluable partner regarding genomic sequencing of different production strains and the analysis of a range of different impurities and related compounds including screening for new types of antibiotics. Xellia and SINTEF-BTN are currently cooperating in a project as part of the newly established SFI Industrial Biotechnology.

In short, the collaboration has contributed to making important lifesaving medicines more available and have significantly contributed to the steady improvement in competence at Xellia and SINTEF-BTN as well as improved instrumentation at SINTEF-BTN.

Knut Danielsen
Sr Director R&D API

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SINTEF, Department of Biotechnology and Nanomedicine
v/ Håvard Sletta
Strindvegen 4
7034 Trondheim

January 30th, 2023

Collaboration between Nykode Therapeutics AS and SINTEF, Department of Biotechnology and Nanomedicine

Nykode is an Oslo-based clinical stage biopharmaceutical company founded in 2006. Our aim is to generate game-changing therapeutics to treat cancers and infectious diseases with a high unmet medical need. Our unique modular vaccine technology is a DNA plasmid encoding a protein consisting of three functional units, targeting antigens to antigen presenting cells in a dimer format, which is essential for inducing rapid, strong and long-lasting immune response.

To explore the technology in clinical studies and to develop products suitable for commercialization, a robust manufacturing process of high quality pDNA is crucial. Sintef has been an important partner in the development of a fermentation process for production of DNA plasmid, included early phase screening of host strains, fermentation media and process parameters, optimization, robustness and scale up studies. Further, Sintef has contributed to the transfer of the process to Contract Manufacturing Organizations (CMOs), carried out studies to modify the process to the CMOs and troubleshooting of production related events. The result is a high yielding, robust platform fermentation process that has been scaled up to large production scale, validated with a large range of plasmids and gives the opportunity to transfer new products candidates to production rapidly and with reduced risks of failure. Another valuable benefit is Nykode ownership to the fermentation process which increases flexibility of the production of comparable drug quality among different CMOs and without licencing costs.

Additionally, Sintef has carried out essential screening experiments to evaluate product candidates before transfer to CMOs for production to clinical trials to reduce risk of failure in a project with extreme tight timelines. The screening results were among other important selection criteria for the choice of plasmid and secured that the clinical study could be initiated as planned.

In sum, the long experience of Sintef in the field of fermentation development and the very trustful and reliable collaboration efforts have given Nykode opportunities to develop highly valuable data and technology at a time where the company has not had any in-house capabilities to do it on its own.

Elin Bjerknes

Head of Product Development

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Collaboration between Sintef (Department of Biotechnology and nanomedicine) and Vectron Biosolutions AS

Vectron Biosolutions AS was founded by professor Svein Valla and Dr. Trond Erik Vee Aune in 2008, as a result of Valla's research into microbial gene-expression at NTNU. The company delivers technology and services in bacterial strain development to the life science industries, including helping the biopharmaceutical industry develop innovative human therapeutics.

SINTEF Biotechnology was an important collaboration partner to Professor Valla as his gene-expression technologies were developed. SINTEF Biotechnology's unique expertise and know-how in microbial cultivation, which is unsurpassed in Norway, was a necessity to develop the gene-expression technology and verify its industrial applicability.

Throughout Vectron's history, SINTEF Biotechnology has continued to be a very important partner to Vectron with its know-how and infrastructure that greatly compliments Vectron's bacterial gene-expression expertise. This strong partnership has manifested itself in co-publication of scientist results, collaboration on many RCN-funded projects with the objectives of improving and developing new technologies and services, and working together to service Vectron's customers across the life science industries.

Without SINTEF Biotechnology, it is unlikely Vectron would ever have been founded and it is unlikely Vectron would have grown to become recognized as a leading industrial player in microbial strain development. SINTEF Biotechnology will remain a preferred partner that compliments Vectron's expertise and will remain vital for the company's continued growth and success.

Trondheim, January 30, 2023

Trond Erik Vee Aune, CEO of Vectron Biosolutions AS

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beyond the skin

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January 31, 2023

Yield Improvement Program for Fusidic acid

To whom it might concern

SINTEF has been collaborating with LEO Pharma A/S for development of a new production strain and suitable process for production of the antibiotic Fusidic acid. Fusidic acid is one of the most important products from LEO Pharma A/S and is used for treatment of gram-positive bacterial infections (e.g., *Staphylococcus* and *Streptococcus*). The new strain and process is currently being implemented in production scale in LEO Pharma's new dkk1.5B manufacturing plant in Ballerup, Denmark.

In this work, SINTEF contributed with competence in strain development, molecular biology and bioprocess development. The work relied heavily on SINTEFs competence and efficient labs and infrastructure for High Throughput Screening (HTS) and fermentation capacity.
The work was financed by LEO Pharma A/S.

Yours sincerely,

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Chapter 2
Expression Platforms for Functional Metagenomics: Emerging Technology Options Beyond *Escherichia coli*

Anna Lewin, Rahmi Lale, and Alexander Wentzel

Abstract *Escherichia coli* is the prime workhorse for various metagenomic applications due to the multitude of efficient tools available for genetic manipulation and controlled heterologous gene expression. However, metagenome-based bioprospecting efforts continuously target a wider spectrum of ecological niches in order to harvest new enzymes and bioactive compounds for industrial and medical applications from the enormous pool of natural microbial diversity. Consequently, the development of robust and flexible screening platforms that allow functional evaluation of an expanded fraction of the highly diverse metagenomic information is widely addressed in Functional Metagenomics research. The heterologous recognition of transcriptional regulators and promotors, diverse codon usages among environmental microorganisms, and sufficient supply of precursors for secondary metabolite formation are major challenges that are addressed by an increasing spectrum of alternative expression and host systems. This includes optimized broad host-range transfer and expression vectors, screening hosts for improved gene expression and metabolite formation, as well as cell-free expression systems to cover proteins that due to toxicity are inaccessible by in vivo screening methods. In this chapter, we provide a current overview of the state of the art of selected expression systems and host organisms useful for functional metagenome screening for new enzymes and bioactive metabolites, as emerging options beyond what is currently available in and for *E. coli*.

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T.C. Charles et al. (eds.), *Functional Metagenomics: Tools and Applications*,
DOI 10.1007/978-3-319-61510-3_2

2.1 Introduction

Metagenomics has since its introduction in the late 1990s (Handelsman et al. 1998) proven to be a powerful tool for describing microbial communities and their metabolic potentials irrespectively of cultivability. Over the years, both sequence- and function-based screening approaches have led to the discovery of numerous new enzymes and metabolites fulfilling various academic and industrial needs (Ferrer et al. 2015; Fernandez-Arrojo et al. 2010; Novakova and Farkasovsky 2013). The pipeline for Functional Metagenomics spans from sampling, isolation of high-quality environmental DNA (eDNA), and its cloning (including vector design) to metagenomic library construction (including host transformation and transfer), heterologous gene expression, and production of functional molecules in amounts sufficient for detection in high throughput screening (Fig. 2.1). The function-based screening route of metagenome-based bioprospecting therewith complements the sequence-based route, in which eDNA is sequenced using next-generation sequencing methods and resulting sequence datasets mined bioinformatically for genes of interest (Lewin et al. 2013).

Irrespective of the chosen screening route, successful bioprospecting of a metagenomic library starts with the isolation of the eDNA. Its quality and quantity are of major importance for the achievable number of clones of the constructed library and consequently the representation of biodiversity in an environmental sample (Zhou et al. 1996). In order to capture as much of the biodiversity as possible, the applied DNA isolation procedures need to be highly effective in sampling from the diverse microorganisms inhabiting the selected environment (Kakirde et al. 2010). In addition, isolated DNA needs to have a high degree of purity and be free of contaminating substances, such as humic acids that are often present in soil and hamper efficient library construction (Tebbe and Vahjen 1993). Several studies document eDNA isolation procedures that resulted in contamination-free high molecular weight (HMW) DNA (Zhou et al. 1996; Brady 2007; Liles et al. 2008; Pel et al. 2009; Cheng et al. 2014). Contaminating compounds co-isolated with the eDNA can also be successfully removed by gel electrophoretic methods, including conventional (Craig et al. 2010), pulse-field (Cheng et al. 2014), or nonlinear electrophoresis (Pel et al. 2009), followed by size selection of the random fragmented DNA, prior to cloning.

New and improved enzyme discovery is currently the largest field of application for Functional Metagenomics tools. Aside from the catalytic function itself, beneficial properties like robustness under harsh conditions or high activity at low temperatures are often required in industrial applications. Consequently, dependent on the aims of a bioprospecting approach, different environments might serve as eDNA sources (Taupp et al. 2011). The microbial habitat to be sampled usually reflects the desired properties, i.e., subjecting a metagenomic library originating from a thermal vent or a hot deep subsurface oil reservoir to thermostable enzyme screening is likely to have a higher success rate compared to subjecting a

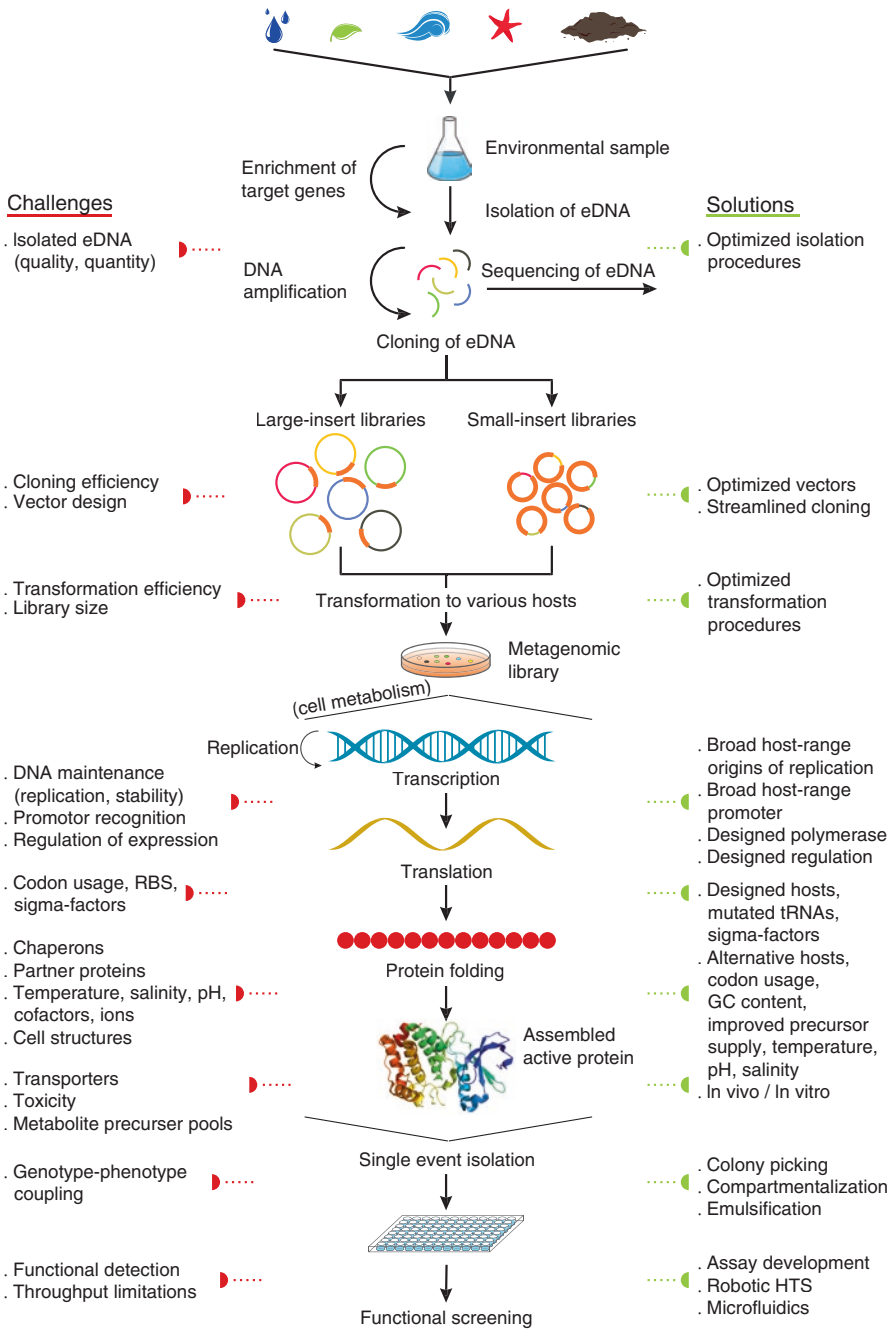

Fig. 2.1 Graphical representation of the Functional Metagenomics biodiscovery pipeline with its key challenges and potential solutions

glacier or permafrost soil-originating library to the same screening. In many examples such directed metagenomics sampling strategies aiming to increase probability of finding the desired properties have proven successful (Vester et al. 2015; Taupp et al. 2011). Selected examples are, among many others, a cold-adapted esterase enzyme from Antarctic desert soil (Hu et al. 2012), hydrolytic enzymes from cow rumen metagenome (Ferrer et al. 2007), and thermostable lipolytic enzymes from water, sediment, and biofilm samples from the Azores, Portugal (Leis et al. 2015a). However, due to often lower microbial density within some, particularly extreme environments, sufficient DNA yields may not be readily obtainable (Kennedy et al. 2008; Vester et al. 2015; Kotlar et al. 2011). In such cases, isolated metagenomic DNA can be subjected to isothermal amplification (like Phi29 whole genome amplification, WGA) in order to increase DNA yields prior to cloning (Rodrigue et al. 2009; Zhang et al. 2006). However, the challenge of this technology with respect to the formation of amplification artifacts, like chimeras, duplications, and inversions, needs to be considered. Therefore it is well suited for small-insert libraries for the purpose of enzyme discovery, but less suitable for large-insert library cloning where intact biosynthetic gene clusters are targeted.

Following sampling and successful isolation, the eDNA is usually either sequenced directly or cloned in suitable vectors for functional screening approaches (Sect. 2.2). The choice of the vector usually depends on the envisioned eDNA insert sizes, as well as the screening targets and methodology. However, for successful expression of genetic information contained in metagenomic DNA libraries, several additional factors need to be taken into account (Fig. 2.1). Suitable vector systems need to carry host-compatible selection markers, replicate stably and autonomously (ideally in combination with the possibility to control the copy number), may contain functional gene regulatory elements like inducible promotors for high level expression, and preferably enable vector transfer to other host organisms. Suitable host organisms in turn need to provide functionality of the vector elements involved in the production of functional products and allow efficient transcription and translation (Sect. 2.3). In addition, proper folding, possible cofactor supply, sufficient precursor availability for metabolite product formation, as well as means for nontoxic product localization, like secretion mechanisms, are needed. In order to meet the different demands for functional expression, such as codon usage, different assay temperatures, precursor requirements, etc. (Lam and Charles 2015; Uchiyama and Miyazaki 2009), different approaches can be applied in order to maximize the probability of successful expression (Fig. 2.1). *E. coli* systems designed and optimized for this purpose have so far been most widely used and extensively covered elsewhere (Guazzaroni et al. 2015). The scope of this chapter is therefore to summarize developments of various hosts and heterologous expression systems for functional metagenome screening beyond the common systems available for *E. coli* only.

2.2 Cloning and Expression Vectors for Environmental DNA

Selection of a suitable vector system for random metagenomic library construction will largely be guided by (1) the expected DNA size encoding the targeted compound of interest, (2) the envisioned subsequent screening approach involving one or more expression hosts, (3) the desired design of the library to be established, as well as in some occasions (4) the quantity of DNA available (Sect. 2.2.1). For approaches to identify new enzymes, small-insert libraries with eDNA sizes of 5–10 kb will in most cases be sufficient to obtain a sufficiently large number of complete gene sequences. Isolation of DNA for small-insert libraries is normally straightforward, since DNA shearing is not a major concern. However, it needs to be considered that a library with an average insert size of 10 kb will require 3–20 times more clones compared to a library with inserts of 30–40 kb to cover the same amount of genetic potential (Sabree et al. 2009). Hence comparably larger amounts of DNA are needed. To identify encoded functions that rely on single genes or small gene loci (e.g., enzyme function or genetic determinants of antibiotic resistance (Riesenfeld et al. 2004)), small-insert libraries are normally sufficient (Kakirde et al. 2010; Sabree et al. 2009). However, in cases where a desired function depends on multiple gene products, libraries harboring larger inserts are needed. These are normally constructed as cosmid, fosmid (30–40 kb), or bacterial artificial chromosome (BAC) libraries (up to ≥100 kb). The construction of comprehensive large-insert libraries can be very laborious, both with respect to the isolation of HMW DNA and successful cloning and transformation of the host. In addition, the lower stability of large inserts in the generated library needs to be considered. Also, the aspect of a higher degree of degradation of low guanine + cytosine (G + C) content DNA and some DNA modifications, which can impair cloning of HMW DNA, can result in a bias within large-insert libraries (Danhorn et al. 2012).

The choice of suitable vector systems is usually also related to the available expression host organism for subsequent screening experiments (Sect. 2.3). Moreover, for some targets, screening in multiple hosts can increase the hit rates (Mullany 2014). Hence library transfer and broad host-range capabilities of an expression vector (Sect. 2.2.2) can be desired characteristics (Craig et al. 2010; Aakvik et al. 2009; Kakirde et al. 2010).

2.2.1 Small- and Large-Insert Random Cloning Vectors

Cloning vectors useful for small-insert metagenomic library construction usually contain a defined promoter for transcription of the inserted DNA sequence. In some cases they are even equipped with two promoters (dual promotor vectors), flanking both sides of the cloning site in order to achieve gene expression regardless of insert orientation (Lammle et al. 2007). The promoters can have different, independent

induction mechanisms in order to achieve expression in only one direction at a time to prevent potential mRNA duplex formation that may result in lower protein production (Lale et al. unpublished). For cloning and construction of small-insert metagenomic libraries in *Escherichia coli* as primary host organisms, standard cloning vectors, such as pUC derivatives, pBluescript SK(+), and pTOPO, or their derivatives (Mullany 2014; Sabree et al. 2009) are frequently used.

In order to allow metagenomic library clones to cover entire biosynthetic pathways, like secondary metabolite clusters, large-insert libraries are required. Such libraries can be generated as cosmids or fosmids based on phage packaging of the eDNA ligated to a respective vector fragment or for very large inserts (up to 100 kb or more) as BACs (Kakirde et al. 2010; Danhorn et al. 2012). Fosmid and cosmid cloning vectors carry inserts of 30–40 kb, and both approaches utilize phage-based transfer of the cloned DNA into the host, usually *E. coli*. Consequently, the resulting library clones carry inserts within a narrow size range, determined by the packing capacity of the phage particle, and generally rely on gene expression from promoters included in the cloned insert. Cosmids are hybrid plasmids containing *cos* sequences from the λ phage, whereas fosmids are based on the F-factor replicon from *E. coli*. Compared to cosmids, fosmids are more tightly regulated with respect to copy number and are hence more stable (Kim et al. 1992; Kakirde et al. 2010). Both cosmids and fosmids are designed to carry antibiotic resistance markers and have broad host-range capabilities (Craig et al. 2010; Cheng et al. 2014; Aakvik et al. 2009; Wexler et al. 2005). Due to the frequent use of both cosmid and fosmid systems for metagenomic library construction, several variants (including commercial ones) are available (Lam et al. 2015; Mullany 2014; Kim et al. 1992; Parks and Graham 1997; Li et al. 2011; Terron-Gonzalez et al. 2013).

For random cloning of very large inserts, 40–100 kb and above, BACs are normally used, relying on the F-factor replicon (Danhorn et al. 2012; Shizuya et al. 1992). BAC vectors have been used in several metagenomic studies (Brady 2007) using, e.g., soil samples (Rondon et al. 2000) and murine bowel microbiota (Yoon et al. 2013). Similar to fosmids and cosmids, there are different BAC systems available, with some of them allowing inducible high copy numbers (Mullany 2014; Warburton et al. 2009; Wild et al. 2002) and/or having broad host-range capability (Mullany 2014; Aakvik et al. 2009; Kakirde et al. 2010). The US-based company Lucigen Corp. (Madison, WI; www.lucigen.com) has developed dedicated broad host-range vector systems for use in Functional Metagenomics. The pBAC-SBO and pSMART-BAC-S vectors both attribute efficient library construction in *E. coli* and are transferable to both Gram-positive and Gram-negative hosts. They have features allowing selection in several host organisms and gene expression from both insert-flanking regions, and are inducible in copy number (see Chap. 1). pSMART-BAC-S vector provides integration in the host genome only, whereas the pBAC-SBO vector allows both chromosomal integration, as well as extrachromosomal propagation in the recipient.

For DNA experiencing superhelical stress due to, e.g., regions dense in tandem and/or inverted repeats, cloning into circular plasmids can be challenging. In such cases, linear plasmids, such as the pJAZZ vector series (Lucigen), have been

designed which can carry large DNA inserts and contain features like transcriptional terminators flanking the cloning site to hinder vector-insert transcriptional interference (Godiska et al. 2010).

2.2.2 Broad Host-Range Expression Vectors

Depending on the desired activity, functional screening in different (or several) hosts can be of high value. As mentioned, *E. coli* is the most commonly used host both for library construction and functional screening. However, for certain screening activities, such as thermostable enzymes, or for bioactive secondary metabolite production, hosts like *Thermus thermophilus* (Angelov et al. 2009) and *Streptomyces* (or other *Actinobacteria*), respectively, might be beneficial due to their inherent features (Kakirde et al. 2010; Martinez et al. 2004) (see Sects. 2.3.1 and 2.3.2). Metagenomic libraries can be constructed directly in the host where they will be screened. However, the number of transformants obtained is often much lower in such hosts compared to the number of clones that can be obtained in *E. coli*. Thus, the common method is to utilize shuttle and/or broad host-range vectors for library construction in *E. coli*, which allows library transfer and screening in the host organism of choice. There are various such vectors available, both for small and large inserts. *E. coli–Bacillus subtilis* shuttle systems (plasmid and BAC) have been used for screening soil metagenomes for antimicrobial activities (Biver et al. 2013), and the pMDB14 vector (McMahon et al. 2012) can be shuttled between *E. coli*, *Pseudomonas putida*, and *Streptomyces lividans*, allowing gene expression in different hosts, similar to other systems reported (Sosio et al. 2000; Martinez et al. 2004). For development of psychrophilic expression systems, *E. coli* shuttle vectors such as a pGEM derivative and a pJRD215 derivative have been constructed, allowing the transfer of constructed libraries from *E. coli* to, e.g., *Psychrobacter* sp. and *Shewanella livingstonensis* (Cavicchioli et al. 2011; Miyake et al. 2007; Tutino et al. 2001). Also, *E. coli–T. thermophilus* shuttle systems have been designed (Angelov et al. 2009; Leis et al. 2015b). Apart from these, several other broad host-range systems have been developed. The pUvBBAC system supports replication in both Gram-positive and Gram-negative bacteria and allows functional screening in *Listeria* hosts (Hain et al. 2008). pGNS-BAC-1 presents opportunities for a copy induction in *E. coli*, as well as replication and functional screening in a broad spectrum of Gram-negative species (Kakirde et al. 2010). The pRS44 plasmid system (Aakvik et al. 2009) has been constructed both as fosmid and BAC system, which enables induction based on control on the vector copy number in *E. coli* and conjugative transfer into other hosts. In addition to the transferable BAC systems, several broad host-range cosmid vectors have also been reported (Craig et al. 2010; Cheng et al. 2014; Wexler et al. 2005).

In order to exploit the benefits of metagenomic library screening in several hosts with complementary features (Martinez et al. 2004; Leis et al. 2015a, b), efficient library transfer between host strains is of high importance. Though library vector

isolation by simple plasmid DNA extraction followed by re-transformation into the alternative host is possible, conjugation is in most cases the transformation method of choice. This is generally regardless of whether the library originally was constructed as a cosmid (Wexler et al. 2005; Craig et al. 2010; Cheng et al. 2014), fosmid (Aakvik et al. 2009), or BAC (Kakirde et al. 2010). The conjugative transfer of abovementioned vectors requires the full set of *tra* genes to be present in the donor (F positive) strain. Libraries to be transferred are often large, and therefore library transfer is preferably done in a high throughput fashion, similar to the high throughput conjugation procedure described by Martinez and co-workers (Martinez et al. 2004).

2.3 Expression Host Organisms

As previously mentioned, *E. coli* is presently the most commonly used expression host in metagenomic functional screening efforts (Ekkers et al. 2012; Kennedy et al. 2008; Aakvik et al. 2009; Rondon et al. 2000; Parachin and Gorwa-Grauslund 2011). Several dedicated tools for metagenomic library screening have also been developed for *E. coli*, such as engineered strains suitable for stable replication and copy control of large vectors. These remain at one single copy prior to screening to minimize the potential toxic effects of insert-encoded proteins or other produced metabolites (Taupp et al. 2011). *E. coli* strains have been modified for optimized heterologous expression, e.g., by expression of heterologous sigma factors that allow recognition of a wider range of promoter structures than *E. coli* wild-type strains (Gaida et al. 2015), and for heterologous expression of polyketide synthase (PKS) encoding secondary metabolite gene clusters and production of derivable natural products (Zhang et al. 2015). However, even engineered *E. coli* strains are not in all cases the best-suited hosts with respect to expressing metagenome-encoded functions. This accounts particularly for screening of metagenomic libraries harboring eDNA from extreme environments at conditions not compatible with *E. coli*'s natural lifestyle as a mesophilic human commensal, like very high or low temperatures. In addition, functional expression of genes from species that are phylogenetically distant from *E. coli* can be challenging (Warren et al. 2008). This can be due to, e.g., the differences in codon usage, improper promoter recognition, lack of transcription and/or translation factors, hampered protein folding, absence of cofactors, gene product toxicity, and absence of precursor metabolites. It has been shown that only approximately 40% of all genes can be heterologously expressed in *E. coli* (Gabor et al. 2004). Therefore, the use of multiple, complementary screening hosts has been proposed to express more of the diversity within a metagenomic library (Liebl et al. 2014). Table 2.1 summarizes the most commonly used as well as high potential future host systems for functional metagenome screening.

Table 2.1 Key features of frequently used as well as high potential future hosts for functional expression and screening of metagenomic libraries

Host organism	Key features	Key references
Escherichia coli	+ Well established as a heterologous expression and screening host	Ekkers et al. (2012), Kennedy et al. (2008), Aakvik et al. (2009), and Rondon et al. (2000), Parachin and Gorwa-Grauslund (2011)
	+ Fully developed toolbox for genetic manipulation, easy manageable	Taupp et al. (2011) and Gaida et al. (2015)
	+ Genetic transfer systems well established	
	+ Designed/optimized strains for different screening purposes available	Gabor et al. (2004) and Zhang et al. (2015)
	− *Only distantly related to many environmental microbes, codon usage*	Warren et al. (2008)
	− *Restricted to mesophilic cultivation an screening*	Liebl et al. (2014)
	− *Limited precursor availability for secondary metabolite formation*	
	− *Restrictions with respect to cofactor availability*	
	− *Potential toxicity effects due to limits in protein secretion, Gram-negative*	
Thermus thermophilus	+ Allows cultivation and in vivo screening high temperatures	Tabata et al. (1993) and Cava et al. (2009)
	+ Natural competence for DNA uptake	Hidaka et al. (1994)
	+ Efficient transformation protocols available	Schwarzenlander and Averhoff (2006)
	+ Thermostable resistance markers and other genetic tools (promoters, origin of replication, etc.) available	Matsumura and Aiba (1985), Liao et al. (1986), Nakamura et al. (2005), and Tamakoshi et al. (1997)
	+ *T. thermophilus–E. coli* shuttle vectors available	Lasa et al. (1992) and Wayne and Xu (1997)
	+ Chromosomal integration well established	de Grado et al. (1999)
	− *Only a limited set of selection markers available*	
	− *Only a few promoter alternatives developed*	
Streptomyces spp.	+ Full set of genetic tools available	Gust et al. (2004), Kieser et al. (2000), and Jones et al. (2013)

(continued)

Table 2.1 (continued)

Host organism	Key features	Key references
Streptomyces coelicolor	+ Optimized strains for heterologous gene cluster expression available	Gomez-Escribano and Bibb (2011, 2012, 2014)
	+ High G + C content, thus complementary to other expression hosts	Gomez-Escribano and Bibb (2014)
	+ Assembly platform for secondary metabolite production machinery	Shima et al. (1996), Okamoto-Hosoya et al. (2000), and Hu et al. (2002)
	+ Natural provision of precursors for sec. metabolite formation	
	+ Gram-positive, efficient protein/enzyme secretion	
	− *Mycelial growth phenotype, advanced cultivation systems necessary*	Wentzel et al. (2012a)
Rhodobacter capsulatus	+ Suitable for expression of membrane proteins	Liebl et al. (2014)
Gluconobacter oxydans	+ Acid tolerant	Liebl et al. (2014)
Burkholderia graminis	+ *E. coli* alternative	Craig et al. (2010)
Caulobacter vibrioides	+ *E. coli* alternative	Craig et al. (2010)
Pseudomonas putida	+ Stress tolerant	Craig et al. (2010) and Troeschel et al. (2010)
	+ Capable of producing secondary metabolites	Loeschcke and Thies (2015)
Ralstonia metallidurans	+ Robustness at extreme conditions, broad screening spectrum	Craig et al. (2010) and Mergeay et al. (2003)
Pseudomonas fluorescens	+ *E. coli* alternative	Aakvik et al. (2009)
	+ Secretion pathway	Retallack et al. (2006)
Xanthomonas campestris	+ *E. coli* alternative	Aakvik et al. (2009)
	+ Increased stabilities of proteins produced	Leza et al. (1996)
Sinorhizobium meliloti	+ *E. coli* alternative	Cheng et al. (2017)
	+ Chromosomal integration established	Heil et al. (2012)
Agrobacterium tumefaciens	+ *E. coli* alternative, plant symbiont	Craig et al. (2010), Troeschel et al. (2010), and Murai (2013)
	+ Chromosomal integration established	Heil et al. (2012)

Table 2.1 (continued)

Host organism	Key features	Key references
Bacillus subtilis	+ Gram-positive model organism	Biver et al. (2013)
	+ Fully developed toolbox for genetic manipulation, easy manageable	
	+ Low GC, thus complementary to other expression hosts	
	+ Secretion pathway (enzyme production)	Wong (1995) and Zobel et al. (2015)
R. eutropha H16	+ *E. coli* alternative	Gruber et al. (2015)
Saccharomyces cerevisiae	+ Eukaryotic (yeast)	Damon et al. (2011)
	+ Fully developed toolbox for genetic manipulation	
	+ Secretion pathway (enzyme production)	Strausberg and Strausberg (2001)
	+ Protein posttranslational modifications, glycosylation	Holz et al. (2003)

2.3.1 *Extremophiles as Expression Hosts for Metagenome Screening*

Similar to sampling and cloning of metagenomic DNA from an environment that matches the desired properties of an enzyme, it appears reasonable to use a heterologous expression host for metagenomic library screening that functions optimally under respective conditions, such as in vivo screening for thermostable enzymes at elevated temperature in a thermophilic host. In terms of thermophilic hosts for heterologous gene expression, the hyperthermophilic, Gram-negative bacterium *T. thermophilus* (*Deinococcus-Thermus* phylum), growing optimally at temperatures as high as 85 °C, is the most well-studied species (Tabata et al. 1993; Cava et al. 2009). Several *T. thermophilus* strains have been genome sequenced, and their natural competence (Hidaka et al. 1994; Koyama et al. 1986) renders them very efficient in taking up external DNA without source discrimination (Schwarzenlander and Averhoff 2006). In addition, *T. thermophilus* has been shown to acquire DNA by conjugation, however, not as effectively as by utilizing its natural competence (Ramirez-Arcos et al. 1998; Cava et al. 2007).

A large number of genetic tools have been developed to genetically amend *T. thermophilus* (Tamakoshi et al. 1997; de Grado et al. 1999). In the 1980s a selection marker in the form of a thermostable version of a kanamycin resistance was developed using mutagenesis (Matsumura and Aiba 1985; Liao et al. 1986), allowing antibiotic-based selection for transformed *T. thermophilus* cells. Since then other selectable markers stable at high temperature have been used such as the bleomycin-binding protein conferring bleomycin resistance (Brouns et al. 2005),

and a hygromycin B phosphotransferase evolved to thermostability (Nakamura et al. 2005). Several plasmids and vectors are available, like the cryptic pTT8 plasmid (Koyama et al. 1990) used to transfer genes into *T. thermophilus*. pTT8 has also been supplemented with the gene providing thermostable kanamycin resistance described above, resulting in the selectable cloning vector pMKM001 (Mather and Fee 1992) and variants thereof. The *Thermus*-compatible plasmids have also been engineered further into *E. coli–Thermus* shuttle vectors by integration of the cryptic *Thermus* vectors with commonly used *E. coli* plasmids from the pUC series, resulting in several variants, e.g., pMY1-3 and pLU1-4 (Lasa et al. 1992; Wayne and Xu 1997). In addition, there are other plasmids available for *Thermus*, like plasmid pTA103 (Chu et al. 2006), the pS4C, and pL4C plasmids harboring both integrase and transposase (Ruan and Xu 2007) as well as the widely used pMK18 vector carrying the multiple cloning site from pUC18 (de Grado et al. 1999).

As a consequence of the available genetic tools for *T. thermophilus*, these strains have been used as thermophilic cell factories to complement production of certain proteins in, e.g., *E. coli*. *T. thermophilus* has been used to homologously produce *Tth* DNA polymerase more efficiently than in *E. coli* (Moreno et al. 2005), as well as for production of an active thermostable Mn-dependent catalase which failed to express in *E. coli* (Hidalgo et al. 2004). *T. thermophilus* has also been successfully used in metagenomic approaches, e.g., by Angelov and co-workers (2009). In their work, large-insert fosmid libraries were constructed in *E. coli* and transferred to a *T. thermophilus* host. Screening was performed in both species, resulting in different hit spectra. This clearly illustrates the benefits of high-temperature screening for thermostable enzymes. The same authors also constructed a pCC1fos derivative (denoted pCT3FK) which carries *T. thermophilus* HB27 chromosomal DNA sequences which allow integration in the host chromosome by homologous recombination (Angelov et al. 2009). This vector has been used in the screening of a metagenomic library for thermostable esterases in both *E. coli* and *T. thermophilus* hosts, resulting in a higher number of thermostable enzyme candidates in the *T. thermophilus* than in the *E. coli* screening (Leis et al. 2015a, b).

On the opposite end of the temperature range, cold environments provide a large understudied biodiversity. Particularly psychrophilic enzymes from such environments are sought due to their unique characteristics, i.e., high activity at low and moderate temperatures, necessitating lower enzyme concentrations to achieve a similar performance compared to higher temperature homologues. Psychrophilic enzymes are considered to be less stable compared to their mesophilic homologues, as their structural flexibility enables them to function at low temperatures and imparts a decreased thermal stability (Feller 2013). However, the biodiscovery of relevant gene functions from these environments is limited to their expression and function in mesophilic hosts. For instance, the utilization of *E. coli* as a host for the expression of psychrophilic enzymes limits the growth temperature to around 15 °C, which presents a significant barrier to their exploitation in biotechnology (Struvay and Feller 2012).

There are several examples where *E. coli* has been successfully used in the production of cold-adapted enzymes (Cavicchioli et al. 2011; Wang et al. 2010; Zhang

and Zeng 2008). However, the total number of such reports is comparably low, reflecting significant challenges. Two strategies to overcome these challenges are (1) low-temperature adaptation of existing mesophilic expression systems and (2) the development of new psychrophilic expression hosts. The former approach includes engineering the mesophilic expression host for sufficient growth at low temperatures to promote correct folding of recombinant proteins. The co-expression of Cpn60 and Cpn10 from *Oleispira antarctica*, cold-adapted homologues of the *E. coli* GroELS chaperonins, provided *E. coli* with an operational folding system at 4–12 °C (Ferrer et al. 2003). This led to improved growth at low temperatures and enhanced solubility of the recombinant proteins produced. Another example is the utilization of cold-shock promoter systems together with solubility partners for psychrophilic genes in *E. coli.* Bjerga and Williamson showed that cspA-driven expression of maltose-binding protein (MBP), thioredoxin (TRX), small ubiquitin-like modifier (SUMO), and trigger factor (TF) encoding gene fusion enabled high level production of soluble protein (Bjerga and Williamson 2015).

Dedicated host-expression systems for the production of cold-adapted products have been developed, such as the pTAUp and pTADw vectors for *Psychrobacter,* found to replicate by rolling circle mechanisms (Tutino et al. 2000). Also, the cryptic replicon plasmid pMtBL from *Pseudoalteromonas* sp. has been used as a psychrophilic expression vector, shown to have a broad host-range profile compatible to not only psychrophiles but also mesophilic species after fusion with a pGEM derivate (Tutino et al. 2001). Other broad host-range vectors for cold-adapted expression include a variant of pJRD215 carrying a regulatory promoter from *Shewanella* and a β-lactamase reporter from *Desulfotalea* (Miyake et al. 2007) and a shuttle vector based on the p54 plasmid originating from a psychrophilic *Arthrobacter* sp. isolated from a Greenland glacier and pUC18. The latter example resulted in a low-temperature expression system transferrable to not only *E. coli* but also some high G + C Gram-positive bacteria (Miteva et al. 2008).

2.3.2 *Actinobacteria as Hosts for Heterologous Natural Product Formation*

The phylum *Actinobacteria* comprises a comprehensive and diverse group of Gram-positive bacteria predominantly with a mycelial lifestyle. They are potent producers of a plethora of natural products with a wide spectrum of medical applications, including antibacterial, antifungal, anthelmintic, and immunosuppressant compounds (Barka et al. 2016). Among them, members of the *Streptomyces* taxon are particularly prolific in this respect, accounting for the majority of antibiotics in medical use today (Hopwood 2007). Actinomycete genomes contain a multitude of secondary metabolite gene clusters (Bentley et al. 2002; Ohnishi et al. 2008; Oliynyk et al. 2007; Udwary et al. 2007) of which, however, only a subset is expressed and the respective compounds produced under laboratory conditions. Hence, the majority of gene clusters remains silent, rendering them cryptic, with functions yet to be

discovered. Also among *Actinobacteria*, cultivable strains represent only a minute fraction of the entire diversity (Maldonado et al. 2005), leaving a vast resource of new potential drug candidates untapped, unless new methods to enable cultivation (Zengler et al. 2002), or efficiently allow the heterologous realization of their genetic potential, become available. In that respect, well-described members of the *Actinobacteria* themselves, like the model species *Streptomyces coelicolor*, have been proposed as hosts for the heterologous expression of natural product gene clusters (Gomez-Escribano and Bibb 2011, 2012). Their versatility with respect to expressing complex biosynthetic gene clusters, their high G + C codon usage, and the provision of important precursors necessary to simultaneously form natural products of different compound classes (like polyketides, non-ribosomal peptides, lantibiotics, etc.) are excellent rationales to select such strains for metagenome screening for new bioactive compounds. In addition, these *Streptomyces* spp. strains might prove useful in accessing the potential of cryptic gene clusters of cultivable strains by heterologous expression. In-depth understanding of gene regulation and precursor supply will be instrumental in optimizing model *Actinobacteria* as functional metagenome screening hosts.

S. coelicolor has been extensively studied with respect to the regulation of secondary metabolite production, and all necessary genetic tools for genetic manipulation, like plasmids and inducible promoters, and large-insert library tools for chromosomal integration (Gust et al. 2004; Kieser et al. 2000; Jones et al. 2013), are fully developed. Also, new tools for fast and efficient genome editing, like the CRISPR/Cas system (Garneau et al. 2010), have been optimized and applied to *Actinobacteria* to make deletions and directed genomic mutations (Tong et al. 2015; Huang et al. 2015; Cobb et al. 2015). Though its applicability to introduce larger gene clusters into the *Streptomyces* genome is currently limited, it can be expected that this technology will develop into a powerful tool for reprogramming *Actinobacteria* for the production of new bioactive compounds. Wild-type *S. coelicolor* produces several antibiotic compounds of different classes, including the polyketides actinorhodin (Act, Rudd and Hopwood 1979) and coelimycin (Cpk, Gomez-Escribano et al. 2012), the prodiginine undecylprodigiosin (Red, Feitelson et al. 1985), the lipopeptide calcium-dependent antibiotic (CDA, Hopwood and Wright 1983), and the plasmid-encoded cyclopentanoid methylenomycin (Mmy, Wright and Hopwood 1976). However, its genome sequence revealed a much larger potential of bioactive compounds, represented by more than 20 different, mostly non-expressed gene clusters for secondary metabolites (Bentley et al. 2002). Extensive research has been performed to detect and study cryptic gene clusters (Medema et al. 2011; Nett et al. 2009; Zerikly and Challis 2009; Baltz 2008) and ultimately activate them for product formation (Ochi et al. 2014; Rutledge and Challis 2015; Yoon and Nodwell 2014; Zhu et al. 2014). However, regulation of antibiotic production by *S. coelicolor* is complex and needs to be understood in depth when considering it as a generic cell factory for heterologous natural product formation.

Several factors are involved in triggering antibiotic production in *Streptomyces* in correlation with the species' life cycle (Bibb 2005; van Wezel and McDowall 2011).

Nutrient depletion and cessation of growth induce morphological differentiation and antibiotic production via the stringent response and guanosine tetra- and pentaphosphate (p)ppGpp (Potrykus and Cashel 2008). Programmed cell death and the release of N-acetyl glucosamine (GlcNAc) trigger the onset of development and antibiotic production via the global regulator DasR (Rigali et al. 2006, 2008). Also, induced mycelial fragmentation by overexpression of cell division activator protein SsgA affects antibiotic production in *S. coelicolor* (van Wezel et al. 2009). From responses of the global regulatory network, information is passed on to pathway-specific activators encoded within biosynthetic gene clusters, usually controlled in a growth phase-dependent manner (Wietzorrek and Bibb 1997). Once produced in sufficient amount, these are solely responsible for all further downstream regulation of the biosynthetic gene cluster expression. Removal of pathway-specific regulators (Smanski et al. 2012) or exchange of native promotors (Du et al. 2013) as well as overexpression of export proteins (Huo et al. 2012) have led to improved production yields of platencin, gougerotin, and bottromycin, respectively.

Taking all the different layers of regulation into account will be the key for developing *Streptomyces* into potent heterologous production platforms for natural product discovery, from both silent gene cluster in cultivable microorganisms and realizing the biosynthetic potential in environmental metagenomes. *S. coelicolor* has been extensively used as heterologous expression platform for antibiotic gene clusters as recently reviewed by Gomez-Escribano and Bibb (2014). By successively deleting the biosynthetic gene clusters for Act, Red, CDA, and Cpk in the plasmid-free (thus Mmy negative) wild-type M145 of *S. coelicolor*, a strain (M1146) was obtained with largely reduced background of bioactive compounds produced and secreted to the medium (Gomez-Escribano and Bibb 2011). In the same work, additional introduction of point mutations in the genes rpoB and rpsL, encoding the RNA polymerase β-subunit and the ribosomal protein S12, respectively, (strain M1154) led to a pleiotropic increase in the level of secondary metabolite production. Each of these mutations had previously been shown to enhance antibiotic production levels in *Streptomyces* without negative effects on growth (Shima et al. 1996; Okamoto-Hosoya et al. 2000; Hu et al. 2002) and has been proposed as a new strategy to activate silent gene clusters for new drug discovery (Ochi and Hosaka 2013). M1146 and M1154 have been successfully applied for the heterologous production of numerous antibiotics of diverse classes (Gomez-Escribano and Bibb 2014).

A further optimization of the existing heterologous host strains of *S. coelicolor* as an optimized Superhost for new antibiotics discovery from environmental metagenomes may be guided by the comprehensive knowledge of physiology and gene regulation of antibiotic production, as well as systems biology understanding of this species. A dedicated fermentation strategy for system scale studies of metabolic switching in *S. coelicolor* has been established (Wentzel et al. 2012a), allowing reproducible cultivations of *S. coelicolor* and high-resolution time-scale sampling for full ‘omics analysis (Battke et al. 2011). The dynamic architecture of the metabolic switch in *S. coelicolor* was studied at the gene expression (Nieselt et al. 2010), the proteome (Thomas et al. 2012) and the metabolome level (Wentzel

et al. 2012b). By studying the effect of different mutations, the complex regulatory interplay of nitrogen and phosphate metabolism was elucidated (Martin et al. 2012; Waldvogel et al. 2011). A genome scale model for *S. coelicolor* is available (Alam et al. 2010), and detailed insight in the structure of the transcription factor mediated regulatory network has been gained (Iqbal et al. 2012).

In addition to *S. coelicolor*, other *Actinobacteria* species have been considered as heterologous expression hosts. *S. avermitilis*, for example, has been engineered as an expression host for heterologous gene clusters (Komatsu et al. 2013), and also *S. lividans* and *S. albus* as well as *Saccharopolyspora* (Baltz 2010) have been used for that purpose. *S. lividans* was used as host organism in successful screening for anti-mycobacterial compounds (Wang et al. 2000), and both *S. lividans* and *S. albus* have been shown to be able to produce products from an introduced Type II PKS pathway (King et al. 2009). *Nonomuraea* sp. ATCC 39727 heterologously produced microbisporicin and planosporicin (Marcone et al. 2010) more efficiently than as *Streptomyces* hosts (Foulston and Bibb 2010; Sherwood and Bibb 2013), indicating potential benefits of using several actinobacterial expression hosts for bioactive compound screening of metagenome libraries. *Streptomyces* spp. have proven to be useful in heterologous gene cluster expression and functional screening for associated bioactivity (Kakirde et al. 2010; Martinez et al. 2005). Screening of a BAC library from soil DNA produced in *E. coli* and transferred to *Pseudomonas putida* (low G + C) and *Streptomyces lividans* (high G + C) resulted in different expression patterns (Martinez et al. 2004), indicating usefulness of the high G + C *Streptomyces* hosts as complement to other metagenome screening platforms for bioactivity, like polyketide production-optimized *E. coli* BTRA (Zhang et al. 2015).

Recently, the "*Tectomicrobia*" candidate phylum including the "*Entotheonella*" candidate genus has been discovered by a combined single cell- and metagenomics-based approach to describe microbial consortia producing bioactive polyketides and peptides in association with the marine sponge species *Theonella swinhoei* (Wilson et al. 2014). This study exemplifies the huge potential of marine environments to identify new compounds produced by non-cultivable microbial strains. The genetic optimization of different actinobacterial model strains for natural product formation will help in establishing a platform of different optimized host strains that in combination can potentially be useful in functional screening also for new natural products from such biodiversity with an increased success rate.

2.3.3 Other Expression Hosts for Metagenome Screening

There are several other species apart from *E. coli* and those discussed above (Sects. 2.3.1 and 2.3.2) that have been considered as hosts for metagenome expression and screening, all with their respective benefits and drawbacks. These species can contribute to building a flexible platform for multi-host expression and screening of microbial metagenomes as suggested before (Liebl et al. 2014).

Mesophilic hosts applied for metagenomic screening, apart from *E. coli* and the *Actinobacteria* covered in detail above (Sect. 2.3.2), include species like *Agrobacterium tumefaciens* (alphaproteobacteria), *Burkholderia graminis* (betaproteobacteria), *Caulobacter vibrioides* (alphaproteobacteria), *Pseudomonas putida* (gammaproteobacteria), and *Ralstonia metallidurans* (betaproteobacteria) that have been used to screen a soil metagenome (Craig et al. 2010). Also, the alphaproteobacterium *Rhizobium leguminosarum* has been used in metagenome screening for alcohol/aldehyde dehydrogenases (Wexler et al. 2005). Other mesophilic host bacteria utilized in metagenomic screening include *Rhodobacter capsulatus* and *Gluconobacter oxydans* (Liebl et al. 2014), where *R. capsulatus* has been shown to be suitable for expression of membrane proteins, and *G. oxydans* to be tolerant to screening at acidic conditions. Also, the low G + C, Gram-positive bacterium *Bacillus subtilis*, widely used for recombinant enzyme production due to its capability to secrete protein in the medium, has been used in metagenome screening (Biver et al. 2013). Similarly, species of *Burkholderia*, *Sphingomonas*, and *Pseudomonas* (Ekkers et al. 2012; Martinez et al. 2004) have been used, and, by using the bacterial symbiont *Sinorhizobium meliloti* as expression host, a greater diversity of clones was found compared to screening in *E. coli* (Lam et al. 2015). In addition, the gammaproteobacteria *Pseudomonas fluorescens* and *Xanthomonas campestris* (Aakvik et al. 2009) as well as integrase-mediated recombination of libraries in hosts *S. meliloti* and *Agrobacterium tumefaciens* (Heil et al. 2012) have been shown to be applicable for functional metagenome screening.

Even though prokaryotic hosts have been applied successfully in screening of metagenomic DNA libraries with content including eukaryotic DNA (Geng et al. 2012), eukaryotic host systems may be an important area for further development of metagenomic tools and expression hosts. Even though much more prokaryotic vector-host systems have been developed and used through history, there are genetic tools available for yeasts such as *Saccharomyces cerevisiae* (e.g., Drew and Kim 2012) and *Pichia pastoris* (e.g., Daly and Hearn 2005), as well as filamentous fungi, for example, *Aspergillus* (Nevalainen et al. 2005). A mutant strain of *S. cerevisiae*, defective in di-/tripeptide uptake, has been used in a functional screening of a soil metagenome library for the identification of novel oligopeptide transporters (Damon et al. 2011), demonstrating the potential of eukaryotic hosts in functional screening of environmental metagenomes.

2.4 In Vitro Expression Systems for Functional Metagenomics

Cell-free protein synthesis (CFPS) covers the in vitro transcription of coding DNA to mRNA and its subsequent translation into polypeptide and functional protein by using cell extracts. CFPS is a field in rapid development with the potential to make large impact in both protein production and screening for new enzyme functions in

the future. The first CFPS system of *E. coli* was already introduced in 1961, with the main purpose of studying the process of translation (Matthaei and Nirenberg 1961). Since then, a multitude of advanced CFPS systems using extracts of organisms from all three domains of life, including from Bacteria, Archaea, fungi, plants, insects, and mammals (Zemella et al. 2015), has been developed. With their open nature, CFPS systems bypass a number of limitations existing in cellular, in vivo expression systems, as they are highly flexible with respect to the physicochemical environment, the reaction conditions, and the reaction format for gene expression to take place. In addition, they allow incorporation of nonnatural amino acids/cofactors, avoid biological background, and are not constraint by cell viability in response to toxic proteins being produced. In the absence of membranes to be bypassed, almost unlimited use of substrates for screening of gene libraries is enabled, and library sizes that are not restricted by transformation efficiency of expression host cells. This renders CFPS an increasingly recognized alternative option to cell-based expression systems for both protein screening and production (Catherine et al. 2013).

Several key challenges associated with CFPS have recently been successfully addressed and mitigated, such as low productivities, quality and quantity constraints of DNA templates, posttranslational modifications, and clonal separation for genotype-phenotype coupling. Low productivity has been a major issue due to the rapid depletion of the chemical energy carrier ATP and stoichiometric accumulation of phosphate, binding vital magnesium ions. The development of ATP regeneration methods, in particular utilization of the intact glycolytic pathway to produce ATP from glucose by oxidative phosphorylation (Jewett and Swartz 2004; Calhoun and Swartz 2007; Kim and Kim 2009), represented a major breakthrough in achieving larger protein amounts. Moreover, in situ supply of glucose by hydrolysis of polymeric carbohydrates like maltodextrin or starch could be implemented to control the ATP delivery rate (Wang and Zhang 2009). Other metabolic functions in crude cell extracts for CFPS were used to be beneficial, for example, for the provision of cofactors for produced target enzymes (Kwon et al. 2013).

Several studies have suggested solutions to the challenges connected to high template amounts required, as well as high exonucleolytic degradation of linear DNA template in crude cell extracts. In addition to sufficient template preparation by PCR-based methods (Sawasaki et al. 2002; Endo and Sawasaki 2004), the use of isothermal DNA amplification in connection with CFPS (Kumar and Chernaya 2009) was shown to enable high throughput protein synthesis based on very small amounts of template DNA. mRNA stabilization by inclusion of the terminal stem-loop structures and depletion of extracts from RNase E led to greatly improved protein production (Ahn et al. 2005). More relevant for expression library screening, the protection of linear DNA templates and improved protein production was shown by inhibiting the RecBCD nuclease in *E. coli* extracts by addition of bacteriophage Lambda Gam (Sitaraman et al. 2004). This was also shown to be achieved by using extracts of *E. coli* in which the endonuclease I gene *endA* was removed and the *recBCD* operon was replaced by the Lambda recombination system (Michel-Reydellet et al. 2005). Also the tethering of linear DNA ends to microbeads in an agarose matrix led to improved DNA template stability (Lee et al. 2012).

For posttranslational modifications during in vitro synthesis of eukaryotic proteins, for example, for pharmaceutical applications, several eukaryotic CFPS systems have been developed as recently reviewed by Zemella and co-workers ((Zemella et al. 2015) and references therein). This includes systems based on *S. cerevisiae*, the fall armyworm *Spodoptera frugiperda*, rabbit reticulocytes, CHO cells, and different human cell lines. The set of well-documented eukaryotic CFPS systems also includes plant systems from tobacco BY-2 and the widely used cell-free expression system based on wheat germ embryos which represents a high yield system with correct folding of many protein types, including disulfide-rich proteins (Takai et al. 2010).

In vitro compartmentalization (IVC) represents one possible solution to the demand for clonal separation and genotype–phenotype coupling in cell-free screening systems. Being early addressed by the SIMPLEX approach (Rungpragayphan et al. 2003) using diluted single-molecule templates for PCR and subsequent CFPS in a microtiter format, emulsion-based approaches bear the possibility of substantial library sizes. Small aqueous droplets are prepared in a continuous oil phase to isolate templates in individual micro-reactors for isothermal or PCR-based amplification (Courtois et al. 2008) and CFPS. This represents a promising platform for enzyme activity screening against a wide array of substrates using either FACS- or microfluidics-based screening and sorting methods (Kintses et al. 2010).

The insight in biodiversity and the huge metabolic potential in nature provided by the recent revolutions in next-generation sequencing have renewed attention in the potential of CFPS. Consequently, key improvements have been triggered, greatly expanding the applicability of cell-free systems to HT gene expression and even large-scale protein production (Zemella et al. 2015). CFPS and suitable screening systems may form an ideal platform for the functional screening of enzymes using genomic and metagenomic DNA, independent of the limitations of cell-based systems. In a recent example, a cow rumen metagenomic library was screened for glycoside hydrolases using cell-free expression and utilizing the energy-providing effect of glucose in CFPS extracts (Kim et al. 2011). Energy generation in this case started with the polysaccharides cellulose, xylan, amylose, as well as a small amount of glucose. Enzymatic substrate degradation in a feedback loop then led to increased glucose amounts, ultimately leading to an indicator-detectable pH drop due to acid by-products (Kim et al. 2011).

This example shows that optimized CFPS systems in combination with smart assay design represent a powerful option for expression screening for microbial enzymes with high versatility, in particular when combined with platforms for ultra-high throughput analysis and sorting. Further developments in this field will likely include expansion of CFPS systems to additional microbial species, including from extreme environments, as eDNA from extreme environments may fail to be transcribed or translated by *E. coli* extracts (Angelov et al. 2009). Hence, "unconventional" microbial systems for functional expression are demanded (Liebl et al. 2014). Pure component systems and extracts have already been described for extremophiles from both Bacteria and Archaea (Endoh et al. 2007; Zhou et al. 2012), including *Thermus*, *Pyrococcus*, *Sulfolobus*, and *Thermococcus* (Hethke et al. 1996; Tachibana et al. 1996; Ruggero et al. 2006), which might be a valuable resource for future systems.

2.5 Outlook

Metagenomics has proven to be a powerful tool to describe environmental microbial biodiversity and exploit it for metabolic functions of relevance for commercial applications. With the ever-advancing throughput of next-generation sequencing technologies, (meta)genomic DNA sequence databases are filling rapidly, and based on that, our insight into the huge and diverse metabolic potential existing in nature has never been deeper. However, identification of useful functions is ultimately still dependent on experimental proof. Though in silico predictions are constantly improving, the field of Functional Metagenomics will continue to develop as it directly and efficiently links desired function to its determining source code, the eDNA.

E. coli and genetic tools developed for this species have been the first choice in Functional Metagenomics research, both with respect to library construction, recombinant expression, and functional screening. However, it is presently obvious that *E. coli* has some shortcomings, especially in the light of the growing spectrum of ecological niches and greater microbial diversity being accessed and a broader spectrum of metabolic functions and properties aimed to be exploited. Therefore, along with *E. coli*, which itself is still being improved further as a screening host for specific target classes, other microbial model systems, potentially more suitable for screenings for particular functions of interest, have emerged in recent years. These include, for example, thermophilic and psychrophilic systems for respective enzyme discovery and actinobacterial systems for secondary metabolite gene cluster expression and bioactive compound formation.

New and better tools are demanded and continuously developed to increase efficiency at the different steps of the Functional Metagenomics biodiscovery pipeline (Fig. 2.1). In addition to dedicated sampling and efficient DNA extraction procedures from diverse natural environments and developments within metagenomic (small- and large-insert) library cloning technology, several other aspects are in focus. Vector development for heterologous expression in and transfer between multiple host species (broad host-range) as well as optimization of different host species to heterologously express genes for bioactive functions will likely continue to converge. In particular, a higher efficiency in large-insert cloning of eDNA and its shuffling between different expression hosts allowing screening in in different organisms with complementary features and capabilities has proven to generate complementary hits (Liebl et al. 2014). It is therefore still highly desired to improve the functional metagenomic pipeline for metagenome-based bioactive compound discovery by means of new expression and screening platforms. Several different host organisms may be included, and shuffling of metagenomic libraries between these, connected to multiple host screening, is of potentially high value. It can be expected that newly developed expression systems aim to be optimal within screening for specific targeted applications (specific enzyme functions or bioactive compounds) or product properties. The concept of specifically accessing environments providing desired properties (e.g., of an enzyme of choice) and subsequently using

screening hosts that perform optimally at similar conditions can be expected to produce further valuable output in the future. In addition, within this concept, the metabolic optimization of the host species from different phyla or even domains (including Archaea and Eukaryotes) may be pursued. The integration of new host species of phyla other than the *Actinobacteria* may expand the options to access biodiversity for medical compound discovery and thus mitigate the threat of antibiotic resistance, as well as help fighting deadly diseases, including cancer.

System biology understanding, the application of new genome editing tools, and synthetic biology principles will guide new approaches to optimize host strains for heterologous expression of metagenomic genes and formation of new natural products. Optimized Superhosts for bioactivity screening based on different model *Actinobacteria* will enable heterologous expression of biosynthetic gene clusters and compound formation from uncultured bacteria. Well-established thermophilic and psychrophilic host species will be good candidates for further optimization with respect to high- and low-temperature screening. Thereby, optimal hosts should attribute, among others, stable cloning vector maintenance, sensitivity toward relevant antibiotics for selection purposes, and suitable transcription and translation machinery. In addition, they should ensure correct folding, cofactor provision and insertion, relevant precursor supply, as well as counteract toxic effects from product formation (e.g., by product export mechanisms).

In vivo systems for Functional Metagenomics come with their inherent set of challenges, like limitations in achievable library sizes and the spectrum of usable substrates for screening. Consequently, cell-free (in vitro) expression systems have lately emerged as a potential alternative in functional metagenome screening for enzymatic functions (Sect. 2.4). In vitro expression systems still have their own limitations, in particular regarding large-scale production, which, however, is not very relevant for screening and biodiscovery, requiring only small amounts of product. Key challenges are constantly being addressed with new research, and solutions to key bottlenecks have already been found. An expanded spectrum of CFPS species, similar to the diversification of in vivo expression systems, as well as hybrid systems combining beneficial components of different species, can be expected to become available soon. Thus, in combination with ongoing developments of compartmentalization and miniaturization of screening technology, as achievable by, e.g., using advanced microfluidics devices, in vitro systems may become a potential future alternative to in vivo systems in Functional Metagenomics.

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Article

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Mannuronan C-5 Epimerases Suited for Tailoring of Specific Alginate Structures Obtained by High-Throughput Screening of an Epimerase Mutant Library

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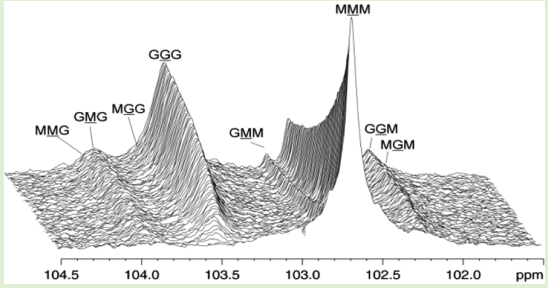
Supporting Information

ABSTRACT: The polysaccharide alginate is produced by brown algae and some bacteria and is composed of the two monomers, β-D-mannuronic acid (M) and α-L-guluronic acid (G). The distribution and composition of M/G are important for the chemical-physical properties of alginate and result from the activity of a family of mannuronan C-5 epimerases that converts M to G in the initially synthesized polyM. Traditionally, G-rich alginates are commercially most interesting due to gelling and viscosifying properties. From a library of mutant epimerases we have isolated enzymes that introduce a high level of G-blocks in polyM more efficiently than the wild-type enzymes from *Azotobacter vinelandii* when employed for in vitro epimerization reactions. This was achieved by developing a high-throughput screening method to discriminate between different alginate structures. Furthermore, genetic and biochemical analyses of the mutant enzymes have revealed structural features that are important for the differences in epimerization pattern found for the various epimerases.



INTRODUCTION

Alginate is a family of linear polysaccharides with numerous present and potential future application areas ranging from food, textile, and printing industry to biomedical and biopharmaceutical as well as electrochemical products.[1−5] The polymer is synthesized by brown algae and by *Azotobacter* and *Pseudomonas* species,[6,7] and currently all commercial production is based on extraction from algal resources. The alginate monomers β-D-mannuronic acid (M) and α-L-guluronic acid (G) are arranged in M-, G-, and MG-blocks along the polymer chain, and the length and distribution of these blocks determine the physicochemical properties of the polysaccharide.[8] Alginates containing G-blocks are dominating in commercial use due to their ability to form hydrogels. The polymer is first produced as mannuronan (polyM); subsequently, the block structure results from the activity of a family of mannuronan C-5 epimerases catalyzing nonrandom epimerization of β-D-mannuronic acid to α-L-guluronic acid at the polymer level.[9] *Azotobacter vinelandii* encodes a family of seven secreted mannuronan C-5 epimerases, AlgE1-AlgE7, involved in the cellular differentiation of the bacterium.[10−13] These enzymes display a modular structure being composed of one or two catalytic A-modules and from one to seven regulatory R-modules. Although highly homologous, these enzymes each create characteristic M/G patterns producing alginates with different properties. AlgE4, which is the smallest epimerase containing one A-module and one R-module, makes predominantly alternating M/G structure acting on polyM by a processive mode of action.[14−16] AlgE1, AlgE2, AlgE3, AlgE5, and AlgE6 make G-blocks of varying lengths, and AlgE6 is the epimerase able to make the longest G-block structures when acting on polyM.[17] AlgE7 encompasses dual functionalities in also having alginate lyase activity.[18] The 3D structure of the A- and R-module of AlgE4 has been reported, and the catalytic residues in the active site have been determined.[19,20] Given the similarity in amino acid sequence of the A-modules of the epimerases, it is clear that very minor and to a large extent unpredictable changes in the primary structure can lead to alterations in the epimerization pattern.

The microscopic structure, that is, the monomeric sequence distribution of biopolymers like alginate, determines the chemical and physical properties of the molecules and thereby the spectrum of possible applications.[21,22] Being able to structurally design biopolymers is therefore highly desirable for obtaining biomaterials with controllable and targeted properties.[2] For alginates this can be approached by utilizing mannuronan C-5 epimerases with defined properties in in vitro epimerization processes of, that is, polyM.[23,24] As opposed to current manufacturing strategies from algal resources, this enzymatic route would reduce batch-to-batch variations as well

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as introduce possibilities for obtaining reproducible alginate structures that are not readily obtainable from algae. Additionally, in vitro epimerization with specific enzymes could also offer a route for so-called upgrading of algal alginate, that is, increasing the level of G-blocks.[17] This strategy could represent a valuable supplement to the global alginate market, which is facing a shortage of G-rich alginates due to the lack of algal raw material.

In the present study, our main goal has been to develop mannuronan C-5 epimerases that can be used for efficient in vitro epimerization of either bacterially produced mannuronan or alginate substrates of algal origin. On the basis of the *algE1-algE6* genes from *A. vinelandii*, a mutant library was constructed by gene shuffling and subsequent error-prone PCR. A high-throughput screening protocol was developed that enabled discrimination of epimerised alginate based on the M/G content in the samples, that is, the microscopic structure of the polymers created by the mutant epimerases. To our knowledge, high-throughput screening studies based on biopolymer structure have not been previously performed. By this approach, we have obtained novel mannuronan C-5 epimerases that are more efficient in epimerizing polyM to high levels of G-blocks than any of the wild-type enzymes. Time-resolved NMR spectra indicate that at least one of these enzymes has altered enzyme kinetics compared with wild-type AlgE6. Furthermore, results obtained in this work indicate that the R-modules of the mannuronan C-5 epimerases play a role also in determining the epimerization pattern, a property that has previously been attributed only to the catalytic A-modules.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and DNA Manipulations. *Escherichia coli* strains DH5α (Bethesda Research Laboratories), JM109 (New England BioLabs), and XL1-Blue (Stratagene) were used as general cloning hosts, whereas XL10-Gold (Stratagene) was used for establishing the mutant library. *E. coli* strains were routinely grown at 37 °C in LB medium (yeast extract, 5 g/L; tryptone, 10 g/L; and NaCl, 10 g/L) or on LB agar (LB medium supplemented with 20 g/L agar). For protein expression, strains were grown in triple-strength LB medium (3 × LB; yeast extract, 15 g/L; tryptone, 30 g/L; and NaCl, 10 g/L). For growth in 96-well plates, a reduced Hi-Ye medium with the following composition was used: $Na_2HPO_4{\cdot}2H_2O$, 12.3 g/L; KH_2PO_4, 4.29 g/L; NH_4Cl, 0.43 g/L; NaCl, 0.71 g/L; glucose, 2.86 g/L; yeast extract, 2.86 g/L; citric acid, 1.43 g/L; $MgSO_4$, 1.86 mM; Fe(III)-citrate, 118 μM; H_3BO_3, 21.0 μM; $MnCl_2$, 37.6 μM; EDTA, 9.86 μM; $CuCl_2$, 3.86 μM; Na_2MoO_4, 4.29 μM; $CoCl_2$, 4.71 μM; and Zn-acetate, 17.3 μM. Cultures were induced for protein expression using an induction solution containing: glycerol (99%), 25.8 g/L; yeast extract, 24 g/L; and isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.25 mM. When appropriate, ampicillin (200 μg/mL) was added to the growth media. Standard recombinant DNA techniques were performed, as described elsewhere.[25] Plasmids were purified by the WizardPlus SV Minipreps DNA purification system (Promega) or the QIAGEN Plasmid Plus Midi Kit (QIAGEN). Transformation of XL10-Gold ultracompetent cells was performed according to instructions given by the manufacturer and for other *E. coli* strains according to RbCl transformation protocol (New England BioLabs). DNA sequencing was performed by Eurofins MWG Operon. Construction of vectors expressing epimerases with site-specific mutations and mutant A-modules combined with the R modules of AlgE6 is described in Table S1 in the Supporting Information.

Construction of an Epimerase Gene Library by Staggered Extension Process (StEP) and Error-Prone PCR. Vectors used and constructed are listed in Table S1 in Supporting Information, whereas sequences of primers utilized are given in Table S2 in Supporting Information. The sequences encoding the A-modules of AlgE1−6 (eight in total) were cloned as *Nco*I-*Xma*I fragments into *pTrc99A*, and the resulting vectors were used as templates in the StEP reaction. Primers StEP fwd (located 257 bp upstream of *Nco*I) and StEP rev (loacated 145 bp downstream of *Xma*I) were used for amplification of PCR fragments, and the reaction mixtures used were as follows: 12 ng of each template vector, 30 pmol of each primer, 0.2 mM dNTP, 1 × Taq 2000 buffer, and 3.75 U Taq 2000 DNA polymerase. PCR conditions used were as follows: 2 min at 96 °C, 80 cycles of 30 s at 95 °C, and 3 to 4 s at 40−45 °C. The StEP procedure was repeated several times, and because DNA sequencing of the PCR products revealed a predominance of the gene AlgE5A in the recombined sequences for the A-modules, the vector encoding this A-module was omitted from some of the StEP reactions. PCR reactions were treated with *Dpn*I to degrade template DNA, and fragments with correct size (1.1 kb) were isloated from agarose gels. The recombined fragments were digested with *Nco*I-*Xma*I and ligated into the same sites of pBL5 creating libraries of hybrid epimerase genes consisting of recombined A-modules (376 amino acid residues) and the R module (177 amino acid residues) from AlgE4. The ligation mixtures were transformed into XL-10 gold cells, and the resulting transformants were pooled together, grown for a few generations in LB medium, and used for plasmid isolation. Plasmids from each StEP reaction were mixed together and used as template for error-prone PCR. Random mutations were introduced into the recombined library either by using the GeneMorph PCR mutagenesis Kit from Stratagene (method 1) or by decreasing the fidelity of the *Taq* polymerase by manipulating the M_n/M_g ratio and the nucleotide concentration in the reaction mixture (method 2). Method 1: Conditions were chosen to give a mutation frequency of 3−7 mutations/kb: 8.5 ng template DNA (corresponding to 1 ng target DNA), 30 pmol each of primers StEP fwd and EU20 (located 52 bp downstream of *Xma*I), 0.8 mM dNTP, 1× mutazyme buffer, and 2.5 U mutazyme in a final volume of 50 μL. Method 2: 78 ng template DNA, 30 pmol each of primers StEP fwd and EU20, 0.2 mM dNTP, 5 μL 5 × PCR-buffer (300 mM Tris-HCl, pH 8.5, 75 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$), 1.25, 2.0, 2.5, or 3.0 μL of 10× mutagenic buffer (8 mM dTTP, 8 mM dCTP, 48 mM $MgCl_2$, 5 mM $MnCl_2$) and 5 U *Taq* polymerase (Promega) in a final volume of 50 μL. PCR conditions used for both methods were as follows; 1 min at 96 °C, 4 cycles of 30 s at 96 °C, 30 s at 55 °C, and 2 min at 72 °C and then 27 cycles of 30 s at 96 °C, 30 s at 60 °C, and 2 min at 72 °C. The five PCR reactions mixtures (one by method 1 and four by method 2) were treated with *Dpn*I, and fragments of correct size (1.1 kb) were isolated from agarose gels, digested with *Nco*I-*Xma*I, and ligated into the corresponding sites of pBL5 to creating recombined, mutated libraries of epimerase genes. The ligations mixtures were transformed into XL10-Gold cells, and the transformants were pooled together before the addition of glycerol and storage (−80 °C) of the library.

Production of Alginate Substrates and Alginate Lyases. High-molecular-weight mannuronan (polyM) was isolated from a mutant strain of *Pseudomonas fluorescens* NCIMB 10525.[26] ^{13}C-1 labeled mannuronan was produced by growing *P. fluorescens* on a minimal media with 99% D-^{13}C-1 fructose as carbon source. The obtained mannuronan was selectively enriched to 59% with ^{13}C at carbon position C-1.[15,27] Alginate with a strictly alternating structure (polyMG; F_G = 0.47 and F_{GG} = 0) was prepared by epimerization of polyM with recombinant mannuronan C-5 epimerase AlgE4 and characterized by NMR as described previously.[27] G-blocks (polyG; F_G = 0.94 and DP = 18.5) were prepared from *Laminaria hyperborea* stipes, as described elsewhere.[28,29] Production of M-M-specific M-lyase (AlxM), G-M, and G-G-specific G-lyase (AlyA) and G-G-specific GG-lyase (AlyA5) by fermentation of recombinant *E. coli* strains was performed as described elsewhere.[30]

Robotic Screening of the Mutant Recombined Epimerase Library. The *E. coli* library was plated on LB agar in 25 × 25 cm Petri dishes (Corning CLS431301) and incubated overnight at 37 °C. Colonies were picked using a Genetix Q-Pix2 robotic colony picker and transferred to 96-well microplates (Greiner M3186) containing 80 μL of reduced Hi-Ye medium. The microplates were incubated at 30

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°C, 900 rpm (3 mm amplitude) and 80% relative humidity. After 24 h, 40 μL of induction solution was added to the microplates using an Asys Hi-Tech Flexispence microplate dispenser. The microplates were incubated at 37 °C, 900 rpm, and 80% relative humidity for 7 h after induction and were frozen at −40 °C prior to analysis.

After thawing, to the microplates was added 30 μL of B-per II solution (Pierce) (with $CaCl_2$ to a concentration of 25 mM) per well; they were shaken for 30 s (900 rpm, 3 mm amplitude) and incubated at room temperature for 1 h. After incubation, the microplates were shaken (850 rpm, 3 mm amplitude) for 10 min and then centrifuged for 30 min at 3500*g*. For epimerization of alginate, 10 μL of enzyme extracts was added to 190 μL of assay buffer (40 mM MOPS, 20 mM NaCl, 2 mM $CaCl_2$, pH 6.8) containing polyM alginate (0.1 mg/mL). The plates were sealed after the addition of enzyme extract using sterile sealing film (Nunc 236366) and incubated at 37 °C for 48 h. Microplates with epimerized alginate were frozen at −40 °C prior to analysis.

For analysis of G-content in epimerized alginate samples, we transferred 30 μL samples of alginate in assay buffer to wells in 384-well microplates (Corning CLS3675). To the wells was then added 10 μL of assay buffer containing the AlyA enzyme (0.14 U/mL on MG alginate), and it was shaken at 1700 rpm for 1 min. The microplates were then incubated at 25 °C for 5 h. The absorbance at 230 nm (A230) was read in a Beckman Coulter DTX880 microplate reader prior to the addition of alginate shortly after mixing and after incubation. The increase in absorption during incubation was calculated, and ΔA230 ($A230_{t=5} - A230_{t=0}$) was used for estimation of the total G content of the epimerized alginates.

To be able to discriminate between alginate samples containing MG- and GG-blocks, we developed a two-step protocol using an M-lyase and two alginate lyases with different specificity toward G-block and polyMG alginates. We transferred 30 μL of epimerized alginate in assay buffer to wells in 384-well microplates (Corning CLS3675). To each of the wells was added 5 μL of assay buffer containing M-lyase, and the microplates were shaken at 1700 rpm for 1 min and incubated at 25 °C for 12 h. To each of the wells was then added 5 μL of assay buffer containing either the AlyA enzyme or the AlyA5 enzyme (0.2 U/mL on polyG for both enzymes), and the microplate was shaken at 1700 rpm for 1 min. After mixing, the microplates were further incubated at 25 °C for 12 h. The absorbance at 230 nm (A230) was read in a Beckman Coulter Paradigm microplate prior to the addition of enzymes, shortly after mixing, and then each hour after addition enzymes. The difference in absorbance between the two first time points (ΔA230 = $A230_{t=1} - A230_{t=0}$) was used for evaluation of the G content and structure of the epimerized alginates. All liquid and microplate handling was performed by a Beckman Coulter Core system robotic liquid handling workstation.

Protein Expression and Purification. Epimerase expressing strains were grown in 100 mL of 3× LB medium in 500 mL baffled shake flasks at 37 °C for 3 h before induction with 0.5 mM IPTG. Growth was continued for 4 h at the same temperature before harvesting the cells by centrifugation. For preparation of protein extracts, the cells were sonicated in 10 mL of 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), 5 mM $CaCl_2$, pH 6.9, and then centrifuged for 30 min at 20 000*g*. The supernatant was filtered (0.2 μm) and applied on a 5 mL HiTrap Q HP column, and proteins were eluted using a stepwise NaCl gradient (0 to 1 M) in the same buffer as above. Protein-containing fractions were tested for epimerase activity by NMR (see below), and the total protein content was measured by the Bio-Rad microassay procedure using bovine serum albumin as standard. Purity of protein fractions was determined by SDS-PAGE.

End-Point and Time-Resolved NMR Analysis of Epimerised Alginate Samples. All experiments were recorded on a BRUKER Avance 600 or DPX 400 spectrometer equipped with a 5 mm cryogenic CP-TCI z-gradient probe and 5 mm z-gradient DUL (C/H) probe, respectively. End-point analysis of epimerised samples was recorded at 90 °C, while time-resolved NMR recording of the epimerization reaction was performed at 40 °C. To reduce the viscosity of the alginate samples prior to NMR measurements, the samples were depolymerized by mild acid hydrolysis to a final average DP_n ∼30 residues.[31] 3-(Trimethylsilyl)-propionic-2,2,3,3-*d*4 acid sodium salt (Aldrich, Milwaukee, WI) was used as an internal standard for the chemical shift and triethylenetetra-amine hexa-acetate (Sigma-Aldrich) was added to chelate residual calcium ions in end-point epimerised samples. For the time-resolved NMR analysis of epimerization reactions, a stock solution of 22 mg/mL ^{13}C-1-enriched polyM (average DP_n ∼70) in 5 mM MOPS, pH 6.9 with 75 mM NaCl in 99.9% D_2O was prepared. Purified enzyme fractions from ion exchange chromatography were subject to buffer exchange and upconcentrated (final concentration of 1.1 to 2.3 mg/mL) by spin columns (VivaSpin, Sartorius Stedim Biotech) with molecular cutoff 10 kDa. Samples were washed with 5 mM MOPS, pH 6.9 with 75 mM NaCl and 27.5 mM $CaCl_2$ in 99.9% D_2O. Protein concentrations were determined with a Nanodrop ND-1000 to ensure similar enzyme concentration in the epimerization reaction. 500 μL of ^{13}C-1-enriched polyM stock solution was preheated in the NMR instrument and 1D proton and carbon spectra were recorded to ensure that the sample has not undergone any degradation or contamination prior to the time-resolved NMR experiment. 50 μL of enzyme solution was added to preheated substrate and mixed by inverting the sample two to three times. The sample was then immediately inserted into the preheated NMR instrument and the experiment was started. The recorded spectrum is a pseudo-2D type experiment recording a 1D carbon NMR spectrum every 15 min. The recorded 1D carbon spectrum (using inverse gated proton decoupling) contains 8K data points and has a spectral width of 80 ppm, 64 scans with a 30° flip angle, and relaxation delay of 1 s (total recording time of 91s). The NMR data were processed and analyzed with Bruker XwinNMR 3.5, TopSpin 2.1, and TopSpin 3.0 software.

Bioinformatics Analysis of Epimerase Mutants. The experimental 3D structure of the A-module from AlgE4 with and without mannuronan trisaccharide bound (Protein Data Bank code 2PYH and 2PYG, respectively) in the substrate binding groove were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank.[32] The structures were used as template input for the SWISS-MODEL platform[33−35] modeling the mutant A-modules identified from the library. The structures were visualized and analyzed with PyMol.[36] The alignment of mutant and wild-type protein sequences was done with ClustalX[37] and visualized with TreeView.[38]

RESULTS AND DISCUSSION

Construction and Characterization of a Mannuronan C-5 Epimerase Mutant Library. To obtain mannuronan C-5 epimerases with improved properties for in vitro epimerization, we used the genes encoding the secreted epimerases from *A. vinelandii* as the basis for construction of a mutant library. The catalytic site of the epimerases is located in the A-modules of the enzymes, and the gene sequences encoding these modules of AlgE1-AlgE6 (E1A1, E1A2, E2A, E3A1, E3A2, E4A, E5A and E6A) were therefore used as templates in the staggered extension process (StEP). DNA encoding AlgE7 was not included in the recombination reaction due to the combined epimerase and lyase activity of this enzyme.[39] The recombined A-module sequences were ligated into expression vectors containing the R-module sequence of AlgE4 creating complete epimerase genes. The resulting plasmids were transformed into XL10-Gold cells creating a library of about 120 000 clones. To test the diversity in the library, we sequenced the plasmids from 48 random clones to analyze the degree of recombination between the different A-module sequences, and the epimerases encoded by the same plasmids were also tested for epimerase activity. Sequence alignment showed that 33 plasmids (69%) encoded shuffled A-modules, and of these, 26 (78%) encoded epimerases displayed activity. To further increase the diversity of the DNA sequences, we performed error-prone PCR on the recombined A-module genes from the first library. Conditions

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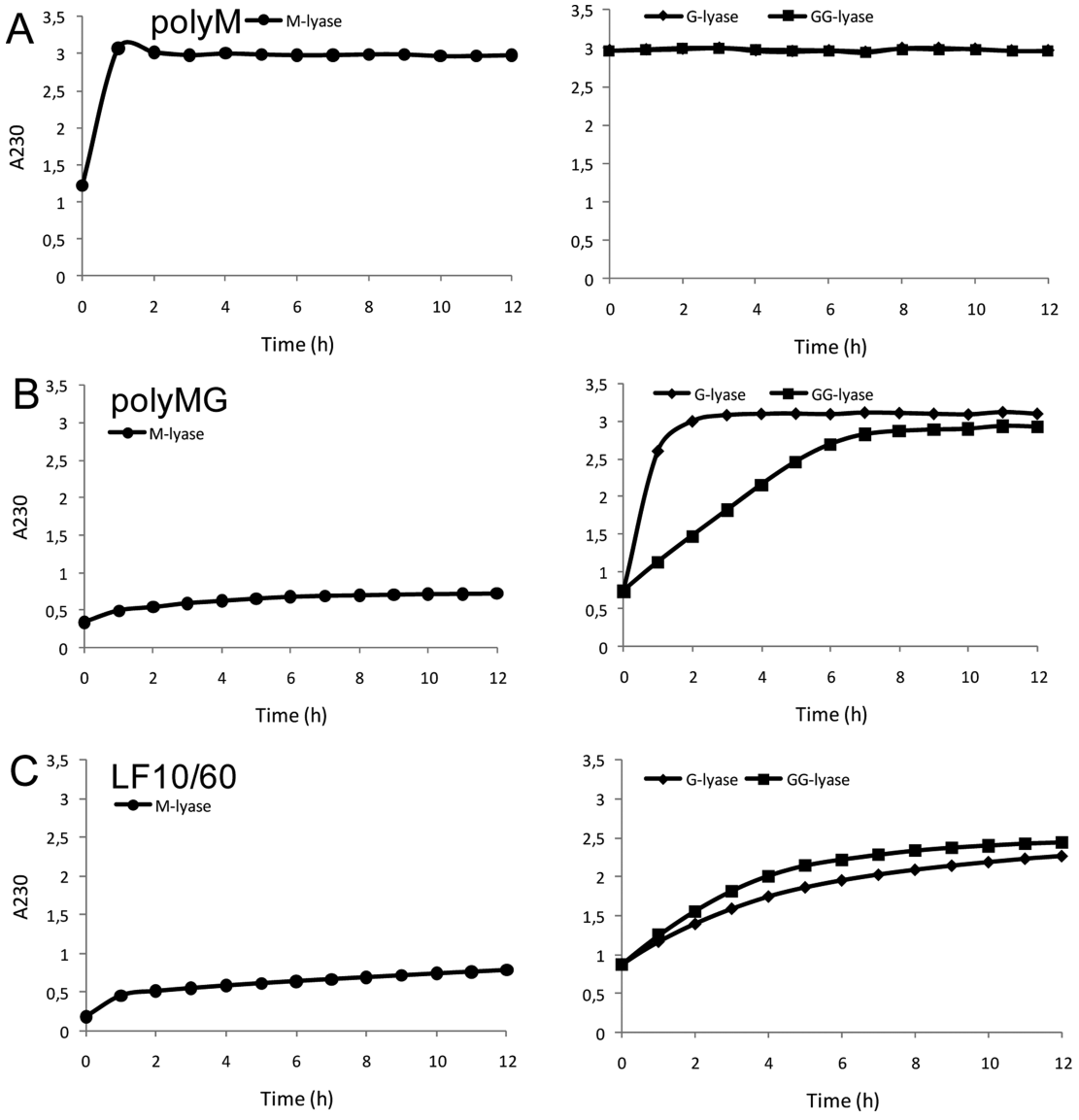

Figure 1. Degradation of polyM (A), polyMG (B), and LF10/60 (C) with M-lyase (left) and G- or GG-lyase (right) measured by monitoring A_{230}. To two parallel samples of the alginate substrate (1 mg/mL in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$) was added M-lyase (0.5 U/mL on polyM), and they were incubated for 12 h with A_{230} readings every hour. Then, G- and GG-lyase (0.2 U/mL on polyG) were added to parallel samples and incubation and A_{230} reading continued for an additional 12h. Degradation reactions for each sample were performed in 384-well plates in 96 parallels, and the curves shown are representatives of each kind. The standard deviation of the mean for the measurements is below 15%.

were adjusted to achieve a mutation frequency of about 3−8 nucleotide changes per gene. The mutagenized A-modules were ligated into expression vectors containing the R-module sequence from AlgE4, and the resulting plasmids transformed into XL10-Gold cells, creating a final library of about 100 000 clones. To verify the mutation frequency, we sequenced plasmid DNA from 56 random clones, and 18 of the mutant enzymes encoded by these plasmids (32%) were found to display epimerase activity. This final library containing epimerases with recombined and mutated A-modules was used for screening for enzymes with high G-block-forming activity.

Screening of the Mutant Library and Isolation of Enzymes That Are Able to Epimerise Mannuronan to High Levels of G-Blocks. An initial screening was performed by randomly picking 11 000 colonies from the final library, followed by cultivation and induction for epimerase expression with IPTG in 96-well microtiter plates. Cell-free extract from each culture was prepared and used for epimerization of polyM. To evaluate the G content in each epimerised sample, we degraded the resulting alginates using a G-lyase, which cleaves G-M and G-G linkages almost equally well (polyMG/polyG activity ratio of 1.2).[40−42] End-point measurements of A_{230} detecting the unsaturated uronic acid residues resulting from lyase degradation made it possible to identify samples containing epimerised alginate among the ones that were not epimerised, that is, containing polyM. However, by this method, it turned out to be difficult to distinguish between samples of alginates with medium G content (~45%) in alternating MG structure and high G content (~80%) in block structure. This is due to the similar amounts of linkages available for lyase degradation in the two structurally very different alginates. A two-step degradation protocol utilizing three alginate lyases with different specificities was therefore developed. The strategy was to first use an M-lyase to specifically degrade all of the M-M linkages in the alginate

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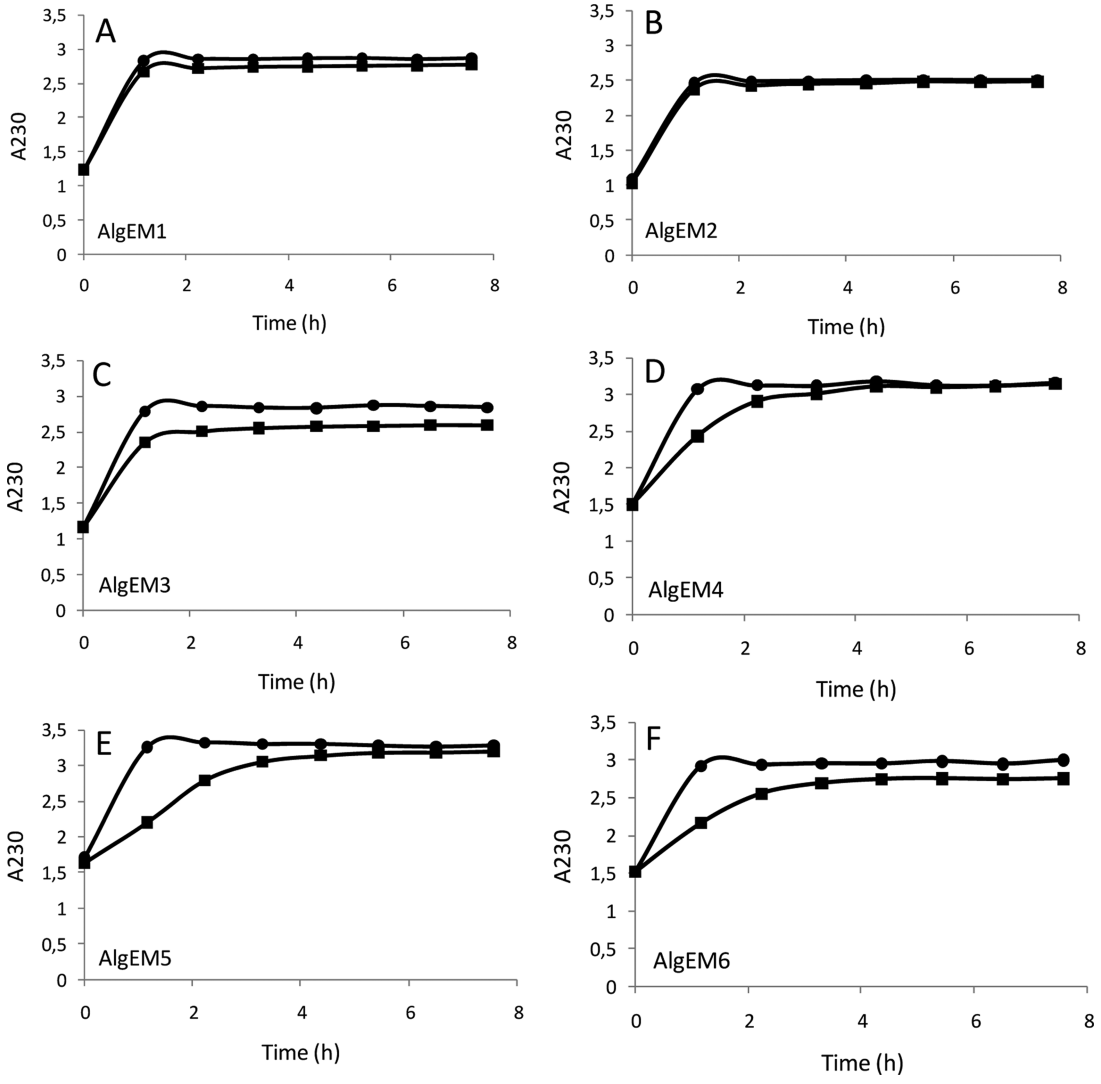

Figure 2. Degradation of polyM epimerised with mutant epimerases AlgEM1−6. Two parallel samples of each were first degraded with M-lyase for 12 h (not shown in the Figure); then, to samples were added G- (circles) and GG-lyase (squares), and incubation and A_{230} reading continued for additional 12h. The Figures show the A_{230} measurements only up to 8 h because after that there was no change in absorbance. The reaction conditions and enzyme concentrations used and the experimental uncertainty for the measurements were as described in the legend to Figure 1.

samples.[43] Samples containing high or low M would then be subject to extensive or limited degradation by this enzyme, respectively. The next step was to further degrade two parallels of the same samples with G-lyase and GG-lyase, with the latter enzyme displaying increased specificity toward G-G linkages (polyMG/polyG activity ratio of 0.1).[42] The GG-lyase was expected to display lower activity toward a sample containing alginate molecules with alternating MG structure than the G-lyase, whereas the two enzymes were expected to have similar activity toward samples rich in G-G linkages. To take full advantage of the different specificities of these two enzymes, it was necessary to follow the kinetics of alginate degradation. End-point measurements would give nearly the same A_{230} for both enzymes because the GG-lyase has some residual activity toward G-M linkages. The protocol was first tested on alginates with known composition: polyM (F_M = 1, F_G = 0), polyMG (F_G = 0.47, F_{GG} = 0), and LF10/60 (F_G = 0.66, F_M = 0.34, F_{GG} = 0.55, F_{MG}/F_{GM} = 0.12, F_{MM} = 0.22). First, M-lyase was added to the samples and degradation was followed by measuring A_{230} every hour for 12 h. Then, two parallel samples were added, G- or GG-lyase, and incubation continued with A_{230} monitoring for another 12 h. As expected, polyM is completely degraded in the M-lyase step; that is, there is no further increase in A_{230} by the addition of G- or GG-lyase (Figure 1A). Furthermore, the kinetics of degradation of polyMG and LF10/60 was clearly different in the second step (Figure 1B,C), and the largest difference in activity between the G- and GG-lyase was as expected obtained on the alternating substrate (Figure 1B). This showed that it was possible to perform a screening of the library based on discrimination between differences in the resulting polymer microstructures created by the mutant enzymes. To our knowledge, this kind of high-throughput screening study has not been previously performed. Because of the increasing applications and need for high-G alginates, we targeted the current screening approach to isolate mutant enzymes giving high content of G-blocks when epimerising polyM.

Alginate epimerised by 960 randomly selected mutants was evaluated using the two-step degradation protocol described above (9% of the amount in the initial screen). PolyM was epimerised with protein extracts from the randomly selected mutants and subjected to lyase degradation, as described for the

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test samples. From data analysis of the degradation kinetics of each sample, we identified three for which the degradation with G- and GG-lyase was almost identical (Figure 2A,B) or very similar (Figure 2C), indicating a high level of G-blocks in the epimerised alginate. Furthermore, around 25 samples displayed degradation kinetics similar to polyMG, indicating an F_G of ∼0.45 (three examples shown in Figure 2D−F). To test whether the observed degradation kinetics corresponded to the expected sample properties, we performed a preliminary characterization of crude protein extracts obtained from a total of 11 strains (including those shown in Figure 2). Strains were grown in shake flasks, and cell-free protein extracts were used to epimerise polyM for NMR analysis. These analyses showed that alginates with F_G in the range of 0.65 to 0.80 were obtained for the samples shown in Figure 2A−C and 0.40−0.45 for the remaining samples. Taken together, this confirmed the validity of the screening method for identification of samples with a specific composition. Furthermore, it also indicated that ∼0.2% of the mutants in the library encoded epimerases that were introducing such a high level of G-blocks into polyM.

It should be pointed out that the kinetic measurements performed to obtain the data underlying the isolation of mutant enzymes are time- and resource-demanding. If the aim was to isolate as many mutants as possible, then an alternative strategy allowing for screening of a larger portion of the library could be considered. As a first step, the entire collection of alginate samples would be degraded with M-lyase and end-point A_{230} measurements used to sort out the ones containing polyM (inactive enzymes) and low G (enzymes with low activity). The remaining samples, that is, with A_{230} below 1 like in Figure 1B,C, would then be chosen for further characterization following the kinetics during degradation with G- and GG-lyase. From the original library of about 100 000 clones, one would then expect to obtain around 200 mutants resulting in high G when starting with polyM.

Mutant Epimerases AlgEM1 and AlgEM2 Epimerise polyM to Higher G-Content than Wild-Type Epimerase AlgE6. To characterize enzyme properties in detail, we show the mutant epimerases corresponding to samples in Figure 2 (designated AlgEM1−AlgEM6), which were partially purified by ion exchange chromatography and used for epimerization of polyM. As a control, polyM was also incubated with AlgE6, which is the wild-type epimerase known to give the highest F_G.[17] The monomer composition and the diad and triad frequencies in the different alginate samples are shown in Table S3 in the Supporting Information. Two of the mutants, AlgEM1 and AlgEM2, epimerised polyM to F_G = 0.85 and 0.83, respectively, which is higher than what was obtained with AlgE6 (F_G = 0.77) under the conditions utilized. AlgEM1- and AlgEM2-epimerised alginate also display high F_{GG} (0.78 and 0.74) and low F_{MGM} (0.030 and 0.027), indicating that the majority of guluronic acid residues are present as blocks and that there is very little alternating structure present. Compared with AlgE6, these two enzymes produce alginates in which a larger fraction of the total guluronic acid residues are present as blocks. AlgEM3−5 resulted in alginates with F_G in the range of 0.54 to 0.67 and where the guluronic acid residues introduced are present both in blocks (F_{GG} = 0.22−0.44) and in alternating structures (F_{MGM} = 0.15−0.25). AlgEM6 epimerised polyM to F_G = 0.4, and most of the guluronic acid residues are present in alternating structures (F_{GG} = 0.039 and F_{MGM} = 0.33), which is very similar to wild-type AlgE4.

The availability of effective enzymes that can epimerise polyM in a single reproducible reaction step is valuable in in vitro design of alginates. Another approach for enzymatic preparation of specific alginates is to perform epimerization of algal alginates with the aim of increasing the G content, and we therefore wanted to test mutants AlgEM1 and AlgEM2 on a predefined complex alginate isolated from leaves of *L. hyperborea* to elucidate the efficacy on this kind of substrate. This natural substrate has F_G = 0.50, with G-residues present as both blocks and alternating structures. NMR analysis showed that AlgE6 and AlgEM1 acted very similar on this substrate, yielding alginates with F_G = 0.76 and 0.75, respectively, whereas AlgEM2 reached F_G = 0.69. This indicates that the enzymes act quite differentially on various substrates and that in screening for epimerases with targeted properties, the choice of substrate is very important.

AlgEM1 and AlgE6 Display Different Epimerization Kinetics on polyM. The results shown in Table S3 in the Supporting Information are end-point measurements of the final composition of polyM epimerised with the different enzymes. To reveal possible differences in the kinetics of epimerization for the mutant enzymes, we compared AlgEM1 and AlgEM2 with AlgE6; continuous NMR-spectra were recorded using ^{13}C-1-enriched polyM. AlgEM1 (Figure 3B) and displayed significantly different epimerization kinetics than AlgE6 (Figure 3A), whereas AlgEM2 displayed a spectrum very similar to AlgE6 (spectra not shown). AlgEM1 showed an almost immediate and fast introduction of G-blocks (evident as increase in peak marked G<u>G</u>G) into the substrate. This is accompanied by a simultaneous rapid decline in the content of M blocks (M<u>M</u>M) as well as a slow accumulation of alternating blocks (G<u>M</u>G and M<u>G</u>M). Moreover the G<u>G</u>M peak that signifies the number of G-blocks remains constant after the initial phase, indicating that the G residues are introduced predominantly as elongation of existing G-blocks. For AlgE6, the formation of G-blocks lagged behind the introduction of G residues in alternating sequences, indicating that AlgEM1 has higher affinity for the alternating polyMG structure than polyM compared with AlgE6. There are, in principle, two modes of action that could account for a predominant G-block formation; a processive mode where the enzyme slides along polyM, carrying out repetitive epimerization reactions without dissociating from the substrate, or a preferred attack mode where the enzymes affinity is higher for M-G than for M-M. In both cases, subsite −1 (by definition, epimerization takes place at subsite +1) must preferentially accommodate a G residue. A processive mode where consecutive residues are epimerized appears to be unlikely because the uronic acid residues are rotated 180° with respect to each other and the enzyme would then have to rotate while sliding around the polymer chain. We have previously demonstrated processivity for AlgE4 acting on polyM generating long alternating MG stretches or for AlgE6 when acting on polyMG. In both of these cases the enzymes act in processive modes, where every second M is converted while the enzymes slide along the polymer; however, this does not require the enzyme to rotate. Whether the properties of AlgEM1 are due to an increase in processivity or a result of an enhanced affinity for pre-existing G residues is not possible to conclude from the present experiments.

Alignment of Mutant and Wild-Type A-Modules Elucidates Sequence Properties Mediating Changes in Enzymatic Activity. To reveal possible structural features in the mutants underlying the enzymatic properties described

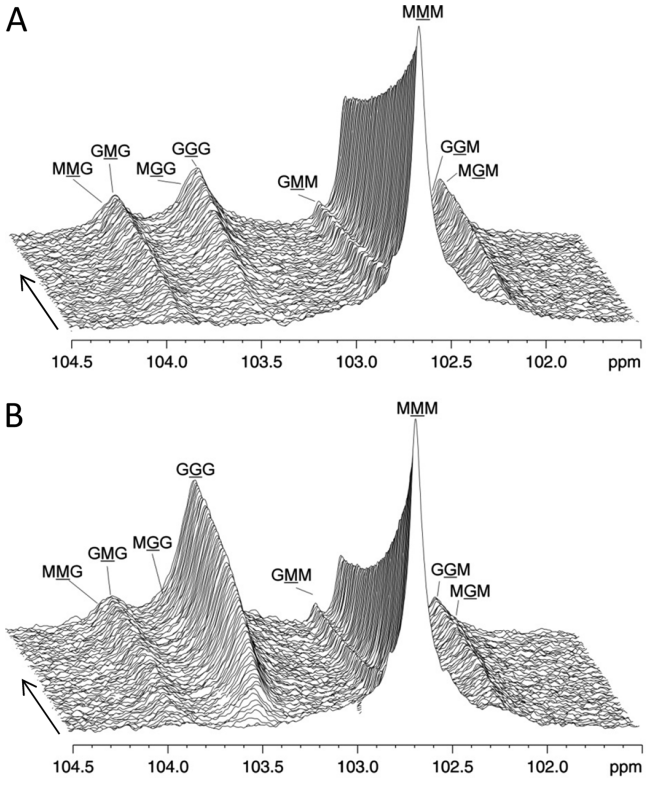

Figure 3. Continuously recorded NMR spectra showing epimerization of ^{13}C-labeled polyM with AlgE6 (A) and mutant enzyme AlgEM1 (B). Substrate (20 mg/mL) and enzyme were mixed and immediately inserted into the NMR instrument before recording of spectra every 15 min. Reactions were performed in MOPS, pH 6.9 with 75 mM NaCl and 2 mM $CaCl_2$. The position of each of the eight possible triads in the spectra is indicated, and the M or G moiety giving rise to the signal is underlined. Arrows indicate increasing reaction times. It should be noted that the enzyme reactions were not run to complete epimerization of the substrate, so the end composition of the resulting alginates in this experiment is not directly comparable to results given in Table S3 of the Supporting Information.

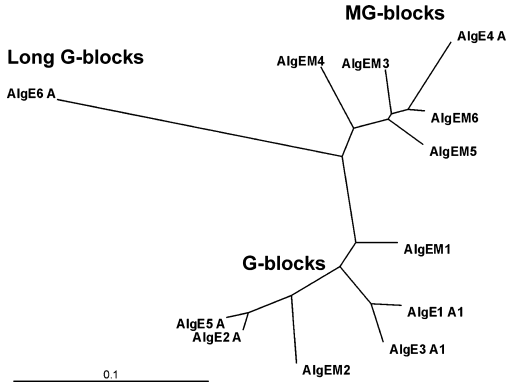

Figure 4. Phylogenetic tree displaying the relationship between the wild-type and mutate A-modules in relation to their function on alginates. Alignment of mutant and wild-type protein sequences was done with ClustalX[37] and visualized with TreeView.[38]

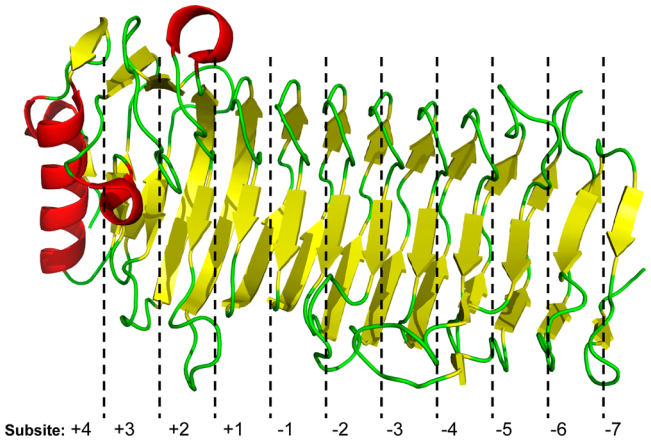

Figure 5. Ribbon structure of the A-module from AlgE4 with subsites for the substrate indicated. The division of the subsites is based on prolongation of the mannuronan trisaccharide bound to A-module from AlgE4 (Protein Data Bank code 2PYH) in the substrate binding groove. Subsite +4: Met1-Glu51, +3:Pro52-Ser91, +2: Ala92-Asn123, +1: Gly124−156+Asp178, −1: Thr157-Asp178+Leu228, −2: Tyr179-Leu228, −3: Glu229-Lys255, −4: Met256-Tyr278, −5: Gly279-Tyr318, −6: Thr319-Arg343, and −7: Asn344. The structures were visualized and analyzed with PyMol.[36]

above, we sequenced the genes encoding the A-modules of mutants AlgEM1−6, and the resulting protein sequences were compared with wild-type A-modules. Sequence properties, that is, similarity to wild-type A-modules and introduction of new amino acid residues due to error-prone PCR, are shown in Table S4 in the Supporting Information. The relationship between the A-modules is visualized in a phylogenetic tree (Figure 4), and alignment of sequences is given as Supporting Information (Figure S1A−C in the Supporting Information). The phylogenetic tree displays three groups, which can be characterized as formers of MG-blocks, G-blocks, and long G-blocks, the latter group comprising only AlgE6. A general tendency found for AlgE6 is that more amino acid residues promoting hydrogen bonding and hydrophobic interactions are identified along the alginate binding groove in the A-module compared with the other G-block-forming enzymes (Figure S1 in the Supporting Information).

Sequence alignment analysis shows that the A module of AlgEM1 is most similar to the G-block-forming A1 modules of AlgE1 and AlgE3 before the position of the active site, whereas it is most similar to AlgE6 after the active site. Furthermore, in AlgEM1 the alginate binding subsite at −3, −4, and −6 seems to have more residues supporting alginate binding through hydrogen bonds and hydrophobic interactions (e.g., Arg, Leu, Ser, Asn) than the other G-block formers (Figure 5, Figure S1 in the Supporting Information). The AlgEM2 A-module is most similar to AlgE2 and AlgE5, which are also both capable of producing G-blocks. Moreover, AlgEM2 has a substitution in subsite −2 (Ala220Asn) that putatively supports stronger alginate binding through hydrogen bonding. Furthermore, some amino acid residues change the N-terminal α-helix interfacing the rest of the protein and lead to minor rearrangement in packing of the hydrophobic core under subsite +1 (Val136Ala) and −1 (Ile200Val). This might result in a deeper substrate binding groove, hereby enlarging the contact surface to the alginate polymer. Because both AlgEM1 and AlgEM2 possibly have improved alginate binding properties compared with AlgE6, this can partially explain their ability to form long G-blocks. The mode of action for the G-block forming A-modules except for AlgE6 acting on polyMG has been characterized as a preferred attack mechanism,[44] and accordingly the N-terminal part before the catalytic site of AlgEM1 and AlgEM2 seems to originate from these A-modules.

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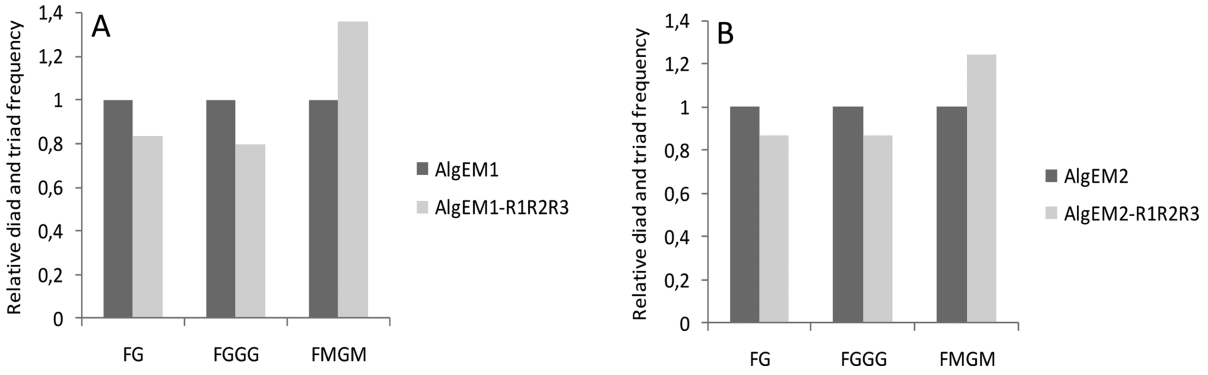

Figure 6. Relative frequencies of F_G, F_{GGG}, and F_{MGM} obtained by epimerization of polyM with mutant enzyme AlgEM1 and AlgEM1-$R_1R_2R_3$, that is, A-module from AlgEM1 and R-modules from AlgE6 (A). Corresponding results for AlgEM2 and AlgEM2-$R_1R_2R_3$ (B). Epimerization was performed with polyM (10 mg) and enzyme (0.2 mg) in MOPS, pH 6.9 with 100 mM NaCl and 1.5 mM $CaCl_2$.

Combining the preferred attack mode of action with better alginate-binding properties, that is, improved processivity may then have resulted in the high G-block-forming epimerases AlgEM1 and AlgEM2. The mutants AlgEM3−6 (Table S4 in the Supporting Information) have almost all mutations at the N-terminal part before the catalytic site, probably resulting in the ability to form G-blocks. After the active site they are almost identical to the AlgE4 A-module, presumably mediating the ability to create alternating structures (Figure S1 in the Supporting Information).

Introduction of R-Modules from AlgE6 ($R_1R_2R_3$) Behind Mutant A-Modules from AlgEM1 and AlgEM2 Modulates the Epimerization Pattern of the Enzymes. As a consequence of the construction strategy (see Materials and Methods), all mutants should contain the same C-terminal end, that is, 177 residues constituting the AlgE4 R-module. However, for AlgEM2 and AlgEM6, a deletion in the *Xma*I restriction site used for cloning leads to a frame shift and translation into a 52 residue long C-terminal end following the A-module. It has been previously shown that the A-modules alone are sufficient for epimerization,[45] and apparently the addition of 52 random residues C-terminally does not affect the enzymes detrimentally. Although it has been shown that the R-modules are Ca^{2+}-binding and stimulate the activity of the A-modules when present,[45] the function of the R-modules is not fully understood. AlgE6 is an efficient G-block forming epimerase with three R-modules ($R_1R_2R_3$), and we wanted to explore the effect of combining these R-modules with the mutant A-modules from AlgEM1 and AlgEM2. DNA sequences encoding $R_1R_2R_3$ were synthesized and cloned into the vectors encoding the two mutant A-modules, resulting in the expression of hybrid enzymes, AlgEM1-$R_1R_2R_3$ and AlgEM2-$R_1R_2R_3$. As previously described, polyM was epimerised, and the resulting alginate structures were analyzed by NMR. For both enzymes, the F_G obtained was lower for the hybrids containing $R_1R_2R_3$ than for the original mutants isolated from the screen (Figure 6). It also appeared that the level of alternating MG structures increased with a concomitant decrease in G-blocks. Furthermore, time-resolved NMR spectra recorded from the epimerization of polyM with AlgEM1-$R_1R_2R_3$ (Figure S2 in the Supporting Information) showed that this enzyme behaved kinetically like AlgE6 and not like AlgEM1. These results show that the R-modules modulate not only the epimerization rate as previously thought but also the epimerization pattern of the individual enzymes. This might be connected to individual differences between the R-modules in binding affinity for the epimerized substrate, which will influence the number of residues that are epimerized before the substrate is released from the enzyme.

Residue Asp119 in the A-Module of the Epimerases Is Important for the Epimerization Pattern. AlgEM3−5 displayed similar properties in introducing both MG- and G-blocks, although being up to 92% identical to AlgE4, which does not make G-blocks at all. Of these three mutants, AlgEM3 was found to have one substitution that was considered to be of particular interest (Table S4 in the Supporting Information). The tyrosine substituting the aspartic acid residue found at position 119 in all wild-type enzymes might influence the catalytic activity due to being in the vicinity of catalytic site (Figure 7A). To elucidate whether this residue is somehow determinative for the epimerization pattern, we made targeted substitutions at position 119 in both AlgE4 and AlgEM3. Site-specific mutations were introduced in the corresponding genes,

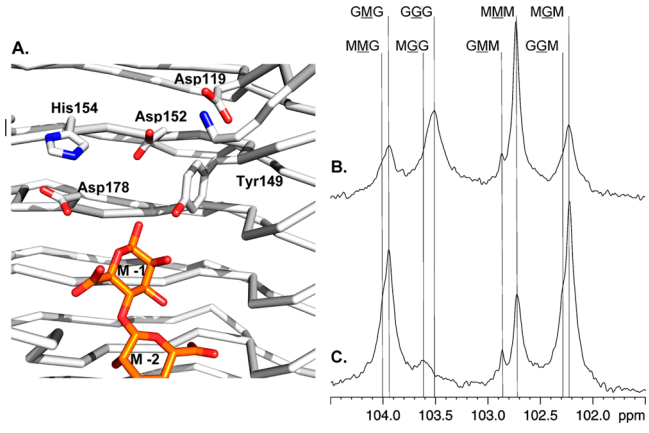

Figure 7. View of the active site with substrate-bound (PDB code: 2PYH) and ^{13}C NMR spectra for end-point products from the epimerization reaction. (A) Bound mannuronan trisaccharide (only two (M-1 and M-2) of the sugar units are shown on the figure), the catalytic residues (Tyr149, Asp152, His154, and Asp 178), and ionic pair (Lys117, Asp119) are shown in stick representation. The ionic pair is involved in the coordinate of the carboxyl group on the mannuronan at subsite +1. The structure was visualized with PyMol.[36] (B) End-point products as a result of epimerization with the GG-block forming epimerase AlgE6. (C) End-point products as a result of epimerization use mutant epimerase AlgEM3. This clearly shows that the mutant epimerase AlgEM3 is not able to form more than only GG-block and not polyG and fill in a G in a GMG sequence.

dx.doi.org/10.1021/bm4005194 | *Biomacromolecules* XXXX, XXX, XXX−XXX

and epimerization of polyM was performed with purified protein samples. Results from NMR analysis of the epimerized polyM are given in Table S5 in the Supporting Information. AlgE4 is forming strictly alternating MG-blocks, but by substitution of Asp119 to Tyr, Phe, or Ala, the resulting enzymes introduce a low level of two sequential G residues (F_{GG} = 0.044 to 0.089). Furthermore, a closer inspection of the end-point ^{13}C spectrum from the time-resolved NMR experiments shows that AlgEM3 (Figure 7C) is not able to produce GGG triads. This might indicate that GG-formation takes place because the enzyme moves only one residue forward instead of two before making the next epimerization reaction, and hereafter AlgEM3 dissociates from the alginate polymer. This points to residue 119 as one of probably many that are directly involved in determining epimerization pattern and also indicates that a negative charge on the side chain might be essential for obtaining the strictly alternating MG structure, as is the case for AlgE4. Effects on the epimerization pattern are also found for AlgEM3 when substituting Tyr119 to Asp or Arg, which in both cases leads to an increase in the frequency of MG. Again, this points to charged residues as being determinative for epimerization pattern.

CONCLUSIONS

In the present study, we have constructed a library of mutant mannuronan C-5 epimerases by gene shuffling and error-prone PCR. Furthermore, a screening method was developed that enabled the identification of specific alginate sequences created by the mutant enzymes. By screening nearly 1000 mutant strains we were able to isolate two epimerases that are more efficient in introducing G-blocks in polyM than the naturally occurring enzymes, and one of these apparently acts kinetically different than the G-block former AlgE6. Such mutant epimerases with new or improved functionalities can be valuable tools in future in vitro design of alginate structures and especially in manufacturing G-rich alginates, of which there is inadequate supply in the global alginate market. The results obtained also emphasize the need for careful design of the screening protocol, in that the AlgEM1 and AlgEM2 did not display superior properties to AlgE6 in epimerizing an alginate substrate with a complex composition. For isolation of robust enzymes with an industrial potential for upgrading of algal alginates the current method can be expanded to screen for enzymes efficient in epimerizing algal alginates under conditions of, for example, defined pH, temperature, ionic strength, and salinity that are relevant for the actual process.

ASSOCIATED CONTENT

Supporting Information

Vectors used and constructed and primers utilized are listed in Tables S1 and S2. Results from NMR analysis of polyM epimerized with AlgE4, AlgE6, and the epimerase mutants are shown in Table S3 and S5. Sequence properties of the A-modules from epimerase mutants AlgEM1−6 are shown in Table S4. Multiple sequence alignment of epimerase A-modules from *A. vinelandii* and the mutant A-modules for AlgEM1−6 is shown in Figure S1. Continuously recorded NMR spectra for epimerization of polyM with AlgEM1-$R_1R_2R_3$ are shown in Figure S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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[UM] [1]

Institution: University of Bergen
Administrative unit: University Museum of Bergen
Title of case study: The impact of collection-based marine biodiversity studies
Period when the underpinning research was undertaken: 2011 - 2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011 - 2021
Period when the impact occurred: 2011 - 2021

1. Summary of the impact

The scientific collections acquired from various species inventories are the "working capital" to which our staff adds scientific value by taxonomy-based processing work at different levels, making material available for in depth studies by internal and external specialists. Various sorts of knowledge obtained from such studies will transform to biological insights with impacts beyond academia. We particularly wish to highlight our work addressing individuals and institutions involved in monitoring, management, and political decisions on environment and natural resources. Our work with DNA barcoding is directly relevant for the application of molecular methods in ecosystem management and monitoring.

2. Underpinning research

The research focus within marine biodiversity at UM is taxonomy, systematics, and animal evolution. Active collection work has made our museum an attractive facility for guest researchers and for loan of zoological study-material. Our collection services are therefore in high demand from international, as well as local clients. The fact that substantial numbers of academic publications are published by authors in *external* institutions, based on the collections facilitated by a museum material, unfortunately is rarely acknowledged when scientific production is scrutinized by policy makers. We modestly claim an important role in the respect, especially in recent years when we have established a reference collection for the bulk of DNA-barcoded marine animals I Norway. Co-operation with the National Institute of Marine Research (IMR) made us a partner in the MAREANO endeavour, the EAF Nansen project of FAO, and recently in Antarctic surveys conducted by the Norwegian Polar Institute. We persuaded these programmes to preserve samples in alcohol. A substantial change was then obtained with the opportunities to do DNA-studies on the material. We have taken the role as a core repository of material that was collected with expensive ship time, and that would often be discarded with loss of voucher documentation and options of further studies. Much of this material has been used by us in the international "Barcode of Life" initiative, in which we have coordinated marine barcoding and processed than 25 000 specimens for the Bold database. Most of the funding for the barcoding was provided by the Norwegian Barcode of Life (NorBol), as a contribution to the biodiversity infrastructure of BOLD. DNA-barcoding of W. African shelf benthos was also an integral part of our in-kind provisions (MIWA) to the EAF Nansen programme. We funded the MIWA work with a grant from JRS Biodiversity Foundation. Since the formal termination of NORBOL as a RCN project, we have continued DNA-barcoding while surveying specific target taxa in 31 of the 35 marine projects funded by the Norwegian Biodiversity Information Centre (NBIC). These projects, along with the Research School in Biosystematics (ForBio), have also been instrumental in communicating insights to a spectre of target groups via social media, citizen science, identification workshops, methodology and taxonomy courses. Among the more transparent examples direct interactions with environmental authorities are two co-authored reports on the use of metabarcoding in environmental monitoring, commissioned by Miljødirektoratet, an atlas of Arctic marine fish fauna from CAFF/Arctic Council, the many reports from species mapping projects, providing records in Artskart, the co-authored "Red List for Norway", and two issues of the status reports summarizing the state of knowledge of Norwegian biodiversity. Our marine scientists also participates in exploration of extreme environments, associated with

UoB's Centre for Deep Sea Research). Insights from this research are in a co-authored (Tandberg) report underpinning political decision on marine mining. Several references to publications of new species described by authors from our research group are in this report.

T. Alvestad – Head Engineer
N. Budaeva – Associate Professor
I. Byrkjedal – Associate Professor
F. Carvalho – Head Engineer
A. Hosia – Associate Professor
K. Kongshavn – Head Engineer
J. A. Kongsrud – Senior Engineer
M. Malaquias - Professor
L. Martell – Researcher
E. Willassen – Professor
C. Rauch – Head Engineer
A.H. Solberg Tandberg – Researcher
N. Straube – Associate Professor

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Hektoen MM, Willassen E, Budaeva N. 2022 Phylogeny and Cryptic Diversity of *Diopatra* (Onuphidae, Annelida) in the East Atlantic. *Biology,* 11:327. https://doi.org/10.3390/biology11020327

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Mortimer K, Kongsrud JA, Willassen E. 2022. Integrative taxonomy of West African *Magelona* (Annelida: Magelonidae): species with thoracic pigmentation, *Zoological Journal of the Linnean Society*, 194:1134–1176 https://doi.org/10.1093/zoolinnean/zlab070

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4. Details of the impact

- DNA-barcoding for the Bold database

DNA sequencing has become a standard tool in our research and sequence analyses are an integral part of our published systematic work. While the societal influences of taxonomic and systematic published research are difficult to assess, new work practices and ideas about the units of biodiversity have clearly influenced the knowledge field beyond academic context. Species identification with DNA-barcodes was quickly adopted by authorities to reveal fraud with food and other commercial products. A fresh example of application is a paper by Straube and colleagues, revealing shortcomings of CITES implementations in Germany. The paper inspired a resolution of restricted shark trade at the CITES Panama conference, 2022 (9). We have produced several thousand barcodes for Boldsystems and believe that our combined work with species inventories has considerably improved the knowledge of marine metazoan

diversity. Impacts from this work can be observed in databases like Artskart, the World Register of Marine Species, the "Red List for Norway". Our contributions to BOLD have revealed many suspected new species. Accumulation of named reference sequences has prepared the ground for metabarcoding of marine metazoans. Evaluation of advantages as compared with traditional approaches has been delivered in two reports to the Norwegian Directorate of Environment and a paper from an application (Willassen et al 2022).

- **MIWA activities** https://miwa.w.uib.no/

Cooperation with the EAF Nansen project was initiated by the late professor C.F. Schander. We received the first materials in 2007 from FAO commissioned cruises, covering waters from Angola to Tunisia includingthe island states. Willassen obtained a grant of 300000$ for sorting, identification, and training of personnel associated with fisheries management in African countries. In 36 months, we hosted a series of workshops in Bergen, identified and barcoded 650 species. We established cooperation with several national research institutes and had about 40 working visits from 12 countries in the project. Our African partners had a good learning experience, particularly in taxonomy, and were involved in publication and presentation of result at international conferences. Four of them are currently seniors in national environmental research and administration. Material from MIWA is still being worked on and new species of gastropods and annelids have been published recently.

- **Artskart NBIC species inventories**

UM has been involved in 31 of the 35 marine species mapping projects funded by NBIC, usually as host institution, but also as cooperating partner and provider of research material. The projects have a focus on particular taxonomic groups or the fauna on particular biotopes. Several projects have engaged amateurs, professional UW photographers, students, and guest researchers in sampling, documentation, and identification workshops, giving the inventories a clear character of citizen science, mediation, lab experience, and trans-generation learning. DNA-barcoding of marine species is a substantial part of the efforts in these projects, following BOLD standards for documentation and metadata. Results and species occurrences are reported to NCBI where data are ported to Artskart and GBIF. A substantial number of event communications have been published on internet, an interactive example presents activities and results from the "Hard bottom fauna inventory".

- **Red list, taxon names, taxonomic databases**

Reliable, curated databases on accepted species names are an important source of knowledge for the management and general education as well as for academic science. UM employees are editors in both the national Artsnavnebasen and the international WoRMS taxonomic databases. WoRMS includes data from GBIF/OBIS, photos of taxa, uploaded reference literature and lists of vernacular names in different languages, as well as ecological traits for the taxa. The national red list for species is co-authored by several scientists from the UM, and two red-list-committees (marine invertebrates and marine fish) were lead/co-lead by UM scientists.

- **Marine protected areas**

Researchers from our group are represented in OSPAR work for nomination of NACES (North Atlantic Current and Evlanov Seamount) as a marine protected area (MPA). In 2020-21, we sorted and identified benthos collected in Antarctic Kong Haakon VIIs Hav. These taxonomic data, alongside video recordings and other oceanographic observations has changed Norway's position, in ongoing negotiations in CCAMLR, from dismissive to supportive of an MPA in the Weddell Sea.

- **Pelagic ecosystems**

Our researchers participate in international working groups promoting and providing new tools for species-level taxonomic analysis of the pelagic ecosystem, including the ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT), as well as SCOR working group #157 and UN Ocean Decade Action No. 102.2 MetaZooGene.

- **Social media, popular publications, exhibits, citizen science, and fair events.**

Our activities are frequently communicated in blog posts and other media (https://invertebrate.w.uib.no/ ; https://evertebrat.w.uib.no). Research results have been presented in many professional conferences, but also at several research-fairs with live and preserved specimens, "bar-coding"-games for children, public lectures (Passion for Ocean, Forskningsdagene, UiB-anniversary, Barneuniversitetet, "Forsk & Fest", etc). Popular science

is regularly published in the museum's yearbook and other publications, including newspapers. New public exhibits opened in 2019 in a renovated museum are all scientifically curated by people from UM. Up to 20 000 visitors per months have been recorded in the museum exhibits.

- **ForBio and open courses (relevance to management and conservation)**

UM coordinates the "marine node" of the Research School in Biosystematics (ForBio) which aims at training a new generation of biosystematists to meet the society's needs for biodiversity expertise in the Nordic countries. Since 2016, the UM ForBio node organized 20 courses with more than 600 participants including students, environmental monitoring consultants and professionals outside academia. Several courses particularly targeted the environmental monitoring agencies and contributed to building taxonomic expertise and facilitating interchange of ideas between academia, environmental agencies, and nature management. Many of our master- and PhD candidates are currently employed as environmental consultants in public or private enterprises.

5. Sources to corroborate the impact

Kunnskapsstatus for bruk av molekylære verktøy i kartlegging og overvåkning av biologisk mangfold i marine miljø
https://munin.uit.no/handle/10037/22291

Finstad AG, …. Willassen E. 2020. Kriterier for lagring av miljø-DNA prøver og data, herunder henvisning til referansemateriale. Criteria for depositing eDNA samples and data, including vouchered specimens.
https://www.miljodirektoratet.no/globalassets/publikasjoner/m1638/m1638.pdf

Redlist of Norwegian Species in 2021 experts:
https://artsdatabanken.no/rodlisteforarter2021/ekspertkomiteene

Elven H., Søli G. (editors) 2020. Kunnskapsstatus for artsmangfoldet i Norge 2020. Utredning for Artsdatabanken 01/2021.
https://www.artsdatabanken.no/Files/41806/Kunnskapsstatus_for_artsmangfoldet_2020_(pdf)

Fagutredning mineralressurser i Norskehavet https://tinyurl.com/2xrk636t

Særlig verdifulle og sårbare områder (SVO) i norske havområder. - Miljøverdi", chapter9
https://tinyurl.com/msb9v9xk

Lowther, A., von Quillfeldt, C., Assmy, P. *et al.* A review of the scientific knowledge of the seascape off Dronning Maud Land, Antarctica. *Polar Biol* **45**, 1313–1349 (2022).
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Hardbunnsfauna https://www.tinyurl.com/hardbunnsfauna

A link to the workshop on marine monitoring by ForBio
https://www.forbio.uio.no/events/courses/2019/Marine_monitoring_workshop

Artsdatabanken marine projects including those conducted by UM
https://artsdatabanken.no/Pages/195803/Hav

[UM] [2]

Institution: University of Bergen
Administrative unit: University Museum of Bergen
Title of case study: Research based exhibitions in Museplassen 3
Period when the underpinning research was undertaken: 2010-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2010-2021
Period when the impact occurred: 2019-2021

1. Summary of the impact

Only knowledge that is shared has true impact. The renovation of Norway's oldest museum building, Muséplassen 3 (MP3) aimed to preserve the look and atmosphere of the listed building from 1865 – while making it technical suitable as a modern museum building – giving the audience access to high quality research-based exhibitions. The University Museum's communication of our researcher's knowledge production has become a strong brand of the University of Bergen, with a clear impact. Since re-opening in 2019, we have had an overwhelming response from the public and reviewers, and from our academic colleagues, doubling our yearly number of visitors.

2. Underpinning research (indicative maximum 500 words)

Throughout the museum project from 2016-2019 it was a clear goal that the 19 new exhibitions were to present our researchers different ongoing research projects. All scientific staff members in biology and several technicians, took part in the exhibition project to some extent, and the exhibitions are based on their research. For this case study we will highlight five exhibitions and six findings from our researchers that the public can explore and learn from through our exhibitions.

1)THE UNKNOWN LAND, A DEEP-SEA EXHIBITION

In the exhibition "The Unknown Land", our researchers give the audience an understanding of an environment that is completely unknown for most people. The deep-sea hydrothermal vent field "Loki's Castle" at 2,350 m depth north of the island Jan Mayen (70°N) is the only known black smoker field from the Arctic Ridge system. This vent field holds a unique fauna clearly distinct from vent sites along the Mid-Atlantic Ridge south of Iceland. Amongst other findings, the audience gets to learn about a new species discovered by Anne Helene Tandberg in 2012. The exhibition gives people insight into this extreme and, for most, unknown environment and gives them access to discussions of what happens to this environment if society starts mining the sea floor.

2) THE WHALE HALL – AN EXHIBITION ABOUT THE NORWEGIAN OCEAN

The iconic Whale Hall at the University Museum discusses several of our researchers' projects. The audience is given access to research from the open ocean to the seashore and the fjords, the oceans impact on the ongoing climate changes, consequences of plastic pollution on animal life, and insight into what a phylum is and understandings of the different phyla in the Norwegian Ocean. One of the research findings discussed in the exhibition is Manuel Malaquias research on snail penises from 2013. Also,

3) PLANTS – CLASSIFICATION AND STRAWBERRIES

In our plant exhibition our researchers give our audience insight into how plants have evolved and how they differ from other organism groups. Torsten Eriksson and Jenny Smedmark present their ongoing research findings and give our audience access to understanding botany

and the evolutionary process better, through something the audience think they know well, namely the strawberry. The findings were published in 2022.

4) BIRDS – THE DINOSAURS AMONGST US

One clear difference in the new exhibitions, is that we no longer are making exhibitions of birds or mammals, but about birds and mammals. Through his research on the migration of birds from 2015, Terje Lislevand lets the audience in on the understanding that one cannot understand other organism groups by understanding humans.

5) POLLEN AND BONE – THE SMALLEST OBJECTS TELL THE LONGEST STORIES

Through the exhibition Kari Loe Hjelle and Anne Karin Hufthammer give the audience access to the actual sources that they use when they produce new knowledge. Through a multidisciplinary approach using several methods, they show the audience how they collect and read pollen and bones from the past and how they can see how both climate and humans have changed the flora and fauna in Norway through time; Climate through 115.000 years and human impact increasingly the last 6000 years. Some findings are published in two papers from 2018.

- Anne Helene Solberg Tandberg – Researcher
- Manuel António E. Malaquias – Professor
- Lars Torsten Eriksson - Associate Professor
- Jenny Smedmark – Associate Professor
- Terje Lislevand – Associate Professor
- Kari Loe Hjelle – Professor
- Anne Karin Hufthammer – Professor

3. References to the research

Amorim, A., Oliveira, E., Malaquias, M.A.E. et al. “New insights into the functional morphology of the male copulatory apparatus of bullid gastropods” 2013 Zoomorphology 132, 145–155 https://doi.org/10.1007/s00435-012-0179-7

Eriksson, Torsten., Persson, N. L. and J. E. E. Smedmark. “What is Potentilla? A phylogeny-based taxonomy for Potentillinae (Rosaceae)”. 2022 Taxon 71: 493-505. https://doi.org/10.1002/tax.12679

Hjelle, Kari Loe, Halvorsen, LS, Prøsch-Danielsen, L, et al. «Long-term changes in regional vegetation cover along the west coast of southern Norway: The importance of human impact”. J Veg Sci. 2018; 29: 404– 415. https://doi.org/10.1111/jvs.12626

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Lislevand, Terje, Chutný, Bohumír, Byrkjedal, Ingvar, Pavel, Václav, Briedis, Martins, Adamik, Peter & Hahn, Steffen “Red-spotted Bluethroats *Luscinia s. svecica* migrate along the Indo-European flyway: a geolocator study”, 2015 Bird Study, 62:4, 508-515, https://doi.org/10.1080/00063657.2015.1077781

Tandberg, A.H., Rapp, H.T., Schander, C. et al. “Exitomelita sigynae gen. et sp. nov.: a new amphipod from the Arctic Loki Castle vent field with potential gill ectosymbionts”. 2012 Polar Biology 35, 705–716 https://doi.org/10.1007/s00300-011-1115-x

4. Details of the impact: A University Museum of the 21st Century, Mission and Vision

Impact: Dissemination of knowledge to different stakeholders and communities

Developing the museum project, we found that our museum's key role is not only to produce new knowledge but also to share the overall purpose of our research and facilitate its role in society. Our museum has now become a natural meeting place where our researchers engage with a wider public, where things are debated, where they share their knowledge production as they are doing it and discuss the role of science; the museum is a place where our researchers listen as much as they talk, where questions are posed as much as answers given. This new societal role is one of the most significant societal impacts of our research in the period 2016-2019.

Even though the University Museum had to close its doors for a long period due to Covid-19, we see that our museum project and our main goal of giving our target groups access to our researchers' knowledge production, has had a significant impact on society. In the period from the museum opening on the 14th of October 2019 to 31st of December 2021 a total of 162.810 visitors have experienced and learned through our research-based exhibitions, events, and school programs. In 2022 we set a new visitor record with 72.000 visitors.

We recognize that our colleagues at Norway's other university museums are inspired by our success, our vision and research communication methods and are seeking to collaborate with us to reach even further with our research dissemination. We also see how the University of Bergen's staff and students make the University Museum their own as an integrated part of the University of Bergen campus. We are winning national (the Norwegian Museum of the Year Award 2021) and international awards (European Museum of the Year Award "The Portimão Museum Prize for Welcoming, Inclusion and Belonging 2022") and are proud of the societal impact the project has had.

Impact: Understanding of a research process

We firmly believe that only shared knowledge can have an impact and be employed to meet the natural and societal challenges we face. To understand what impact a university museum can create through research, and science communication, we need to reflect on what a university is. Our goal was to let society in on the understanding that a university is not an ivory tower, where researchers are out of touch with society, but that it is a construction hall, where researchers produce new knowledge and tools for society, so that we together can tackle current and future natural and societal challenges. As a University Museum we do not only present the tools, or the results of ongoing research, but we engage the public in questions about how our researchers are producing their research findings, which sources and methods they are using, how they are collaborating across research fields, which questions they are asking, and what impact they are achieving.

By doing this we give the public a true understanding of why society uses tax-payers money to fund research, and we see that the public likes this effort of the research communities to give something back. Through our research-based exhibitions, events, and school programs we give our audience access to the knowledge production in itself. The audience meets our researchers and see them demonstrating what research is in their respective fields, through various experiences and methods, thus, the public gets an understanding of what research is and why it is necessary for society to produce new knowledge. Enabling this understanding is a democratization of knowledge and leads to an empowered public with trust in its researchers.

Impact: Diversity Education/Dissemination of knowledge/Recruitment to higher education

We planned the new natural history exhibitions for the curious, those who wonder, who want to know. Through the museum project in MP3, we reach out to our diverse audience and

seek to contribute to this empowerment of people from all backgrounds through facilitating this sharing of knowledge, presenting research in a societal context, thereby strengthening public discourse, and offering a contribution to the very democratization of knowledge.

Regardless of social background, class or ethnicity, all children and young people in the region takes part in our school programs. This is important both to facilitate recruitment to higher education, but also to give everyone access to the same knowledge and understanding. Through our exhibitions, events, and school programs our researchers inspire young and old and, thus, contributes to the recruitment of the new generations of scientists needed if society is to rise to the challenges ahead. Through our high visitor numbers, the meeting with young people through collaborations with Norwegian Schools and through our presence in different media, we see that people are responding to our research findings and that it has an impact.

5. Sources to corroborate the impact

Museum of the Year Award 2021: "The winner is a museum which, through innovative thinking and new ways of communicating history and research, has carried out an extensive rehabilitation of buildings and exhibition facilities. Just as important as opening up the rooms and establishing new meeting places is the way in which, through their innovative and bold communication, they welcome future generations without breaking ties to history and traditions. Those who know the museum from before will still feel at home in what is an important cultural and historical symbol for the city and the region in which it is located. But in the modern thinking around knowledge and the important interaction between research and curiosity, the social upheavals and the needs of our time for playful seriousness. This is how the winner becomes a tradition bearer who stands firm in his future without losing sight of perspectives and future goals" https://museumsforbundet.no/nyheter/universitetsmuseet-i-bergen-er-arets-museum-2021/ (Translated from Norwegian 16.01.2023)

European Museum of the Year Award "The Portimão Museum Prize for Welcoming, Inclusion and Belonging 2022": "This museum does not only present the results of research but engages the public in questions about how they are achieved and how knowledge is produced. The exhibition combines innovative, openminded presentations which deliver a high-level scientific message. It shows how artefacts which serve to build science can constantly lead to new interpretations. Envisioned as a platform for dialogue and discussion with the public, the intellectual framework of the museum is supported by a deep reflection on what the new missions and visions of a university museum should be." https://emya2022winners.europeanforum.museum/ (16.01.2023)

Some of the exhibtions you can visit at the University Museum, Natural History:

- *Biodiversity - Wonders of Diversity*
- *Birds – The dinosaurs among us*
- *Crystals – Look, they are growing*
- *Evolution – A science for the future and The power of selection*
- *Herptiles – The first terrestrial vertebrates*
- *Insects – A million stories*
- *Mammals – Your closest relatives*
- *Nature of time – Perception of time*
- *Our Bedrock – The rise and fall of a mountain range*
- *Plants – Giving life*
- *The Deep Sea – The Unknown land*

- *The Globe Room – Our dynamic Planet*
- *The Whale Hall – The great swarm*
- *Worldviews – Knowledge that shapes society*

https://www.universitetsmuseet.no/nb/utstillinger/44 (16.01.2023)

Administrative unit IBV, UiO Impact case 1

Societal impact

Institution: University of Oslo

Department of Biosciences

Title of case study: Extending the serum half-life of IgG therapeutics and albumin fused biologics

Period when the underpinning research was undertaken: 2007-2022

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed:
Jan Terje Andersen (PhD 2008) Stian Foss (PhD 2016) Algirdas Grevys (PhD 2019) Kine M Knudsen Sand (PhD 2017) Malin Bern (PhD 2017) Jeannette Nilsen (PhD 2019) Muluneh Daba (PhD 2012) Kristin S Gunnarsen (PhD 2012)	All were MSc students, then PhD students and postdoctoral fellows after graduation. Jan Terje Andersen is a professor at the Medical Faculty since 2020.	Muluneh Daba left in 2016, Kristin S Gunnarsen in 2018, Kine M Knudsen Sand in 2021 and Malin Bern in 2022. Foss and Nilsen remain, as part of the Andersen lab at the Medical Faculty.

Period when the impact occurred: 2012-present

1. Summary of the impact

We have revealed the biological mechanism responsible for the long serum half-life of albumin, which is 3-weeks. The long half-life is due to the interaction of albumin with the neonatal Fc receptor (FcRn), which also binds the antibody IgG to a separate binding site. FcRn binding rescues both molecules from degradation, while all other serum proteins degrade within hours or a few days. The receptor also transports both ligands across cell layers, albumin preferentially from the outside and into the body across mucus membranes. Furthermore, we have designed albumin and IgG variants with improved binding to FcRn, with even longer half-life and more efficient uptake. The new knowledge inspires therapeutic applications, where vaccines or biologics are fused to the albumin variant, as well as design of therapeutic IgGs. Long serum half-life allows for reduced dose size and dosing frequency of therapeutics, which increase patient compliance and reduce cost. Importantly, albumin fused vaccines can be given in a needle free manner.

2. Underpinning research

Most proteins in blood degrade within a few hours or days, but the two most abundant - IgG and albumin - are rescued from degradation and have half-lives of 3 weeks. We have given important contributions to the understanding of how binding of these proteins to FcRn regulates their serum half-life and biodistribution via cellular recycling or transcytosis. We have thus obtained fundamental new insights that have tremendous implications for the understanding of the biology of IgG antibodies and albumin. As both albumin and target specific IgGs are increasingly used as therapeutics, this new knowledge has paved the way for our design of antibody and albumin molecules with tailored FcRn binding and transport properties.

We have designed an IgG variant with increased FcRn binding and half-life, which induces effector functions on a level on par with or better than natural, non-engineered IgG molecules *(Grevys, J Immunol 2015).* Antibody variable sequences with charged patches contribute to FcRn binding and have

a pronounced effect on cellular transport and plasma half-life *(Grevys, iScience 2022).* Thus, the half-life of 3 weeks observed for serum IgG is an average.

We have also dissected the interaction between FcRn and albumin (*Andersen, Nat Commun 2012*), and our studies of the interaction with albumin fused to other protein sequences have given information on how long half-life and efficient transport can be conferred upon albumin-fused therapeutics *(Andersen, J Biol Chem 2013)*. We have also designed albumin variants with increased binding to FcRn at acidic pH that have increased half-life and are transported more efficiently *(Andersen, J Biol Chem 2013). (Andersen, J Biol Chem 2014) (Bern, Sci Transl Med 2020).*

FcRn is expressed intracellularly, and binds IgG as well as albumin taken up by fluid-phase endocytosis. The receptor then directs the ligands to the surface of the opposite side of the cell (transcytosis) or to the side of entry (recycling). We studied both processes using the natural ligands as well as engineered variants. A new *in vitro* recycling assay designed by us allows us to predict the behavior of designed FcRn-binding molecules *in vivo* in animal models *(Grevys, Nat Commun 2017).* Furthermore, we found that albumin is transcytosed most efficiently from the apical to the basolateral side (from the outside and into the body). This observation holds great promise for needle free mucosal delivery of albumin-based vaccines and therapeutics *(Bern, J Control Release 2015).* We demonstrated that transport efficiency correlates with the strength of the FcRn interaction, and thus, our engineered albumin with improved FcRn binding is transported more efficiently. We have obtained very encouraging results from studies in mice, where such albumin variants fused to several different viral antigens given intranasally give complete protection from deadly doses of virus (*manuscript in revision for Nature Comm*).

FcRn is the only Fc receptor required for transport of IgG across cellular barriers and placenta *(Mathiesen, Blood 2013).* We have unpublished data demonstrating that IgG is transported in an FcRn-dependent manner across the human placenta, whereas albumin is not, which is in line with a study in humans from 1964 using radioactive ligands. This is important, since monoclonal IgGs are increasingly used in therapy, and the fetus is exposed when the patient is a pregnant woman. The use of albumin as a fusion partner for antibody fragments and other biologics may well be a safer treatment option.

Furthermore, we have found that albumin is transcytosed efficiently from the apical to the basolateral side (from the outside and into the body). This observation holds great promise for needle free mucosal delivery of albumin-based vaccines and therapeutics. The transport efficiency is greatly increased when albumin is engineered for enhanced FcRn binding.

3. References to the research

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4 and 5. Details of the impact and sources to corroborate the impact

Long serum half-life is important for the therapeutic success of IgG antibodies, and thus, there is an intense interest in increasing the half-life even further. The IgG variant with increased FcRn binding and extended half-life designed by us, is described in a patent (*PCT/IB2017/000327).* The patent has been licensed nonexclusively by *Tillotts Pharma*, a large international drug development company, for use in therapy against inflammatory bowel disease. This is further described in WO 2019/057564. Other companies may well sign such license agreements with *Inven2*.

The therapeutic efficacy of small proteins, peptides, and chemical drug candidates is often hampered by short serum half-life, which is the main reason why they fail *in vivo*. Thus, strategies to tailor their serum persistence and biodistribution are highly needed. An attractive approach is to link them to albumin, genetically or chemically, and thereby take advantage of the exceptionally long half-life of albumin. Our research has resulted in launching of the Veltis® technology by *Novozymes Biopharma A/S*, where any drug of interest is genetically fused or conjugated to wild type or engineered albumin variants designed by us, that have extended half-life. In 2016, Novozymes separated its albumin activity to form *Albumedix A/S* as an independent pharma company based on our results (*granted patent US 8822417 B2*). In 2018 *Novartis* initiated a program to explore our technology across multiple therapeutic areas and against multiple targets, and Albumedix was then acquired by Sartorius in 2022. Furthermore, Inven2 has outlicensed our most recent and superior albumin technology (WO2015063611) nonexclusively to Neutrolis Inc, a Boston based biotechnology company developing therapeutics that target neutrophils, and neutrophil extracellular traps (NETs).

Furthermore, Jan Terje Andersen and Inger Sandlie have established the company JTIS A/S that is in the process of finalizing the licensing of this recent albumin technology patent from Inven2 and is actively

negotiating with the Neutrolis founders and Boston Venture to extend the collaboration to other therapeutics. Lastly, Andersen and Sandlie are among the cofounders of Authera A/S, a newly established company that form partnerships with other companies to guide their design and selection of FcRn binding molecules. Authera also has two lead preclinical development programs.

Administrative unit IBV, UiO Impact case 2

Institution: University of Oslo (UiO)
Administrative unit: Department of Biosciences (IBV)
Title of case study: Chronic Wasting Disease (CWD) management
Period when the underpinning research was undertaken: 2016-2021 (and ongoing)
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021 (see Section 2, Personnel)
Period when the impact occurred: 2016-2021 (and ongoing)

1. Summary of the impact

The discovery of chronic wasting disease (CWD) in reindeer in 2016 in Norway made headlines as the first case of the infection in Europe. CWD is a contagious and lethal prion disease in cervids widely distributed and still spreading in North America. The geographic expansion of CWD to Europe represented a significant biodiversity and economic concern.

Cervids play important roles in ecosystem functioning, and they have high associated cultural and economic values. Europe harbours an estimated 2.4 million red deer, 9.6 million roe deer, 30,000 reindeer and 437,000 moose, in addition to exotic cervids and more than 1.0 million semi-domestic reindeer. The emergence of CWD is one of the greatest challenges ever experienced in nature management in Norway.

We developed a general approach of “proactive hunting surveillance” aimed at detecting wildlife diseases at early epidemic stages as an alternative to preemptive culling.

Our research has led to a direct change in management of wild reindeer for more effective surveillance and mitigation of this serious wildlife disease.

2. Underpinning research

Atle Mysterud is as an internationally recognized researcher on ungulate ecology, and he was in this role invited to the Norwegian Scientific Committee for Food and Environment (VKM) developing plans for management of CWD in 2016. Mysterud saw the necessity of cooperation between ecologists at the Norwegian Institute for Nature Research doing population monitoring of cervids (since 1991), and the veterinary side sampling tissues for CWD testing. Mysterud had worked on tick-borne diseases since 2009 in collaboration with a veterinary epidemiologist (Hildegunn Viljugrein), having a 20% position at CEES. Mysterud contacted Viljugrein, and her main employer – the Norwegian Veterinary Institute – put her in charge of CWD epidemiology modelling. Mysterud also contacted a cervid ecologist (Christer Moe Rolandsen) at the Norwegian Institute for Nature Research (NINA-Trondheim). Viljugrein and Rolandsen were both positive towards collaboration, and Mysterud has since played a key role for flow of data, analysis and leading research and development of novel surveillance tools (Mysterud et al. 2019, 2020, 2021, 2023, Viljugrein et al. 2019, 2021).

Mysterud has been a member of the expert groups of the Norwegian Food Safety Authority and the Norwegian Environmental Agency from the start and is hence familiar with the different views of the Veterinary and the Ecology/Conservation side.

The main idea of proactive hunting surveillance, later proven successful, came after the Norwegian Veterinary Institute first tried to convince local management to raise their quotas without having a selective harvest. Mysterud contacted Viljugrein and also the head person for CWD at the Norwegian Food Safety Authority, Kristin Ruud Alsveike. Alsveike adopted the principles for the management season of 2019, and became coauthor of the paper (Mysterud et al. 2020). Hence, this is a case of *co-design and co-production of knowledge* with stakeholder groups. Further, we then sat down with the secretary of the local management (Svein-Erik Lund) of the largest population at Hardangervidda, being in charge of the actual quota setting and important for actually

convincing local landowners and hunters to implement it. This was hence a case of successful *participatory modelling*. Later, we co-produced with Lund a paper showing how to set quotas to obtain a specific offtake, since quota filling can vary hugely from year to year; 12-48% (Mysterud et al. 2021).

Aims for determining absence of CWD in adjacent populations to the first infected, is set by the Norwegian Food Safety Authority. Based on their aims, Mysterud led development of effective harvesting strategies to enable early detection or more rapid establishment of absence of CWD, yet, being less invasive. This included both novel surveillance methods for wild reindeer through hunting and semi-domestic reindeer through slaughter.

Personnel at IBV

- Professor Atle Mysterud (1999-present)
- Researcher Hildegunn Viljugrein (2008-present)

3. References to the research

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4. Details of the impact

Intensive harvesting is often used to control wildlife disease outbreaks, but is invasive and often in conflict with other management objectives. For diseases with latent stages or extended periods of low prevalence leading to low detectability during the early epidemic stages, mitigation may involve "preemptive culling" as a proactive measure by removing contact herds before the disease is detected. Although the use of preemptive culling is widespread for livestock and beneficial from a disease mitigation perspective, this measure may not be a politically feasible option for wildlife species of conservation concern. Reindeer face population declines across the northern hemisphere. **The affected populations in Norway are part of the southern European**

conservation region for wild reindeer, and preemptive culling is therefore not desirable from a conservation viewpoint and politically challenging.

CWD surveillance relies on testing hunter-killed cervids. To increase the chance of detecting the disease in its early stages by harvesting, massive sampling is required, but it does not usually involve strategic plans for selective harvest. Unless well planned, this massive harvesting may be unsustainable. The targeted sampling of specific demographic groups may enhance the probability of disease detection. 1) We documented a 2.7 times higher prevalence of CWD in adult males compared to adult females in the reindeer population with the first outbreak (Mysterud et al. 2019). 2) For polygynous species, males are typically not limiting for population growth, unless the sex ratios are extreme, and increasing the harvest of specific demographic groups may be used to reduce impacts on population growth. Based on this, **we developed the general approach of "proactive hunting surveillance" aimed at detecting wildlife diseases at early epidemic stages as an alternative to preemptive culling** (Mysterud et al. 2020).

The idea of creating a detailed plan for harvest quotas to selectively hunt for particular demographic groups to enhance disease detection but simultaneously avoid undesirable population declines was novel. We determined, by aid of a simulation model, the optimal size and demographic composition of the harvest when the aim is to rapidly substantiate freedom from infection, without causing a notable population decline. Our approach hence merged traditional wildlife management principles of selective harvesting with concepts of freedom from infection and risk-based surveillance coming from veterinary epidemiology.

The Norwegian Food Safety Authority and the Norwegian Environmental Agency used this new principle to set harvesting quotas for two reindeer populations adjacent to the first infected. We documented in later work (Mysterud et al. 2021) that this strategy was effective, raising the harvest of adult males from 15-20% to 47% in a single year.

Similarly, novel surveillance tools have been implemented for adjacent semi-domestic reindeer (Viljugrein et al. 2019).

5. Sources to corroborate the impact

Aftenposten newspaper articles:
Mysterud, A., Viljugrein, H. & Strand, O. 2019. Friskmelding av villrein med gevær. Aftenposten (Viten), 11. april 2019.
Mysterud, A., Viljugrein, H., Hopp, P., Rolandsen, C.R. & Nilsen, E.B. 2017. Utbruddet av skrantesyke er et tidsskille i norsk naturforvaltning. Aftenposten (Viten), 10. aug. 2017.
Norwegian Veterinary Institute (NVI) – ongoing research on CWD: https://www.vetinst.no/forskning-innovasjon/pagaende-forskningsprosjekter/cwd-forskning-pa-skrantesjuke-cwd
Norwegian Institute for Nature Research (NINA) report:
Mapping and monitoring of chronic wasting disease (CWD) 2020. https://hdl.handle.net/11250/2735743

Administrative unit IBV, UiO Impact case 3

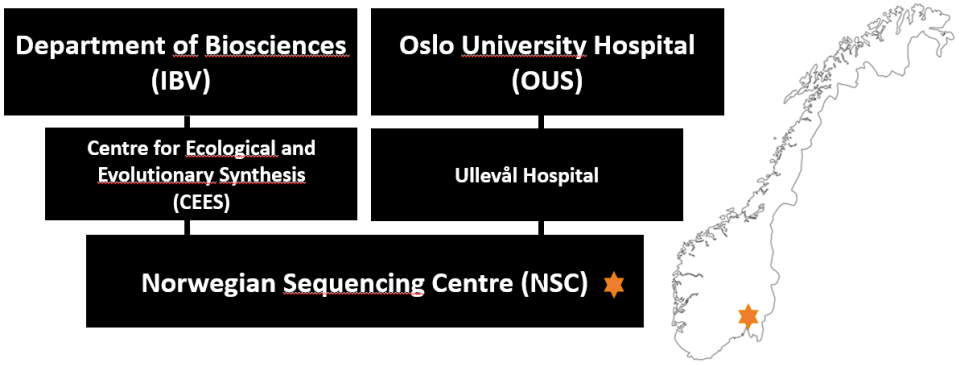
Institution: University of Oslo (UiO)
Administrative unit: Department of Biosciences (IBV)
Title of case study: The Norwegian Sequencing Centre (NSC) as a national resource in COVID-19 whole genome sequencing
Period when the underpinning research was undertaken: from 2009 to 2019
Period when staff involved in the underpinning research were employed by the submitting institution: Throughout the period from 2009 - 2022
Period when the impact occurred: 2020 and ongoing

1. Summary of the impact

The science- and engineering competence built up at the Norwegian Sequencing Centre (NSC) over a long period (from 2009 and until now) was used to develop a national health care whole genome COVID-19 sequencing unit during the pandemic. The COVID-19 sequencing is still ongoing. This illustrates the impact of having an already established – through several rounds of funding from the Research Council of Norway (RCN) – state-of-the-art infrastructure that can tackle a pandemic on short notice. Approximately 90% of all whole genome sequenced COVID cases in Norway so far have been performed at the NSC.

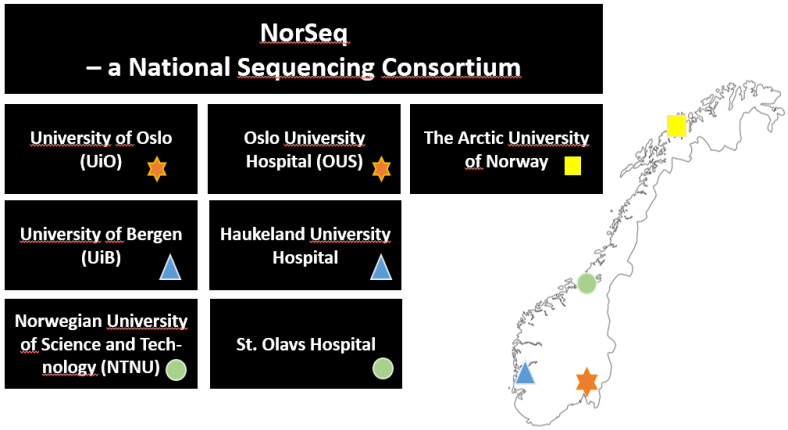
2. Underpinning research

In 2008 Next Generation Sequencing (NGS) was established at the Centre for Ecological and Evolutionary Synthesis (CEES, IBV, UiO) and the Ullevål Hospital (under the Oslo University Hospital (OUS)) as the first sites in Norway (see figure). NGS provides magnitudes of faster and less expensive sequencing than the traditional Sanger method. In 2009 the Norwegian Sequencing Centre (NSC) was established through support from RCN (Infrastructure program) – as a joint effort between CEES and Ullevål. This initiative was taken by professor Kjetill S. Jakobsen (CEES) and professor Dag Undlien (Ullevål).



In the years to follow whole genome sequencing of bacteria (Rounge et al. 2009), vertebrates (Star et al. 2011, Malmstrøm et al. 2016) and humans (see Undlien 2012; early overview) was established. A highlight that caught attention across the world was sequencing of the Atlantic cod genome – the first fish genome assembled with a pure NGS approach (Star et al. 2011). NSC developed amplicon sequencing, transcriptomics and viral approaches, mutation detection/GWAS approaches and methylome sequencing. Using amplicon sequencing we demonstrated that there is a diverse microbial community in urine from healthy humans implying that it is not sterile (Siddiqui et al. 2011). As the main sequencing centre in Norway, NSC obtained several rounds of funding from the Infrastructure program (RCN) and from UiO and OUS (equipment and positions).

The last RCN infrastructure grant (in 2016) led to the establishment of NorSeq a national sequencing consortium involving UiO, OUS (Ullevål and Radiumhospitalet), UiB/Haukeland, NTNU/St Olavs and UiT/UNN. NorSeq is currently on the National Roadmap for Infrastructures (NSC was on the Roadmap prior to establishment of NorSeq).



In other words, when the COVID pandemic hit in 2019, NSC (CEES and Ullevål) had a long-time experience with all kinds of sequencing and possessed a large capacity in Illumina (short read) and PacBio (long read) sequencing. Importantly, we had experience with large projects and had established a safe pipeline for generating and storing human data (TSD – Tjenester for Sensitive Data). Having established a safe bioinformatic pipeline based in the supercomputer infrastructure of Sigma2/NRIS (https://www.sigma2.no) as well as strong support from USIT (the IT department at UiO) were critical factors. Furthermore, NSC had experience in performing sequencing services across Norway (Universities, Research Institutes, Health Institutions and industry).

Personnel at IBV

Kjetill S. Jakobsen – Professor (1994 – present)
Thomas F. Hansen – Professor (2005 – present)
Nils C. Stenseth – Professor (1980 – present)
Tone F. Gregers – Associate professor (2008 – 2020)
Bastiaan Star – Researcher/Associate professor (2008 – present)
Alexander J. Nederbragt – Senior Engineer/Senior Lecturer (2013 – present)
Ave Tooming-Klunderud – Senior Engineer (2007 – present)
Morten Skage – Senior Engineer (2008 – present)
Mari Espelund – Senior Engineer (2008 – 2011)
Spyros Kollias – Head Engineer (2014 – present)
Sissel Jentoft – Researcher (2009 – present)
Michael Matschiner – Researcher (2013 – 2017)
Unni Grimholt – Researcher (2010 – 2013)
Walter Salzburger – Researcher (2012)
Trine B. Rounge – Postdoctor (2008 – 2012)
Karin Lagesen – Postdoctor (2010 – 1013)
Ole K. Tørresen – PhD-student/Researcher (2011 – present)
Martin Malmstrøm – PhD-student/Researcher (2008 – 2017)
Helle T. Baalsrud – PhD-student/researcher (2012 – present)
Monica H. Solbakken – PhD-student/Researcher (2008 – 2022)
Huma Siddiqui – PhD-student (2008 – 2012)
Paul R. Berg – PhD-student (2010 – 2015)

3. References to the research

Rounge TB, Rohrlack T, **Nederbragt AJ**, Kristensen T and **Jakobsen KS** (2009). A genome-wide analysis of nonribosomal peptide synthetase gene clusters and their peptides in a *Planktothrix rubescens* strain. *BMC Genomics*, 10:396 https://doi.org/10.1186/1471-2164-10-396

Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL and **Jakobsen KS (**2011). Assessing

diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiol* 11:244.
https://doi.org/10.1186/1471-2180-11-244

Star B, Nederbragt AJ, Jentoft S, Grimholt U, Malmstrøm M, Gregers TF, Rounge TB, Paulsen J, **Solbakken MH**, Sharma A, Wetten OF, Lanzén A, Winer R, Knight J, Vogel J-H, Aken B, Andersen Ø, **Lagesen K, Tooming-Klunderud A**, Edvardsen RB, Tina KG, **Espelund M**, Nepal C, Previti C, Karlsen BO, Moum T, **Skage M, Berg PR**, Gjøen T, Kuhl H, Thorsen J, Malde K, Reinhardt R, Du L, Johansen SD, Searle S, Lien S, Nilsen F, Jonassen I, Omholt SW, **Stenseth NC, Jakobsen KS** (2011). The genome sequence of Atlantic cod reveals a unique immune system. *Nature* 477: 207-210.
https://doi.org/10.1038/nature10342

Siddiqui H, Lagesen K, Nederbragt AJ, Jeansson SJ, **Jakobsen KS** (2012) Alterations of microbiota in urine from women with interstitial cystitis. *BMC Microbiol* 12: 205.
https://doi.org/10.1186/1471-2180-12-205

Malmstrøm M, Matschiner M, Tørresen OK, Star B, Snipen LG, **Hansen TF, Baalsrud HT, Nederbragt AJ**, Hanel R, **Salzburger W, Stenseth NC, Jakobsen KS, Jentoft S** (2016). Evolution of the immune system influences speciation rates in teleost fishes. *Nature Genetics* 48 (10): 1204-1210 (22 August)
https://doi.org/10.1038/ng.3645

Undlien D.E. (2012). Individuell genomsekvensering. *Tidsskriftet Den Norske Legeforening* nr. 3, 2012; 132. doi: 10.4045/tidsskr.11.1490
https://tidsskriftet.no/2012/02/leder/individuell-genomsekvensering

4. Details of the impact

The Norwegian Sequencing Centre (NSC) – including the associated research team – has had a large impact on Norwegian science exemplified by more than 1000 scientific articles that have been produced based on data generated by NSC in the period 2009 – 2022. It has also promoted and made it possible to establish large projects in Biodiversity Genomics in Norway (Earth BioGenome Project Norway; EBP-Nor) – and for UiO being member of European and world-wide consortia (EBP and European Reference Genome Atlas (ERGA)). A substantial number of results and insights from these research outputs have generated innovations including patents and commercialization of bacterial diagnostics, algal toxins, fungal based biotechnology and aquaculture. Furthermore, NSC has contributed to strengthening sequencing/genome competence at the academic and health institutions in Norway – and taken the initiative to establish the national technology consortium "The Norwegian Consortium for Sequencing and Personalized Medicine" (NorSeq). When the pandemics broke out, NSC had well established protocols, high capacity and competence. Large investments funded by the RCN, UiO and OUS had been put into NSC. In the very beginning of the pandemics, whole genome sequencing of virus variants was only performed to a very small extent at the Norwegian Institute for Public Health (NIPH) using their in-house small capacity instrument. In January 2020 it became clear that NIPH needed to substantially upscale whole genome sequencing of COVID-19 variants. Due to the lack of sequencing capacity at NIPH, we (NSC) were asked if we could perform this task – implying that "thousands" of genomes needed to be sequenced within a short period. Using the competence at NSC we developed and implemented an amplicon-based Illumina sequencing approach within 14 days – adapted to the large capacity our Illumina Novaseq 6000 instruments NSC possesses. The protocol and testing up against other protocols are documented (Lind et al. 2021; Ribarska et al. 2022). So far, over 80.000 COVID-19 genomes have been sequenced at NSC – about 90% of all COVID sequencing in Norway. This has enabled the Norwegian health authorities to monitor the evolution of the pandemic in terms of new virus variants from an in-depth sequence point of view.

Notably, this is the first time whole genome sequencing have been used to monitor the causative agent of a severe illness. The key take-home lesson is the importance of investing for preparedness for unknown and unforeseen pandemics – or other catastrophes for our society. The lessons learned here, should stand as a prime example.

COVID-19 sequencing at NSC has been covered by several main media such as NRK (see point 5).

5. Sources to corroborate the impact

The Norwegian Sequencing Centre (NSC): https://www.sequencing.uio.no and The National Sequencing Consortium: https://www.norseq.org

Biodiversity genomics:
EBP-Nor: https://www.ebpnor.org
EBP: https://www.earthbiogenome.org
ERGA: https://www.erga-biodiversity.eu

Some news items about the "case study" on COVID-19 sequencing:
https://www.vg.no/nyheter/i/2dPXmx/skal-hjelpe-fhi-med-500-analyser-i-uken-dette-er-smaatteri
https://www.nrk.no/norge/fhi-skal-teste-alle-positive-koronatester-i-oslo-for-mutert-virus-1.15349948
https://www.nrk.no/norge/norske-sykehus-overrasket-over-fhi-_-na-vil-de-teste-for-mutanter-selv-1.15344283
https://www.nrk.no/norge/island-tester-tusenvis-av-helt-friske-personer-_-na-skal-norge-folge-etter-1.14963157
https://www.dagbladet.no/nyheter/her-avslores-mutantviruset/73332641

Editorial about human sequencing:
https://tidsskriftet.no/2012/02/leder/individuell-genomsekvensering

Establishing COVID-19 sequencing pipeline:
Lind A, Barlinn R, Landaas ET, Andresen LL, Jakobsen K, Fladeby C, Nilsen M, Bjørnstad PÅ, Sundaram AYM, Ribirska T, Müller F, Gilfillian GD, Holberg-Petersen M (2021). Rapid SARS-CoV-2 variant monitoring using PCR confirmed by whole genome sequencing in a high-volum diagnostic laboratory. Journal of Clinical Virology 141.https://doi.org/10.1016/j.jcv.2021.104906

Ribarska T, Bjørnstad PM, Sundaram AYM and Gilfillan GD (2022). Optimization of enzymatic fragmentation is crucial to maximize genome coverage: a comparison of library preparation methods for Illumina sequencing. *BMC Genomics* **23**, 92. https://doi.org/10.1186/s12864-022-08316-y

Administrative unit IBV, UiO Impact case 4

Institution: University of Oslo
Administrative unit: Department of Biosciences
Title of case study: Discovery of a muscle memory altered the WADA anti-doping code
Period when the underpinning research was undertaken: 2008-2013
Period when staff involved in the underpinning research were employed by the submitting institution: 1996-Present. All authors were employees at the institution at the time of the relevant research.
Period when the impact occurred: 2013-2015

1. Summary of the impact

In 2013 Science News scored the Gundersen group´s research on long-lasting muscle memory after testosterone administration as one of the 25 most important news in all of science. In 2015, World Anti-Doping Agency (WADA) changed its maximum exclusion time from 2 to 4 years for anabolic androgen steroid (AAS) abuse after Anti-Doping Norway presented our research results to WADA.

2. Underpinning research

The group has established that there is a cellular memory in muscle cells. The “memory effect”, is related to the general observation in biology that cell size is related to DNA content, and in case of muscle to the number of myonuclei in the multinuclear muscle fibers (Hansson et al.). During exercise or steroid treatment nuclei are accreted from stem cells in the interstitium, but these new nuclei were previously thought to be removed by apoptosis during de-training. This would prove no memory mechanism. In 2010, Bruusgaard & Gundersen used advanced in vivo time-lapse microscopy and demonstrated that nuclei are not lost, and in a 2010 PNAS paper the group showed that accreted nuclei are permanent. The breakthrough study with respect to doping was Egner et al. 2013, which showed that the memory effects from training could also be achieved after testosterone administration. These findings led to changes in the doping code in 2015.

3. References to the research

Bruusgaard, J.C. and Gundersen, K. (2008) In vivo time-lapse microscopy reveals no loss of murine myonuclei during weeks of muscle atrophy. Journal of Clinical Investigation 118:1450-1457

Bruusgaard, J. C., I. B. Johansen, I. M. Egner, Z. A. Rana, and K. Gundersen. (2010) Myonuclei Acquired by Overload Exercise Precede Hypertrophy and Are Not Lost on Detraining. Proceedings of the National Academy of Sciences 107: 15111-15116

Egner, I.M. Bruusgaard, J.C., Eftestøl, E., Gundersen, K. (2013) A cellular memory mechanism aids overload hypertrophy in muscle long after an episodic exposure to anabolic steroids. Journal of Physiology 591:6221-6230. doi: 10.1113

Psilander N, Eftestøl E, Cumming KT, Juvkam I, Ekblom MM, Sunding K, Wernbom M, Holmberg HC, Ekblom B, Bruusgaard JC, Raastad T & Gundersen K. (2019). Effects of training, detraining, and retraining on strength, hypertrophy, and myonuclear number in human skeletal muscle. Journal of applied physiology: 126, 1636-1645

Hansson KA, Eftestøl E, Bruusgaard JC, Juvkam I, Cramer AW, Malthe-Sørenssen A, Millay DP & Gundersen K. (2020). Myonuclear content regulates cell size with similar scaling properties in mice and humans. Nature Communication 11, 6288

4. Details of the impact

Most anti-doping work is aimed at detection of performance enhancing drugs, in particular during competition. Modern doping abusers in sport rely upon using the drugs away from the limelight, and it has been a worry that previous use of anabolic steroids has long-lasting effects after previous use. The controversy of this issue was expressed by “the second fastest man in history”,

the twice convicted Olympic champion Justin Gatlin. In 2014 he told the British newspaper The Guardian “For the few haters out there, seems like that’s what they want to do, discredit my name and label me with laboratory rats in Oslo, and say, ‘Oh, steroids are in your system for decades and decades,’” (https://www.theguardian.com/sport/2014/oct/13/justin-gatlin-defends-nomination-athlete-of-year).

The Gundersen group´s research in Oslo showed that a brief steroid exposure induced muscle hypertrophy, and upon withdrawal, the hypertrophic effect was gone after 3 weeks. However, when the animals were subjected to overload exercise three months (>15% of the mouse lifespan) after the drug withdrawal, the mice showed a 36% hypertrophy after 6 days, significantly more than controls (never drug-treated mice), which grew their muscle by only 6%.

The seminal paper with respect to the societal impact and doping was Egner et al. 2013. This paper currently has an Altimetric attention score of 597, which is in the top 5% of all research output, and 1% of output of the same age.

All the participants involved were employees of the department at the time of the research.

5. Sources to corroborate the impact

Anders Solheim, CEO, Antidoping Norway anders.solheim@antidoping.no
https://www.bbc.com/sport/athletics/29531360
https://www.sciencenews.org/article/top-25-stories-2013-microbes-meteorites
https://jobruusgaard.altmetric.com/details/1862505#score

Administrative unit IBV, UiO Impact case 5

Institution: University of Oslo (UiO)
Administrative unit: Department of Biosciences (IBV)
Title of case study: Coastal Marine Protected Areas (MPAs) and management of coastal resources
Period when the underpinning research was undertaken: 2011-2021 (and ongoing)
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021 (see Section 2, Personnel)
Period when the impact occurred: 2011-2021 (and ongoing)

1. Summary of the impact

Coastal areas, in particular in the Skagerrak area, are heavily impacted by various human encroachments, including various developments, pollution, traffic, and harvesting (recreational and commercial). Commercial harvesting of species about which we have limited biological knowledge can lead to population collapses, as we have seen in the coastal cod populations.

We have collected and analysed data on the effect of various management protocols for conserving populations of lobster and wrasses. The knowledge has been directly implemented into management rules and regulations. The documented positive effects of implementing Marine Protected Areas (MPAs) as a tool for protecting target species has been instrumental in leading to the establishment of numerous protected areas along the coast.

2. Underpinning research

The PROBLEM: The coastal marine environment constitutes a very important biological, geochemical, and physical milieu. These ecosystems are among the most productive in the world, exceeding that of tropical rain forests. Also, the coastal zone produces disproportionately more ecosystem services relating to human well-being than most others – this is also the case for Norway.

The coastal zone, in particular along the Skagerrak coast, is heavily used for a variety of purposes providing numerous ecosystem services. The coastal waters are used for harvesting of biological resources, recreation, transport, development of the coastal zone, and numerous other developments. It is also the recipient of runoff from terrestrial systems – in particular from agricultural and urbanized areas. All these stressors impact on natural resources, such as fish and crustaceans that are targets for both commercial and recreational harvesting. Unfortunately, most of the coastal (near-shore) fish and crustacean resources has not been the focus for much research up to recently.

Here, we describe research activities that have been performed to remediate this effect, and which has immediately impacted the society through changes in management decisions by the appropriate authorities.

The lobster problem

The lobster, *Homarus gammarus*, has been a long time target for commercial fisheries. In spite of restricted legal seasons and body size limitations, the lobster population along the Norwegian coast are doing badly. Limited to no knowledge about the biology and the population dynamics on this species existed. Together with researches at the Institute for Marine Research (IMR), we initiated a long-term study on the detailed dynamics of lobster on the Skagerrak coast.

Our research team performed population biological studies of the species – involving demographic parameters such as recruitment, survival and dispersal/distribution. We also studied mating preference, morphometry and genetics on this species.

The main objective was to evaluate if the establishment of marine protected areas (MPAs) could be a viable management tool for securing and potentially rebuilding local populations.

The usefulness of lobster MPAs have recently been evaluated in a paper published in Marine Policy (Knutsen et al 2022. Lobster reserves as a management tool in coastal waters: Two decades of experience in Norway). They conclude that the lobster reserves have attracted high public attention and are now considered a credible supplement to traditional fisheries management. In the period from 2002 to 2021, more than 50 lobster reserves have been implemented in Norway. One major effect of the MPAs that we identified is that it restores the original effect of sexual selection working on body size and mating behaviour in the lobster. This evolutionary rescue has a positive effect on the population growth rate inside the MPA, with a potential spill-over effect to the areas where harvesting is still ongoing.

Wrasses harvested for use as cleaner-fish in salmon aquaculture

Wrasses (a total of six species) are presently intensively fished for use as cleaner-fish in the salmon aquaculture. The wrasses are stocked into net pens in order to graze on salmon lice that is a pest. Prior to 2010 such fishing was limited, but increased strongly thereafter.

Unfortunately, very little was known about the ecology of the species and how populations would respond to intensive selective harvesting. Together with researchers at the Institute of Marine Research (IMR), we initiated a long-term study on the ecology of wrasses on the Skagerrak coast. Our main focus was to evaluate basic biology, as well as how populations would respond to the fishery. Also, the efficiency of marine protected areas (MPAs) as a management option was evaluated.

One major finding in our research was the very limited individual movement of the different species. This indicates that the populations has to be managed at a very local scale, and that overfishing may easily occur locally. Detailed investigations of growth rates, maturation rates and survival give indications to what should be the maximum and minimum size limits set for the fishery. Several species have a very complex breeding system, with alternative male phenotypes, nest building and defence by the males, and also sex change in some species. This complicates management and conservation of the various species which highlights the importance of advanced biological knowledge.

Personnel at IBV

- Professor Asbjørn Vøllestad (1991 - present)
- Researcher Halvor Knutsen (2010-2021)
- Researcher Esben Moland Olsen (2011-2015)
- PhD-student Tonje Sørdalen (2013-2019)
- PhD-student Kim Halvorsen (2013-2016)
- Five master students (2013-2021)

3. References to the research

Sørdalen, T.K., Halvorsen, K.T., Harrison, H.B., Ellis, C., **Vøllestad, L.A**., Knutsen, H., Moland, E. & Olsen, E.M. **2018**. Harvesting changes mating behaviour in European lobster. Evolutionary Applications **11**: 963-977. https://doi:10.1111/eva.12611

Sørdalen, T., Halvorsen, K.T., Vøllestad, L.A., Moland, E. & Olsen, E.M.2020. Marine protected areas rescue a secondary sexual trait in European lobster. Evolutionary Applications 13: 2222-2233. https://doi:10.1111/eva.12992

Halvorsen, K.T., Sørdalen T.K., Durif, C., Knutsen H., Olsen E.M., Skiftesvik, A.B., Rustand, T.E., Bjelland R. & Vøllestad L.A. 2016. Male-biased sexual size dimorphism in the nest building corkwing wrasse (*Symphodus melops*): implications for a size regulated fishery. ICES Journal of Marine Science 73: 2586-2594. https://doi:10.1093/icesjms/fsw135

Halvorsen, K.T., Larsen, T., Sørdalen, T.K., Vøllestad, L.A., Knutsen, H. & Olsen, E.M. 2017. Impact of harvesting cleaner fish for salmonid aquaculture assessed from replicated coastal marine protected areas. Marine Biology Research 13: 359-369. https://doi:10.1080/17451000.2016.1262042

Halvorsen, K.T., Sørdalen T.K., Vøllestad L.A., Skiftesvik, A.B., Espeland, S.H. & Olsen E.M. 2017. Sex- and size-selective harvesting of corkwing wrasse (*Symphodus melops*) – a cleaner fish used in salmonid aquaculture. ICES Journal of Marine Science 73: 2586-2594. https://doi:10.1093/icesjms/fsw221

Halvorsen, K.T., Larsen, T., Browman, H.I., Durif, C., Aasen, N., Vøllestad, L.A., Cresci, A., Sørdalen, T.K., Bjelland, R. & Skiftesvik, A.B. 2021. Movement patterns of temperate wrasses (Labridae) within a small marine protected area. Journal of Fish Biology 99: 1513-1518. https://doi:10.0000/jfb.14825

4. Details of the impact

The data and analyses presented in our work described here has almost immediately been used to implement new fisheries regulations and as background arguments for the establishment of new Marine Protected Areas. Our results are annually reported to the Directorate of Fisheries – mainly through annual reports produced by colleagues at the Institute of Marine Research.

Lobster

The lobster fishery has strong local interest, and is executed by both commercial and recreational fishers. Our research has mainly focused on the effect of the fishery itself (degree of selection and potential evolutionary effects) and the potential local and population-level effects of the establishment of MPAs. Local communities in general have taken initiatives to establish new MPAs within their borders, and the borders of these MPAs are later formalised into regulations by the Directorate of Fisheries. A large number of such MPAs are now established along the coast.

Wrasses

The wrasse fishery has developed continuously with the increasing demands for finding alternative methods to handle the problematic salmon lice infections in the salmon aquaculture. **The results presented by us and collaborators are continuously used to update and potentially change fishing regulations**. This has mainly been changes in the seasonal opening and closing times for the fishery for the different species, based on updated information on timing of reproduction of the various species of wrasses. Further, our data on the selectivity of the various harvest methods and on the growth rates and age at maturation have been used to change the fishing regulations. Mainly it has led to changes in the minimum and maximum size limits for the different species. The impact of our research has therefore been almost immediate. There are annual or semi-annual meetings about the regulation of the wrasse fishery between researchers (represented by Institute of Marine Research), the Directorate of Fisheries, and stakeholder (fishers)). In these meetings, a summary of the recent research activity is presented and discussed. Also, results acquired during the different master of science project are presented there. **The transfer of information is therefore directly from the researchers to the Directorate of Fisheries which is responsible for management of these coastal resources**.

Education

In the projects presented here the education part has been of utmost importance. Our candidates are equipped with in-depth and relevant knowledge making them highly relevant for jobs where they can have a societal impact.

At IBV 7 students have been directly involved in the lobster and wrasse projects (several other students have also been educated at our project partner University of Agder). These students have then been given direct access to information on the processes that are involved in the development of new management regulations, in addition they have learned thorough research methods and critical thinking.

5. Sources to corroborate the impact

Lobster:
Established MPAs for lobster in Norway: https://www.fiskeridir.no/Yrkesfiske/Regelverk-og-reguleringer/J-meldinger/Gjeldende-J-meldinger/j-170-2022

Review paper: Lobster reserves as a management tool in coastal waters: Two decades of experience in Norway. Journal: *Marine Policy* 2022
https://www.sciencedirect.com/science/article/pii/S0308597X21005194?via%3Dihub

Review paper: Restoration of Abundance and Dynamics of Coastal Fish and Lobster Within Northern Marine Protected Areas Across Two Decades. Journal: *Frontiers in Marine Science* 2021. https://www.frontiersin.org/articles/10.3389/fmars.2021.674756/full

Wrasses (examples):
Scientific advice for managing the Norwegian wrasse fishery in 2022.
-On request from the Directorate of Fisheries, the Institute of Marine Research (IMR) have reviewed the state of knowledge for wrasses and provides advice for management regulations of the wrasse fisheries for 2021:
https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2021-54

Management advice for Norwegian wrasse fisheries 2021.
-On request from the Directorate of Fisheries, the Institute of Marine Research (IMR) have reviewed the state of knowledge for wrasses and provides advice for management regulations of the wrasse fisheries for 2021:
https://www.hi.no/hi/nettrapporter/rapport-fra-havforskningen-2020-51

Regulation of fishing for wrasse in 2020 preparation for the 2020 season.
-On request from the Directorate of Fisheries, the Institute of Marine Research)IMR) give advice on the take of wrasse for 2020 (total quota, possibly species-specific quotas, geographical distribution of the take and quota at vessel level):
https://www.hi.no/resources/Regulering-av-fisket-etter-leppefisk-i-2020-forberedelse-til-2020-sesongen.pdf

The 2022 wrasse regulation (Directorate of Fisheries):
https://www.fiskeridir.no/Yrkesfiske/Tema/Leppefisk/Leppefisk-reguleringa-2022

UiO-NHM Impact Case 1

Institution: University of Oslo
Administrative unit: Natural History Museum
Title of case study: Mapping and Description of Norwegian Nature's Variation: Nature in Norway
Period when the underpinning research was undertaken: 2005 - present
Period when staff involved in the underpinning research were employed by the submitting institution: 2005 - present
Period when the impact occurred: 2005- present

1. Summary of the impact (indicative maximum 100 words)

NHM's research and expertise for the basis for the development of Nature in Norway (*Natur i Norge*; NiN), the Norwegian system for typification, description and mapping of Norway's nature's, including terrestrial, aquatic and marine ecosystems, landscape types and geomorphological landforms. NiN is based on EcoSyst, a framework developed from basic biological, geological and ecological theory. In 2015, NiN was sanctioned by the Norwegian Parliament as the official system for land-cover mapping in. Currently NiN is the backbone of several national mapping programmes. NHM continues to develop EcoSyst&NiN, including its applications in research, teaching and practical mapping.

2. Underpinning research (indicative maximum 500 words)

NHM's long research tradition in systematics, ecology and biogeography underpins the recent development of Nature in Norway (*Natur i Norge*; NiN), the Norwegian system for typification, description and mapping of all aspects of nature's variation in areas under Norwegian jurisdiction; from the deepest sea floor at –5500 m to the highest summit (2469 m a.s.l.). Founded in 2005, NiN is owned by the Norwegian Biodiversity Information Centre which is also responsible for making it publicly available. NHM at UiO is responsible for the scientific content of NiN, including most of the documentation of the system (https://www.artsdatabanken.no/NiN). NiN version 3.0 will be published November 16, 2023.

NiN is a value and sector neutral systematisation of Nature's variation in Norway that applies EcoSyst; a framework developed from basic biological, geological and ecological theory (Halvorsen et al. 2020). Based on a comprehensive set of standardised variables that address landform, environmental and biotic compositional variation at all scales, criteria-based type systems are developed for terrestrial, aquatic and marine ecosystems, landscape types, and geomorphological landform types including river reaches, lake basins, peat massifs and glacier massifs. Ecosystem types are adapted to land-cover mapping to scales from 1:500 to 1:50 000.

Names of the key researchers and what positions they held at the administrative unit at the time of the research, and when they joined or left the institute

Rune Halvorsen, Associate professor and professor, 20(?) - present
Olav Skarpaas, Associate professor and professor, 20(?) - present
Anders Bryn, Associate professor and professor, 20(?) – present
Anyone else?

3. References to the research (indicative maximum of six references)

Eriksen, E.L., Ullerud, H.A., Halvorsen, R., Aune, S., Bratli, H., Horvath, P., Volden, I.K., Wollan, A.K. & Bryn, A. 2019. Point of view: error estimation in field assignment of land-cover types. – Phytocoenologia 49: 135-148. https://doi.org/10.1127/phyto/2018/0293

Haga, H.E.E.S., Bryn, A., Ullerud, H.A. & Nilsen, A.B. 2018. Opplæring av nye feltkartleggere: ABC-metoden. Kart & Plan 78: 377-382.

Haga, H.E.E.S., Nilsen, A.B., Ullerud, H.A. & Bryn, A. 2021. Quantification of accuracy in field-based land cover maps: a new method to separate different components. – Appl. Veg. Sci. 24: e12578: 1-11. https://doi.org/10.1111/avsc.12578

Halvorsen, R., Skarpaas, O., Bryn, A., Bratli, H., Erikstad, L., Simensen, T. & Lieungh, E. 2020. Towards a systematics of ecodiversity: the EcoSyst framework. – Global Ecol. Biogeogr. 29: 1887-1906. https://doi.org/10.1111/geb.13164

Simensen, T., Erikstad, L. & Halvorsen, R. 2021. Diversity and distribution of landscape types in Norway. – Norsk geogr. Tidskr. 75: 79-100.

Simensen, T., Halvorsen, R. & Erikstad, L. 2022. Gradient analysis of landscape variation in Norway. – Sommerfeltia 40: 1-193. https://doi.org/10.2478/som-2022-0001

Ullerud, H.A., Bryn, A. & Skånes, H. 2020. Bridging theory and implementation - testing an abstract classification system for practical mapping by field survey and 3D aerial photographic interpretation. – Norwegian Journal of Geography 73: 301-317. https://doi.org/10.1080/00291951.2020.1717595

Ullerud, H.A., Bryn, A., Halvorsen, R. & Hemsing, L.Ø. 2018. Consistency in land cover mapping; influence of fieldworkers, spatial scale and classification system. - Applied Vegetation Science 21(2): 278-288. https://doi.org/10.1111/avsc.12368

4. Details of the impact (indicative maximum 750 words)

NHM's research has major societal impacts through EcoSyst and NiN. In 2015, NiN was sanctioned by the Norwegian Parliament as the official system for land-cover mapping in Norway (later affirmed by a governmental white paper). Currently NiN is the backbone of several national mapping programmes. NHM continues to contribute theoretical and methodological developments related to EcoSyst&NiN, and studies that underpin the system and its applications in research, teaching and practical mapping.

5. Sources to corroborate the impact

Natur i Norge website and documentation:
https://www.artsdatabanken.no/NiN

Confirmation that the Norwegian Environment Agency maps Nature according to NiN:
https://www.miljodirektoratet.no/tjenester/natur-i-norge/

Two White papers attached from the Parliamental Committee and government confirming the use of NiN for mapping land-cover in Norway. See for NiN or Natur i Norge for content related to NiN in these two reports.

UiO-NHM Case #2

Institution: University of Oslo
Administrative unit: Natural History Museum
Title of case study: Fighting illegal wildlife trade
Period when the underpinning research was undertaken: 2013-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2013-2021
Period when the impact occurred: 2013-2021

1. Summary of the impact

The Natural History Museum hosts a research program on the use of molecular tools for the identification of traded wildlife – plants and animals. This research has enabled rapid and cost-effective identification of traded wildlife, particularly derivatives of plants. This expertise was further developed in an EU H2020 MSCA-ITN Plant.ID that progressed the field beyond its state of the art. This expertise is used in Norway through the Norwegian Scientific Committee for Food and Environment and the Norwegian Scientific Authority for the Convention on International Trade in Endangered Species (CITES), and additionally globally by CITES in the Conference of Parties and Plant Committee.

2. Underpinning research (indicative maximum 500 words)

Plant phylogenetics had long been laying the groundwork for a paradigm shift from morphology based identification of plants for societal purposes to one based on DNA sequences for more empirical studies (e.g., de Boer et al. 2012 BMC Evol Biol, 2015 J Biogeo, Käfer et al J Evol Biol, Merckx et al, 2015 Nature, Schwallier et al. 2016 Divers). Harnessing molecular biology for organismal identification was not prioritized until Herbert et al. (2003) promoted the concept of DNA barcoding as an innovative way to identify species. DNA-based species identification, i.e., molecular identification, makes it possible to identify species precisely from trace fragments such as pollen (Bell et al. 2019; Hawkins et al. 2015), detecting substitution in herbal pharmaceuticals (Raclariu et al. 2018, 2017), authentication of sustainable tropical timber (Nithaniyal et al. 2014), monitoring invasive alien species (Armstrong and Ball 2005), uncovering illegal international trade in endangered species (de Boer et al. 2017; Ghorbani et al. 2017), making rapid molecular biodiversity assessments (Bohmann et al. 2014; Thomsen and Willerslev 2015), and studying historical biodiversity through sedimentary DNA and ancient DNA (Anderson-Carpenter et al. 2011; Bálint et al. 2018). NHM has been central in the development of the field of DNA metabarcoding (Taberlet et al. 2012 ME; Bellemain et al. 2010 BMC M), especially of ancient environmental DNA to reconstruct past communities (Parducci et al. 2012 Science; Jørgensen et al. 2012 ME; Willerslev et al. 2014 Nature), as well as identification of plants in products and trade (Kool et al. 2012 PLOS One, de Boer et al. 2014 PLOS One, Veldman et al. 2014 Traffic, Osathanunkul et al. 2015a PLOS One, 2015b PLOS One, 2016 Phytomed, Ghorbani et al. 2017 PLOS One). Researchers at NHM were some of the first to tackle the challenges of plant complex mixtures through DNA metabarcoding applied to herbal medicines (Raclariu et al., 2017a Sci Rep, 2017b Front. Pharmacol., 2018 Phytomed). Similarly applying DNA metabarcoding to plants in trade highlighted the potential of molecular identification to uncover and monitor illegal trade (de Boer et al. 2017 Proc B, Veldman et al. 2017 Plants). This pioneering research was expanded into an EU H2020 MSCA-ITN (PI de Boer) with 27 partners and 15 PhD students to expand on this concept of transferring cutting-edge developments in systematics, including target-capture sequencing, skimming, k-mer analysis, multispecies coalescent phylogenomics, deep learning, to societal applications including the molecular identification of pollen, reconstruction of diets of current and past fauna from fresh and permafrost preserved fecal DNA, establishing vegetation types from soil eDNA, historical museomics of plants in colonial trade, assessment of aquatic plants, identification of tropical timber, species authentication in musical instruments and many others

(e.g., Manzanilla et al. 2018 BMC Evol Biol, Polling et al. 2021a Sci Rep, 2021b QSR, 2022 STOTEN, Masters et al. 2020 Biol Cons, 2022 Biol & Cons, Lens et al. 2020, ter Schure et al. 2021 QSR, Anthoons et al. 2021 J Food Sci, Trucchi et al. 2021 Nature Plants, Moiloa et al. 2021 Mol Phyl Evol, Ariza et al. 2022 Meth Ecol Evol, Canales et al. 2022).

Key researchers at NHM for case study #2. Sanne Boessenkool (- 2012, postdoc), Laura Epp (- 2012 postdoc), Galina Gusarova (- 2014 Researcher), Magnus Popp (- 2015 associate professor), Vincent Manzanilla (2014-2018 PhD student), Ancuta-Cristina Raclariu (2013-2021 PhD student), Maria Ariza (2018-2021 PhD student), Margret Veltman (2018-2021 PhD student), Anneleen Kool (2012-2021 Associate Professor), Christian Brochmann (- 2021 Professor), Hugo de Boer (2013-2021 Researcher, Associate Professor, Professor, Research Director).

This is a research field that emerged from the field of plant systematics and phylogenetics with novel applications in studying ecosystems – past and present, dietary analysis of herbivores – extant and extinct, food and drug safety, allergenic pollen and illegal wildlife trade.

3. References to the research

Taberlet, P., Coissac, E., Pompanon, F., **Brochmann, C.** and Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular ecology, 21(8), pp.2045-2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Kool, A., de Boer, H.J., Krüger, Å., Rydberg, A., Abbad, A., Björk, L. and Martin, G., 2012. Molecular identification of commercialized medicinal plants in Southern Morocco. PloS one, 7(6), p.e39459. https://doi.org/10.1371/journal.pone.0039459

Willerslev, E., **Gussarova, G., Boessenkool, S., Epp, L., Brochmann, C.** a.o., 2014. Fifty thousand years of Arctic vegetation and megafaunal diet. Nature, 506(7486), pp.47-51. https://doi.org/10.1038/nature12921

De Boer, H.J., Ghorbani, A., **Manzanilla, V., Raclariu, A.C.**, Kreziou, A., Ounjai, S., Osathanunkul, M. and Gravendeel, B., 2017. DNA metabarcoding of orchid-derived products reveals widespread illegal orchid trade. Proceedings of the Royal Society B: Biological Sciences, 284(1863), p.20171182. https://doi.org/10.1098/rspb.2017.1182

Raclariu, A.C., Paltinean, R., Vlase, L., Labarre, A., **Manzanilla, V.**, Ichim, M.C., Crisan, G., Brysting, A.K. and **de Boer, H.**, 2017. Comparative authentication of Hypericum perforatum herbal products using DNA metabarcoding, TLC and HPLC-MS. Scientific reports, 7(1), pp.1-12. https://doi.org/10.1038/s41598-017-01389-w

de Boer, H., Rydmark, M.O., Verstraete, B. and Gravendeel, B., 2022. Molecular identification of plants: from sequence to species. Advanced Books. 399p. https://doi.org/10.3897/ab.e98875

4. Details of the impact

The development of a strong research program in molecular identification of plants (detailed above) was supported by the Research Council of Norway, the EEA Grants and the European Research Agency (2012-2022). The societal applications of such DNA-based identification are myriad, and led to numerous collaborations to develop and apply these methods in society and industry, e.g., NatureMetrics UK, Medicines and Healthcare products Regulatory Agency UK, Diatomella NL, Nefeloudis Food Additives Company GR, Wildlife Justice Commission NL, Norwegian Medicines Agency NO, Norwegian Food Safety Authority NO, and others (2016-2022). Additionally, invited talks at the European Medicines Agency (EMA) and European Food Safety Authority (EFSA) (2020-2022). These collaborations explored among others, ways to use DNA-based identification of timber in musical instruments, plants in horticultural trade,

illegal wildlife trade, accurate allergenic pollen forecasts, aquatic invasive species, dietary overlap of grazers in protected areas, quality control of herbal medicines, dietary supplements and foods.

This expertise was also adopted by the Norwegian Scientific Committee for Food and Environment (VKM), a commission giving scientific advice to the Norwegian government, when de Boer joined VKM in 2015 as a member of their scientific panel for invasive species and trade in endangered species. In 2020, de Boer became a member of the Norwegian Scientific Authority of CITES. In 2022, de Boer became a member of the general committee of VKM, and leader of the scientific panel on CITES. Additionally, in 2022, de Boer became a member of the global CITES Plant Committee as a representative member for Europe. In VKM, Norwegian CITES SA and the international CITES Plant Committee, de Boer works on questions related to the identification, trade and sustainability of international harvest, hunting and trade of wild species of flora and fauna. In this work we see a direct societal benefit of the knowledge and expertise developed within DNA-based identification at NHM. The research built up at NHM in the period 2012-2022 has translated into direct applications that provide science-based decision making in international and national policy and law.

Some of the impacts of our assessments include: Assessment of the risks to Norwegian biodiversity from the import and keeping of aquarium and garden pond plants (de Boer et al 2016) and Assessment of the risks to Norwegian biodiversity from the import and keeping of terrestrial arachnids and insects (Nielsen et al. 2016), both of which led to the stricter regulation of trade in these species through Regulation FOR-2015-06-19-716 (https://lovdata.no/dokument/SF/forskrift/2015-06-19-716); Assessments of species listing proposals for the CITES Conference of Parties meetings CoP17, CoP18 (2019) and CoP19 (2022) have contributed to the Norwegian position at these meetings, and the subsequent establishment and revisions of the CITES Regulation FOR-2018-06-15-889 https://lovdata.no/dokument/SF/forskrift/2018-06-15-889. Specific risk assessments for the Norwegian Environment Agency has contributed to science-based decision making, amendment of existing laws and new regulations, i.e. a CITES risk assessment for polar bear (Ursus maritimus) (Rueness et al. 2020); Status and trade assessment of parrots listed in CITES Appendix I (Rueness et al. 2020); Compilation of knowledge on the global population of common minke whale (Balaenoptera acutorostrata) (Rueness et al. 2022). Additionally in role of appointed member of the Norwegian delegation to CITES Conference of Parties meetings, de Boer is able to translate scientific knowledge to practice and policy as part of the negotiations among parties for listing of wildlife affected by unsustainable trade.

5. Sources to corroborate the impact

Hugo de Boer, Member of the CITES Plants Committee, https://cites.org/eng/com/pc/member.php

Hugo de Boer, Leader of the Scientific Panel on CITES, and member of the Scientific Steering Committee, https://vkm.no/personsider/hugodeboer.4.2994e95b15cc545071612cef.html

Eli K. Rueness, Kjersti S. Kvie, Erlend B. Nilsen, **Hugo de Boer**, Katrine Eldegard, Kjetil Hindar, Lars Robert Hole, Johanna Järnegren, Kyrre Kauserud, Inger Måren, Anders Nielsen, Eva B. Thorstad, Gaute Velle (2022). Compilation of knowledge on the global population of common minke whale (Balaenoptera acutorostrata). Scientific Opinion of the Panel on Alien organisms and Trade in Endangered Species (CITES). VKM Report 2022:07, ISBN: 978-82-8259-382-3, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Eli K. Rueness, Maria G. Asmyhr, **Hugo de Boer**, Katrine Eldegard, Kjetil Hindar, Lars Robert Hole, Johanna Järnegren, Kyrre Kausrud, Lawrence Kirkendall, Inger Måren, Erlend B. Nilsen, Eva B. Thorstad, Anders Nielsen, Gaute Velle (2020) Status and trade assessment of parrots listed in CITES Appendix I. Scientific Opinion of the Panel on alien orgnaisms and trade in

endangered species (CITES) of the Norwegian Scientific Committee for Food and Environment. VKM Report 2020:15, ISBN: 978-82-8259-354-0 ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Eli K. Rueness, Maria G. Asmyhr, **Hugo de Boer**, Katrine Eldegard, Lars Robert Hole, Kjetil Hindar, Johanna Järnegren, Kyrre Kausrud, Lawrence Kirkendall, Inger Måren, Erlend B. Nilsen, Anders Nielsen, Eva B. Thorstad, Gaute Velle (2020). A CITES Risk assessment for polar bear (Ursus maritimus). Opinion of the Panel on Alien Organisms and Trade in Endangered Species (CITES) of the Norwegian Scientific Committee for Food and Environment. VKM report 2020: 06, ISBN: 978-82-8259-344-1, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Eli K. Rueness, Maria G. Asmyhr, Siobhan Dennison, Anders Endrestøl, Jan Ove Gjershaug, Inger Elisabeth Måren, **Hugo de Boer**, a.o. (2016) Assessment of listing proposals for CITES CoP17. Scientific Opinion on the Panel on Alien Organisms and Trade in Endangered Species (CITES). Opinion of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-228-4, Oslo, Norway.

Hugo de Boer, Maria G. Asmyhr, Hanne H. Grundt, Inga Kjersti Sjøtun, Hans K. Stenøien, Iris Stiers (2016). Assessment of the risks to Norwegian biodiversity from the import and keeping of aquarium and garden pond plants. Scientific Opinion on the on Alien Organisms and Trade in Endangered species of the Norwegian Scientific Committee for Food Safety ISBN: 978-82-8259-240-6, Oslo, Norway.

Vigdis Vandvik (chair), **Hugo de Boer**, Jan Ove Gjershaug, Kjetil Hindar, Lawrence R. Kirkendall, Nina Elisabeth Nagy, Anders Nielsen, Eli K. Rueness, Odd Terje Sandlund, Kjersti Sjøtun, Hans Kristen Stenøien, Gaute Velle (2016). Assessment of risks to Norwegian biodiversity from the import and keeping of terrestrial arachnids and insects. Scientific Opinion on the Panel on Alien Organisms and Trade in Endangered species of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-226-0, Oslo, Norway

Forskrift om innførsel, utførsel, besittelse mv. av truede arter av vill fauna og flora (CITES-forskriften). FOR-2018-06-15-889. https://lovdata.no/dokument/SF/forskrift/2018-06-15-889

Forskrift om fremmede organismer. FOR-2015-06-19-716.
https://lovdata.no/dokument/SF/forskrift/2015-06-19-716

PhyloNorway – impact case study – The Arctic University Museum of Norway

Institution: The Arctic University of Norway and Academy of Arts
Administative Unit:
Title of case study: PhyloNorway – barcoding past and current ecosystems for environmental management
Period when the underpinning research was undertaken: 2011-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2011-2021
Period when impact occurred: 2018-present (ongoing)

1. Summary of Impact

The creation of the ***PhyloNorway*** genetic database by the NEAT research group at The Arctic University Museum of Norway provides a unique resource for environmental managers. It enables DNA sequences from environmental samples to be assigned to species with almost 100% identity - far above that possible with the Global databases. This allows eDNA to be used for ecological surveys with confidence that it has the power to identify all the species in Norway and Polar regions. The resource finally completed in 2020 is currently being used by several environmental agencies including Norwegian Institute of Nature Research, NIBIO and in forensic science.

2. Underpinning research

In 2011 it was realised that the natural history collection at the museum could be used to generate a full database of the Polar/ Norwegian flora and fauna. The funding was provided by the Research Council of Norway and The Norwegian Biodiversity Centre through several rounds from 2013 and onwards. At that time, it had been problematic to find good barcoding regions for plants, and after some years of testing and trying, we were the first in the world to go for large scale genome skimming based on herbarium material. Herbarium material has poorer DNA quality than freshly collected material but makes use of already available resources rather than large scale field campaigns. ***PhyloNorway*** was needed due to the explosion in the use of environmental DNA (eDNA) for both academic research and environmental monitoring. Its value comes from the fact that it is imperative that false negatives are not created due to the lack of a comprehensive database. Before ***PhyloNorway*** a user had to use one of the Global databases such as EMBL which because they are not complete for the Arctic regions, meant that the user could not be sure that a species was absent due to real absence or just because it was not in the database. It took 10 years to complete the work and the database was finally completed in 2020 (Alsos et al. 2020) with further release and use of data in Wang et al. (2021) and Alsos et al. (2022). It is a sister project of ***PhyloAlps,*** which is based on freshly collected material team from LECA, France, with the first data released in Alsos et al. (2020) and Garces-Pastor et al. (2022). Several other similar projects are now underway in China and the UK (Barcode UK). The main users of ***PhyloNorway*** include both environmental managers and academics (as a research tool). It has been used both by us, but also by other groups such as GLOBE Institute Copenhagen and the Alfred Wegner Institute Potsdam (AWI) to investigate the impacts of climate change on terrestrial vegetation. However, its potentially major user group and societal impact is through environmental managers taking eDNA samples for ecological monitoring and environmental impact surveys. The current user group (for example Norwegian Institute of Nature Research, Aquaplan NIVA, the Norwegian Institute of Bioeconomy Research) will grow as the database is free (and easy) due to its original funding being governmental.

The research arose due to the growth in the museum's then Botany Department of research that sought to use DNA metabarcoding for research on the past and present flora of Norway, and the realisation that the small fragments of DNA held in soil and sediment (so called sedaDNA) could be used to produce comprehensive lists of species. However, this required a comprehensive database for the part of the genome that could be used for identification (P6 loop region of the chloroplast trnL (UAA) intron) – hence the realisation that the herbarium could be used to produce just such a database (***PhyloNorway***). The research was not done in a vacuum and went hand-in-hand with the development of genomic research and advanced teaching at the museum. Its later phases of development and use were funded by RCN NorBOL (2014-2019) and RCN topforsk ECOGEN (2016-2023). Its existence was also one of the reasons that an Adv ERC grant was moved from the UK to Norway (TerrACE), and that Alsos obtained an ERC consolidator grant (IceAGenT). Further, it has been the basis for several PhDs both at Tromsø but also externally at Southampton UK (x2), Oslo and Copenhagen.

Staff Involved

Inger Greve Alsos, Professor, (joined 2010)
Marie K. F. Merkel, Senior Engineer, joined 2015
Eric Cossac, volunteer since 2013
Dilli P. Rijal, PhD 2012-2016, then Post doc 2017-2020, and now Assoc Prof. since 2023
Youri Lammers, PhD student, joined 2016
Antony G. Brown, Professor, joined 2019
Iva Pitelkova, Senior Engineer, joined 2017
Peter Heintzman, Researcher 2017-2019, Assoc Prof 2019-2022
Galina Gusarova, Researcher, joined 2015
Sandra Garces-Pastor, post doc, 2018-2022

Contextual Information

This project and also product (the genome-skim database) is the direct result of research in Botany on plant genetics but also the existence of a well curated herbarium collected over 150 years since the foundation of the museum in 1872. Because both the museum and the research making PhyloNorway possible were publicly funded, it was decided that the dataset had to be free to all users and particularly to government departments and not for profit NGOs, rather than being sold as part of a spin-off enterprise as has happened with other research products in this field.

3. References to the research

1 Alsos, I G, Y Lammers, N G Yoccoz, T Jørgensen, P Sjögren, L Gielly and M E Edwards (2018) "Plant DNA metabarcoding of lake sediments: How does it represent the contemporary vegetation" PLOS ONE 13: e0195403. https://doi.org/10.1371/journal.pone.0195403

2 Alsos, I G, S Lavergne, M K F Merkel, M Boleda, Y Lammers, A Alberti, C Pouchon, F Denoeud, I Pitelkova, M Pușcaș, C Roquet, B-I Hurdu, W Thuiller, N E Zimmermann, P M Hollingsworth and E Coissac (2020) "The treasure vault can be opened: Large-scale genome skimming works well using herbarium and silica gel dried material" Plants 9: 432. https://doi.org/10.3390/plants9040432

3 Alsos, I G, D P Rijal, D Ehrich, D N Karger, N G Yoccoz, P D Heintzman, A G Brown, Y Lammers, L Pellissier, T Alm, K A Bråthen, E Coissac, M K F Merkel, A Alberti, F Denoeud and J Bakke (2022) "Postglacial species arrival and diversity buildup of northern ecosystems took millennia" Science Advances 8: eabo7434. https://www.science.org/doi/10.1126/sciadv.abo7434

4 Brown, T, D P Rijal, P D Heintzman, C L Clarke, H-P Blankholm, H I Høeg, Y Lammers, K A Bråthen, M Edwards and I G Alsos (2022) "Paleoeconomy more than demography determined prehistoric human impact in Arctic Norway" PNAS Nexus 1(5). https://academic.oup.com/pnasnexus/article/1/5/pgac209/6751926

5 Rijal, D P, P D Heintzman, Y Lammers, N G Yoccoz, K E Lorberau, I Pitelkova, T Goslar, F J A Murguzur, J S Salonen, K F Helmens, J Bakke, M E Edwards, T Alm, K A Bråthen, A G Brown and I G Alsos (2021) "Sedimentary ancient DNA shows terrestrial plant richness continuously increased over the Holocene in northern Fennoscandia" Science Advances 7(31): eabf9557. https://doi.org/10.1126/sciadv.abf9557

6 Wang, Y, M W Pedersen, I G Alsos, B De Sanctis, F Racimo, A Prohaska, E Coissac, H L Owens, M K F Merkel, A Fernandez-Guerra, A Rouillard, Y Lammers, A Alberti, F Denoeud, D Money, A H Ruter, H McColl, N K Larsen, A A Cherezova, M E Edwards, G B Fedorov, J Haile, L Orlando, L Vinner, T S Korneliussen, D W Beilman, A A Bjørk, J Cao, C Dockter, J Esdale, G Gusarova, K K Kjeldsen, J Mangerud, J T Rasic, B Skadhauge, J I Svendsen, A Tikhonov, P Wincker, Y Xing, Y Zhang, D G Froese, C Rahbek, D B Nogues, P B Holden, N R Edwards, R Durbin, D J Meltzer, K H Kjær, P Möller and E Willerslev (2021) "Late Quaternary dynamics of Arctic biota from ancient environmental genomics" Nature 600: 86-92. https://www.nature.com/articles/s41586-021-04016-x (Alsos co-first author, 39,000 access by 13th of January 2023, release of the first 1540 genomskims of PhyloNorway).

4. Details of the Impact

The major impact that we cite here is the use of PhyloNorway as part of NorBOL for the accurate identification of organisms, often to species level, for the purposes of environmental monitoring and management by environmental organisations/companies. The following agencies are using ***PhyloNorway*** as described below:

Norwegian Institute of Nature Research (NINA): NINA uses NorBOL for the accurate identification of species from eDNA surveys for biodiversity and vegetation surveys. At the moment this is trialling and is being undertaken in tandem with traditional vegetation surveys.

Norwegian Institute of Bioeconomy Research (NIBIO): NIBIO has established a Molecular Ecology Lab at Svanhovd which is currently using NorBOL and specifically PhyloNorway for herbivore diet in order to maintain animal health and manage natural resources in a stable manner.

The development of forensic botany in Norway: following on from both research and cases undertaken by Prof Brown (UMAK, UiT) both pollen and now eDNA are being developed as forensic tools in Norway. More background can be found in the corroborating letter but the newly established Centre for Forensic Genetics at Tromsø now aims to trial the use of eDNA for serious crime work using PhyloNorway. The use of PhyloNorway means that in court a nearly 100% identification accuracy and completeness can be claimed in court greatly increasing evidential value.

Education: we need to educate people in using this new tool. We have arranged several courses in hand-on methods of barcoding for the institute sector and university staff (students, engineers, research staff) through the NorBOL project. Through the ForBio research School in biosystematics (https://www.forbio.uio.no/), we train master and PhD students as well as post docs and general academic staff in the more advanced methods in using the PhyloNorway and other DNA reference libraries for identification of species in environmental DNA. We arrange a course in metabarcoding annually. In addition, several of our research group participate in teaching on an international course in metabarcoding last arranged in Colombia (2019).

In addition, the project output has been used in **outreach to the public and science-communication**. This has included articles in Forskning.no (which are listed below). It has also formed the basis of two exhibitions in the museum for the public (Rijal, Lammers, Heintzmann) and also been used the Museum's annual December-Night outreach event.

5. Sources to corroborate the impact

We have several sources to corroborate the impact as described in the quoted sections from the following letters of support from users and potential users:

NINA: Letter from Dr Frode Fossøy Centre for Biod9iversity Genetics, NINA, Trondheim (10th Jan 2023) "The Norwegian Barcode of Life (NorBOL) project has provided DNA reference resources that makes up part of the core tools for NINAGEN. The high cover of species that are now barcoded in Norway allows a reliable identification of organism in environmental samples across our different ecosystems. The genome skim plant DNA reference library PhyloNorway is of particularly high quality, as this reference library covers a large part of the genome, that increases the probability of accurate detection and identification of species."

Centre for Forensic Genetics: Letter from Assoc Profs Janssen & Berg (13th Jan 2023): "DNA metabarcoding of the p6 loop from the chloroplast has large potential to augment or even replace these standard methods (pollen analysis) of taxonomic plant identification in the future, although this must still be validated before being used in court. It is expected that DNA metabarcoding could provide a superior match due to its ability to record insect pollinated plants and higher taxonomic resolution than pollen. For this purpose, the phyloNorway DNA reference library will be invaluable as it covers the majority of plant species growing in Norway"

NIBIO: Letter from Snorre Hagen NIBIO Svanhovd (11th Jan 2023): "Molecular methods are increasingly used in monitoring and research and are rapidly becoming essential to address a wide range of research problems within the ecological, evolutionary, and environmental sciences. NIBIO therefore has established the Molecular Ecology Lab

at NIBIO Svanhovd, as a tool for management and research, to study northern species, populations, and ecosystems. The basis for any identification of species from environmental samples are DNA reference libraries. Thanks to the NorBOL project, Norway has excellent cover of multicellular organisms. Of special value is the PhyloNorway genome reference database, as this allows us to identify the diet for key ecosystem engineer species as reindeer and elk, as well as key domesticates as sheep and goats. Knowledge of herbivore diet is essential for maintaining animal health as well as sustainable management of natural resources."

The corroborating articles in Forskning.no can be found at:
https://forskning.no/dna-dyreverden-geologi/verdens-eldste-dnaforskere-har-funnet-2-millioner-ar-gamle-rester-av-planter-og-dyr/2116851

https://forskning.no/istiden-klima-planteverden/disse-plantene-var-de-forste-som-dukket-opp-i-nord-norge-etter-siste-istid/2082549

https://forskning.no/istiden-naturvitenskap-planeter/planter-fortsatte-a-spre-seg-mot-nord-tusener-av-ar-etter-klimaendringen-pa-slutten-av-istiden/1893015

Alsos has been rewarded UiT research price:
https://uit.no/nyheter/artikkel?p_document_id=777610

Example of international outreach can be found here:
https://www.discovermagazine.com/environment/a-different-kind-of-climate-refugee

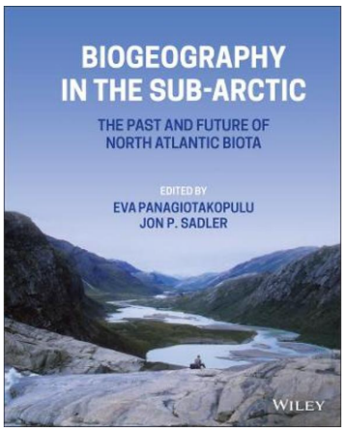
https://sciencenorway.no/animal-kingdom-climate-dna/worlds-oldest-dna-scientists-discover-2-million-year-old-remains-of-plants-and-animals/2127105

https://sciencenorway.no/climate-ice-age-plants/these-were-the-first-plants-to-appear-in-northern-norway-after-the-last-ice-age/2091083

https://sciencenorway.no/animal-kingdom-arctic-genetics/mammoths-and-other-large-animals-survived-in-the-north-much-longer-than-previously-believed/1968247

Youtube:
https://www.youtube.com/watch?v=H8ldpZrf9F4

Text book:



[BFE] [case number:1]

Institution: UiT – the Arctic University of Norway
Administrative unit: BFE (Faculty of Bioscience, fisheries and economics)
Title of case study: The Finnfjord lab (The Finnfjord project)
Period when the underpinning research was undertaken: 2013-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2013-2021
Period when the impact occurred: See below

1. **Summary of the impact** (indicative maximum 100 words)
   This section should briefly state what specific impact is being described in the case study.

The "Finnfjord project" is a strategic UiT project hosted and run by the BFE - as an industry - academic collaboration with Finnfjord AS, a ferrosilicon smelter plant.

In short, the ultimate goals are to use the CO_2 produced during the melting process and released as factory smoke as carbon source for microalgae production while using factory cooling (sea)water as cultivation media and 2) exploit the microalgae for e.g., feed production containing EPA and protein rich biomass and other value-added applications.
Photo: Finnfjord melt plant (from Wikipedia; 10.01.2023).

2. **Underpinning research** (indicative maximum 500 words)

The Finnfjord project "Scaling up cultivation of arctic marine microalgae to industrial scale" was established in 2013 as a project funded by UiT and Troms County. It is a spin-off from the MabCent - SFI centre (2007-2015), where the algae lab at BFE had a central role in bioprospecting, isolation, culture collection and mass cultivation of many microalgae strains. During the collection and cultivation process of microalgae species, strains with superior qualities regarding growth at low light, in tanks and at a wide temperature interval (including very low temperatures) and low pH were found. We still maintain a close collaboration with Marbio, and other BFE research groups such as the Seafood science research team at NFH through several projects.

The industrial part of the project started January 2015, i.e. experimental mass cultivation of northern/Arctic diatoms integrated in the production line at Finnfjord AS. In 2017 the joint UiT-Finnfjord lab officially opened alongside with microalgae cultivation infrastructure comprising bioreactor volumes from 0.1, 1, 10, 150, 6000, 14000 and 300 000 liters (300 000 L - see photo at the left of this cell. (Photo: O.M. Rapp, Klassekampen)).

When the selected diatom species is cultivated with high *p*CO2 (p=partial pressure) they increase both total lipid production and omega-3 levels. In addition, by applying appropriate cultivation procedures, we have achieved >6 months continuous production without contamination or fouling of the photobioractors. Scalability is the key to success, and our production concept, using tanks, is cheaper the bigger the tanks are, and this has opened new avenues of microalgae research - focused on species that can be used in a scaled-up production system. The produced biomass have high nutritional value, comparable to e.g. pelagic fish, making it an ideal substitute of fish feed ingredient. At the same time, marine microalgae are rich sources of biomolecules such as pigments, have alternative uses as nutraceuticals and to increase performance of e.g., solar panels and batteries.

Currently, the collaboration also includes Sintef (Trondheim, Norway) and other industrial partners via the Green Platform project AlgScaleUP/AlgOpti which is an integral part of the ARC (Arctic Centre for Sustainable Energy) at UiT and the UiT´s iCCU (interdisciplinary Carbon Capture and Utilization Centre).

Employees (2021): 1 senior researcher and 3 technicians, 2 PhDs and 1 master´s student – mostly at the Finnfjord plant. In addition, the algae research team located at BFE has two assoc. prof., one post doc, two technicians, one PhD and two master´s students. These frequently commutes to Finnfjord.

It has been published more than 20 scientific peer reviewed articles in the area of microalgae cultivation and products in the period 2011-2021 - by members of the algae group and partners. In addition, we have submitted one Disclosure of Inventions (DOFIs), that UiT has accepted and subsequently overtaken the rights:

Until 2021: Hans Christian Eilertsen, Project leader and group leader, professor, retired (2019). Richard Ingebrigtsen, PhD student 2012-17, Post Doc (2018-2020), Researcher/project leader (2020-) have directly been affiliated to the project. Later, the Microalgae & Microbiome research group has been added by one professor, one ass. Professor together with seven PhDs/post docs and technicians.

3. References to the research (indicative maximum of six references)

Eilertsen, Hans Chr, Edel Elvevoll, Ingeborg Hulda Giæver, Jon Brage Svenning, Lars Dalheim, Ragnhild Aven Svalheim, Birthe Vang, Sten Siikavuopio, Ragnhild Dragøy, and Richard A Ingebrigtsen. 2021. "Inclusion of photoautotrophic cultivated diatom biomass in salmon feed can deter lice." PloS one 16 (7):e0255370. https://doi.org/10.1371/journal.pone.0255370

Eilertsen, Hans Chr, Gunilla K Eriksen, John-Steinar Bergum, Jo Strømholt, Edel Elvevoll, Karl-Erik Eilertsen, Eldbjørg Sofie Heimstad, Ingeborg Hulda Giæver, Linn Israelsen, Jon Brage Svenning, Lars Dalheim, Renate D Osvik, E Hansen, Richard A Ingebrigtsen, Terje Aspen, and Geir-Henning Wintervoll. 2022. "Mass Cultivation of Microalgae: I. Experiences with Vertical Column Airlift Photobioreactors, Diatoms and CO2 Sequestration." Applied Sciences 12 (6):3082. https://doi.org/10.3390/app12063082

Huseby, Siv, Maria Degerlund, Gunilla K. Eriksen, Richard A. Ingebrigtsen, Hans Chr Eilertsen, and Espen Hansen. 2013. "Chemical diversity as a function of temperature in six northern diatom species." Marine Drugs 11 (11):4232-4245. doi: https://doi.org/10.3390/md11114232.
Ingebrigtsen, Richard A., Espen Hansen, Jeanette Hammer Andersen, and Hans Christian Eilertsen. 2016. "Light and temperature effects on bioactivity in diatoms." J. Appl. Phycol. 28 (2):939-950. doi: https://doi.org/10.1007/s10811-015-0631-4.

Osvik, Renate Døving, Richard Andre Ingebrigtsen, Maria Fredrika Norrbin, Jeanette Hammer Andersen, Hans Christian Eilertsen, and Espen Holst Hansen. 2021. "Adding Zooplankton to the OSMAC Toolkit: Effect of Grazing Stress on the Metabolic Profile and Bioactivity of a Diatom." Marine drugs 19 (2):87. **https://doi.org/10.3390/md19020087**

Svenning, Jon Brage, Lars Dalheim, Hans Christian Eilertsen, and Terje Vasskog. 2019. "Temperature dependent growth rate, lipid content and fatty acid composition of the marine cold-water diatom *Porosira glacialis*." Algal Research 37:11-16. https://doi.org/10.1016/j.algal.2018.10.009

4. Details of the impact (indicative maximum 750 words)

The Finnfjord project members and the Finnfjord lab, together with Finnfjord AS engineers and the wider research group - M2RG – Marine Microalgae and Microbiomes research group, has developed expertise in and infrastructure for 1) Mass cultivation of marine microalgae in an industrially relevant environment with a range of monitoring and sensor capabilities in photobioreactors up to 300 000 Liters. 2) Strain isolation and cultivation facilities, and 3) Up-scaling facilities ranging from 0.1, 1, 10, 150, 600, 6000, 14000 and 300 000 Liters.

Figure: Small-scale incubators/tanks for algae production at Finnfjord. Photo: Tommy Hansen, UiT.

The competence by UIT/BFE researchers and the facility and willingness by the Finnfjord AS to develop this strategic alliance has been exemplary. The researchers transferred knowledge and skills from small BFE algae facilities to create the concept.

Feed and food ingredients have been the underpinning main products the project has in mind when aiming for large scale microalgae cultivation facility and production. In Norway, the salmon production and feed industry are constantly met with increasing competition for marine feed resources which have less and less availability in the market. Furthermore, both the authorities and customers in both Norway and at the EU level have stricter requirements to the CO_2 footprint and overall sustainability of aquaculture food production. For future microalgae production, scalability and mass

cultivation are together the main barriers. We have focused on photobioreactor systems that offer a much cheaper alternative compared to other systems - the bigger the tanks are the bigger is the potential. Our choice of cultivation requires microalgae strains and species that are larger than the usual species used in algae cultivation (due to less self-shading). At the same time, they have been less thoroughly biochemically and nutritionally characterised. As such, an important focus is to characterise several algae species. Through several projects, the lipid content has been analysed, new uses have been proposed, and experiments with downstream processing of biomass have been undertaken - such as the Mabit (Marine Biotechnology in Tromsø) projects "HEaT" (2021) and "MabiSurf" (2020) and the regional research fund - Arctic 2030 project "AlgTech" (2020-), wherein biomass extraction methods were the focus.
In a project with NOFIMA (research institute) and Flakstadvåg Laks AS (salmon producer), where salmon were fed a feed with added microalgae, we found indications that those salmon had less salmon lice infestation than the control ("New innovative lice deterring salmon feed" – Innovation Norway 2020 -). This could potentially be interesting for the salmon industry, which spends billions of NOK annually on fighting salmon lice. This aspect will be followed up in the NRC/Innovation Norway - Green platform project "AlgScaleup/Algopti" that started in 2022.
The new plan for the algae factory is to develop the concept into larger facilities, i.e., many large tanks on a larger area. Since the current activity started more than 10 years ago, with continuous milestones and deliverables, with their impacts, it is hard to mention a specific start date for the impact. It is too early to speak of a production volume that calls the needs for the aquaculture industry (mill tons of feed).

Nevertheless, the building of this collaboration has had several impacts on our research and teaching, e.g., the way we collaborate with industrial partners and how we include and learn from researchers outside our own field of research - in e.g., economics, philosophy, sustainable development, and business (e.g., iCCU). In addition, we the research and projects have been disseminated to local press, workers at the factory, middle schools, and high schools in Senja municipality, a municipality where UiT had less contact with the community prior to our project and collaboration with Finnfjord AS. This has been well received. We have also included the lessons learned about sustainability challenges faced by industry in teaching, and often have visiting classes from UiT. We are also engaged in the national graduate research school on photosynthetic research, "Photosyntech." Since Finnfjord is the largest factory in the area it attracts political vigilance. The project staff members can thus talk directly to policy makers about the importance of basic research in tackling future industrial sustainability challenges. From all outreach activities and communication with politician, the algae project at Finnfjord is, in fact, mentioned in a recent parliamentary notice that concerns Northern Norway, job creation and green transition (Meld. St. 9 (2020-2021); https://www.regjeringen.no/no/dokumenter/meld.-st.-9-20202021/id2787429/?q=finnfjord&ch=11#match_2) (In Norwegian only).

5. Sources to corroborate the impact (indicative maximum of ten references)

AlgTech - (2020 -). Optimisation project funded by the Troms County Regional Research Funding program "Arctic 2030", Project No. 20/23857 Total budget 4.9 mill NOK

HEaT – Heating Extends Algal Stability (2021) Commercialisation/optimization project funded by Mabit, Project No. Total budget 1.3 mill NOK

MabiSurf - Novel Marine Biosurfactants for Various Industrial Applications - (2020) Commercialisation/optimization project funded by Mabit, Project No. Total budget 1.2 mill NOK

A new and sustainably produced lice deterring salmon feed (2021) Research and innovation project funded by the Research council of Norway Project No. 321415. Total budget ca. 6 mill NOK

The Finnfjord project - Scaleup of mass-cultivation of marine microalgae to industrial scale (2016-2019). This research was funded by Norwegian Regional Funds Project No. RDA: 551.6. Total budget: 28.9 mill NOK

[BFE] [case number: 2]

Institution:
Administrative unit: BFE
Title of case study: Marbio
Period when the underpinning research was undertaken: 2011-2021
Period when staff involved in the underpinning research were employed by the submitting institution: 2007->
Period when the impact occurred: NA; See below

1. **Summary of the impact** (indicative maximum 100 words)
   This section should briefly state what specific impact is being described in the case study.

Marbio is an analytical platform for natural products drug discovery at UiT - The Arctic University of Norway. It belongs to the Faculty of Biosciences, Economics and Fisheries (BFE), and the Norwegian College of Fishery Science (NCFS). Marbio is the main hub in Norway for marine bioprospecting, and the search for bioactive compounds from marine resources is a priority area at UiT. It is approved as a specialized screening site within the European Research Infrastructure Consortium (ERIC) EU-OPENSCREEN. The platform has incubated its own drug discovery and development projects and has contributed to commercial development of research results from external users.

2. **Underpinning research** (indicative maximum 500 words)

Marbio was established in 2004 as a project funded by NFR, and during the implementation of the Center for Research-based Innovation MabCent (2007-2015), Marbio became the central hub for UiT in the strategic initiative on marine bioprospecting. The expertise at Marbio include fermenting marine microorganisms, screening crude extracts/fractions for different bioactivities and performing bioassay-guided purification to isolate active compounds and hence build up a library of natural products. We explore Arctic and sub-Arctic marine organisms, searching for compounds with activities against bacteria, cancer and diabetes as well as compounds with immunomodulatory and antioxidative effects. Our screening campaign has been based on a classic bioassay-guided fractionation approach, but we are also using metabolomic and genomic analysis in our work to identify the bioactive natural products. We are screening a unique collection of cold-water invertebrates and microorganisms that we have established in collaboration with Marbank, a national marine biobank in Tromsø, Norway. The microorganisms have been collected from diverse marine sources, including sediments, seawater, invertebrates and vertebrates, and they are cultivated under different growth conditions and extracted to build up an extract library for bioactivity screening. Marbio has been successful in receiving support from both regional, national and EU programs. Our research interest and publications within marine biodiscovery has made us an attractive partner in EU applications and industry collaborations. We have achieved funding through the national infrastructure program for our NOR-openscreen consortia (www.openscreen.no). This grant allowed us to invest in new technologies and further enhance UiT's effort within marine bioprospecting and drug discovery.

Positions (2021) 2 professors, 2 assoc.prof., 2 researchers and 2 technicians. In addition, 2 Postdocs, 3 PhD and 3 Master students.

The group has published more than 60 scientific articles in the area of biodiscovery of natural products in the period 2011-2021.
In addition, we have submitted 8 Disclosure of Inventions (DOFIs), that UiT has accepted and taken further/over the rights:

 Names of the key researchers and what positions they held at the administrative unit at the time of the research (where researchers joined or left the administrative unit during this time, these dates must also be stated).

Jeanette Hammer Andersen, head of Marbio since 2004, professor 2016.
Espen Holst Hansen, researcher (2004-2017)- professor (2017)
Kine Østnes Hansen – PhD student (2011-2014) (2015-2018 postdoc), Assoc. prof. (2018-) researcher
Teppo Rämä, postdoc (2015-2018), researcher (2018-2019), tenure track Assoc. prof. (2019-)

3. References to the research (indicative maximum of six references)

Hanssen, K. O., Schuler, B., Williams, A. J., Demissie, T. B., Hansen, E., Andersen, J. H., Svenson, J., Blinov, K., Repisky, M., Mohn, F., Meyer, G., Svendsen, J. S., Ruud, K., Elyashberg, M., Gross, L., Jaspars, M., & Isaksson, J. (2012). A combined atomic force microscopy and computational approach for the structural elucidation of Breitfussin A and B: highly modified halogenated dipeptides from *Thuiaria breitfussi*. *Angewandte Chemie-International Edition, 51*(49), 12238-12241. https://doi.org/10.1002/anie.201203960

Hansen, K. O., Isaksson, J., Bayer, A., Johansen, J. A., Andersen, J. H., & Hansen, E. (2017). Securamine Derivatives from the Arctic Bryozoan *Securiflustra securifrons*. *Journal of Natural Products, 80*(12), 3277-3284. https://doi.org/10.1021/acs.jnatprod.7b00703

Olsen, E. K., Soderholm, K. L., Isaksson, J., Andersen, J. H., & Hansen, E. (2016). Metabolomic profiling reveals the N-acyl-taurine Geodiataurine in extracts from the marine sponge *Geodia macandrewii* (Bowerbank). *Journal of Natural Products, 79*(5), 1285-1291. https://doi.org/10.1021/acs.jnatprod.5b00966

Hansen, K. O., Andersen, J. H., Bayer, A., Pandey, S. K., Lorentzen, M., Jorgensen, K. B., Sydnes, M. O., Guttormsen, Y., Baumann, M., Koch, U., Klebl, B., Eickhoff, J., Haug, B. E., Isaksson, J., & Hansen, E. H. (2019). Kinase Chemodiversity from the Arctic: The Breitfussins. *Journal of Medicinal Chemistry, 62*(22), 10167-10181. https://doi.org/10.1021/acs.jmedchem.9b01006

Hagestad, O. C., Hou, L. W., Andersen, J. H., Hansen, E. H., Altermark, B., Li, C., Kuhnert, E., Cox, R. J., Crous, P. W., Spatafora, J. W., Lail, K., Amirebrahimi, M., Lipzen, A., Pangilinan, J., Andreopoulos, W., Hayes, R. D., Ng, V., Grigoriev, I. V., Jackson, S. A., Sutton, T. D. S., Dobson, A. D. W., & Rama, T. (2021). Genomic characterization of three marine fungi, including Emericellopsis atlantica sp. nov. with signatures of a generalist lifestyle and marine biomass degradation. *Ima Fungus, 12*(1), 23. https://doi.org/10.1186/s43008-021-00072-0

Jenssen, M., Rainsford, P., Juskewitz, E., Andersen, J. H., Hansen, E. H., Isaksson, J., Rama, T., & Hansen, K. O. (2021). Lulworthinone, a New Dimeric Naphthopyrone From a Marine Fungus in the Family Lulworthiaceae With Antibacterial Activity Against Clinical Methicillin-Resistant Staphylococcus aureus Isolates. *Frontiers in Microbiology, 12*, 14. https://doi.org/10.3389/fmicb.2021.730740

4. Details of the impact (indicative maximum 750 words)

Marbio has expertise in and infrastructure for 1) bioactivity testing for a wide range of indications, 2) isolation and chemical characterization of the bioactive compounds, and 3) isolation and cultivation of microorganisms (bacteria and fungi in particular). In screening of extract libraries of natural products, including both terrestrial and marine micro- and macro-organisms, Marbio has successfully characterized several novel bioactive compounds.
The search for bioactive compounds from marine resources is a priority area at UiT, and it is a key activity for CANS – Centre for Antibacterial Strategies, a centre for research, education, innovation and dissemination related to antimicrobial resistance (AMR) at UiT. Marbio also has the capacity to

assess the drug-like properties of the bioactive compounds by testing them for their ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) properties. The platform has been used for characterization and preclinical development for bioactive compounds within the research group. We have also been extensively used by other research groups at UiT as well as other Norwegian Universities for bioactivity and chemical profiling of different products (compounds, extracts and fractions). We have also performed tasks for industrial parties in Norway and internationally on a commercial basis.

In one of our internal projects, we have discovered a family of novel natural products from a sessile, bottom-living Arctic marine animal, and these compounds were found to inhibit the survival of selected cancer cell lines. The scaffold of the natural products we isolated from the marine animal was used as a starting point for production of synthetic analogues. We developed a new set of kinase inhibitors that are more potent and selective, but less toxic compared to the original natural products. We can now target just one specific type of blood cancer by inhibiting a specific mutated variant of a kinase, which is the cause of cancer in ~30% of these patients. This project did the initial optimization of the efficacy and pharmacokinetic properties and could successfully nominate a lead compound for the next development phase. The project has been funded through two RCN projects ('KinSea', optimization project 2015-2017,'TackAML', commercialisation project 2019-2022) and a project funded by the regional biotechnology fund MABIT (MarCan, 2018). A start-up company is established to attract investors to develop this project into a preclinical development candidate. This project has significantly increased the competence in Marbio and UiT within the field of preclinical drug development, and the establishment of a local company (partially owned by UiT) will serve as a tool to incubate other projects emerging from the marine bioprospecting effort at UiT.

As an example of our work with industrial partners from abroad, we would like to mention the development of a new painkiller by the Portuguese company Sea4Us. Through the Ocean Medicine (2015-2019) RISE-MSCA program Horizon2020, researchers from Marbio co-invented with Sea4Us an analgesic compound. A joint project funded from EEA to further develop the painkiller is ongoing (2019-): Bluebiotech4Pain project: Biotechnology from marine bacteria for the treatment of chronic pain. Marbio is responsible for performing toxicity and biotransformation assays with the identified analgesic molecule (and its analogues) and for identifying alternative bioactivities of the candidates.

5. Sources to corroborate the impact (indicative maximum of ten references)

KinSea – Potent and selective protein kinase inhibitors from the sea (2015-2017). Optimisation project funded by the Research Council of Norway, project No 244264. Total budget 11 mill NOK

MarCan – A marine derived compound against cancer (2018). Commercialisation project funded by MABIT, Project No 143. Total budget 1.3 mill NOK.

TackAML – tackling drug resistant acute myeloid leukameia with the next-generation FLT3 inhibitor funded by the Research council of Norway, project no.310097. Total budget 10.3 mill NOK

BlueBiotech4Pain- Sea4Us - Biotecnologia e Recursos Marinhos, Lda to European Economic Area Financial Mechanism. EEA, [Pedido de Pagamento N.º(101721254)].

[BFE] [Impact case no. 3]

Institution: UiT the Arctic University of Norway

Administrative unit: Faculty of Biosciences, Fisheries and Economics (BFE)

Title of case study: Arctic Seasonal Ice Zone Ecology

Period when the underpinning research was undertaken: 2003-2021

Period when staff involved in the underpinning research were employed by the submitting institution:
Entire period: Profs. Paul Wassmann, Marit Reigstad, Raul Primicerio, Michaela Aschan, Bjørn Gulliksen (professor emeritus since 2014)
From 2012: Prof. Jørgen Berge, from 2014: Prof. Bodil Bluhm, Researcher Malin Daase
From 2016: Prof. Rolf Gradinger, Haakon Hop (Prof. II)
Susanne Kortsch (PhD student, 2012-2016), Amalia Keck Al-Hababeh (MSci (2016-2018), and PhD student (2021-))

Period when the impact occurred: 2011-2021

1. Summary of the impact

Sea ice biota and lower trophic level ecosystem knowledge from the seasonal ice zone (SIZ) region, including the ocean surrounding Svalbard, provided important contributions for the Norwegian Ecosystem-based Management plan for the Barents Sea 2010, its update in 2015 and 2021, and underlying scientific reports like the ones on 'Very valuable and vulnerable areas' (Særlig verdifulle og sårbare områder) (2018, 2019 and 2021). The BFE-generated knowledge on both past and, climate change-mediated, present ecosystems enhance the understanding of the current status as well as enable planning for the future. Specifically the impact documents included BFE knowledge on biodiversity and functioning of ecosystem components in ice-covered areas in guiding management of the distinctly different ice-covered areas. Also included are time series results on borealization of fish and benthic communities, shifts in ice algal communities related to sea ice change, and related changes in food webs in the SIZ. BFE biodiversity research was used in the Arctic Council working group Conservation of Arctic Flora and Fauna (CAFF) for their pan-Arctic State of the Arctic Marine Biodiversity Report (2017). The emerging polar night ecology field initiated by BFE researchers is reflected in the Arctic Council Snow, Water, Ice and Parmafrost in the Arctic (SWIPA, 2017) report.

2. Underpinning research

BFE has generated research insights related to the impact in the following theme block.
BFE research provided critical knowledge regarding the varying roles of Arctic sea ice for biological production, diversity and life cycles, needed for ecosystem based management: Bluhm et al. (2017 a,b), Gradinger (2020), and Hop et al. (2020) summarized biodiversity of ice fauna and algae (and other protists) documenting that over 1000 species of eukaryotes inhabit the sea ice itself. Gradinger and Bluhm (2020), analyzing food web and carbon partitioning inside sea ice, showed that both algal or faunal components contribute substantially to ice-derived carbon at varying times of the ice-covered period, yet that fauna consume little of ice algal production, explaining why a large fraction remains available or the pelagic and benthic food webs below. For example, the dominant Arctic copepod *Calanus glacialis* uses the under-ice habitat during part of its life cycle and derives energy from ice-generated production on a pan-Arctic scale (Daase et al. 2013). Berge et al. (2012), in contrast, suggested that the common ice-associated amphipod, *Apherusa glacialis,* may be an example how to use a pelagic phase as a strategy to reduce export from the Arctic via sea ice exported through Fram Strait (later supported by two other BFE-authored articles).

BFE research based on time series in the SIZ and adjacent boreal zone showed that, under climate change, macroalgal cover increased abruptly around the year 2000, facilitating biodiversity change in Arctic fjord coastal zones. The same study stresses biological vulnerability of Arctic coasts by showing that complete community recovery after (mechanical in this case) disturbance can take 1-2 decades (Al-Hababeh et al. 2020). Changes in ice-algal communities over 40 years were detected in pack ice regions, likely related to the shift from

multiyear to first-year sea ice (Hop et al. 2020). For the Barents Sea shelf, Kortsch et al. (2015; 330 citations) demonstrated that increase in boreal food web components (e.g., Atlantic cod) in traditionally Arctic waters shifted food web structure towards more connected food webs that are thought to perpetuate change or disturbance faster than the Arctic, less linked, food web.

BFE research combining physical-biological coupled numerical models with field-based system approaches in the SIZ provided estimates of interannual as well as regional variability of primary production. It further documents the partitioning of fate of primary production between pelagic and benthic consumers and carbon-sequestration in the Svalbard and Barents Sea open water and SIZ (Reigstad et al. 2011). Conceptual models revealed how the seasonal dynamics would change with climate warming and longer productive seasons and how the projected future productivity would increase pelagic consumption and change the pelagic-benthic coupling across the Arctic seasonal ice zone (Wassmann and Reigstad, 2011; cited >300 times).

BFE research has initiated a new research field "polar night ecology" mediated through technological advances and development. Collaborative research detected zooplankton diurnal migration (in response to moonlight), feeding and reproductive activities where hibernation was assumed to be the rule (Berge et al. 2015). Impacts of artificial light during polar night was later also detected.

In summary, the body of work clearly documented that biodiversity and functioning of ecosystem components in ice-covered areas are distinctly different from those in open water areas. Climate change related shifts were detected and scenarios for future system status developed.

Results underpinning the impact were generated from a suite of BFE-led projects including UiT-BFE benthic time series (Gulliksen (1980-2013/ Bluhm 2014-, Norwegian Environment Agency for some period), Cabanera (RCN, 2003-2006), Arctic Tipping Point (EU, 2009-2012), Fram Center Flagship Fjord and Coast (2010-2020), ArcticSIZE (UiT/Tromsø Research Foundation, 2015-2021), Arctic ABC (RCN/ Tromsø Research Foundation, 2016-2025), BarEcoRe (RCN 2010-2013) among others. In addition, results were related group members' activities in the Circumpolar Biodiversity Monitoring Program under the Arctic Council's working group Conservation of Arctic Fauna and Flora (since 2012). Polar night ecology studies were driven by technology development that facilitated observations during the Polar night.

Key researchers and their positions during the relevant reporting time include:
Entire period: Profs. Paul Wassmann, Marit Reigstad, Raul Primicerio, Michaela Aschan, Bjørn Gulliksen (professor emeritus since 2014)
From 2012: Prof. Jørgen Berge, from 2014: Prof. Bodil Bluhm, Researcher Malin Daase
From 2016: Prof. Rolf Gradinger, Haakon Hop (Prof. II)
Susanne Kortsch (PhD student, 2012-2016), Amalia Keck Al-Hababeh (MSci (2016-2018), PhD student (2021-))

Relevant contextual information about the SIZ research includes that BFE has developed strong expertise in conducting successful and safe research in sea ice-covered areas using ice strengthened vessels including the UiT-owned RV Helmer Hanssen, including training personnel for working on sea ice. Investment into technology and modelling infrastructure have been invaluable for BFE SIZ research. Time series data sets is key to climate-change related work, e.g., the photograph-based benthic time series from Svalbard- and North Norwegian fjords since 1980.

3. References to the research (indicative maximum of six references)

1. **Al-Habahbeh, A.K**., Kortsch, S., **Bluhm, B.A**., Beuchel, F., **Gulliksen, B**., Ballantine, C., Cristini, D., **Primicerio, R.,** 2020. Arctic coastal benthos long-term responses to perturbations under climate warming. Philosophical Transactions of the Royal Society A, 378(2181). s. 20190355. https://doi.org/10.1098/rsta.2019.0355

2. **Berge J.**, Varpe Ø., Moline, M.A., Wold, A., Renaud, P.E., **Daase, M**., Falk-Petersen, S. 2012. Retention of ice-associated amphipods: possible consequences for an ice-free Arctic Ocean. Biology Letters 8, 1012-1015. https://doi.org/10.1098/rsbl.2012.0517.
3. **Berge, J.**, Renaud. P.E., Darnis, G., Cottier, F., Last, K., Gabrielsen, T.M., Johnsen, G., **L. Seuthe**, J.M. Weslawski, E. Leu, M. Moline, **J. Nahrgang**, J.E. Søreide, Ø. Varpe, Lønne, O.L., **Daase, M.,** Falk-Petersen, S. 2015a. In the dark: A review of ecosystem processes during the Arctic polar night. Progress in Oceanography, 139:258-271. https://doi.org/10.1016/j.pocean.2015.08.005
4. **Bluhm, B.A**., Swadling, K., **Gradinger, R**. 2017a. Chapter 16: Sea ice as habitat for macrograzersThomas D.N. (ed.). Sea ice 3rd edition, Wiley Blackwell. https://doi.org/10.1002/9781118778371.ch16
5. **Daase, M**., Falk-Petersen, S., Varpe, O., Darnis, G., Soreide, J.E., Wold, A., Leu, E., **Berge J**., Philippe, B., Fortier, L. 2013. Timing of reproductive events in the marine copepod *Calanus glacialis:* a pan-Arctic perspective. Canadian Journal of Fisheries and Aquatic Sciences, 70, s. 871-884. DOI: 10.1139/cjfas-2012-0401
6. **Gradinger, R**. 2020. Life in Arctic Sea Ice. In: Goldstein M.I., DellaSala, D.A. (ed.), Encyclopedia of the World's Biomes, vol. 2. Elsevier, s. 507–514. https://doi.org/10.1016/B978-0-12-409548-9.12431-5
7. **Gradinger, R., Bluhm, B.A**. 2020. First Arctic sea ice meiofauna food web analysis based on abundance, biomass and stable isotope ratios of sea ice metazoan fauna from near-shore Arctic fast ice. Marine Ecology Progress Series 634:29-43 https://doi.org/10.3354/meps13170
8. **Hop, H.,** Vihtakari, M., **Bluhm, B.A**., Assmy, P., Poulin, M., **Gradinger, R**., Peeken, I., von Quillfeldt, C.H., Olsen, L.M., Zhitina, L., Melnikov, I.A. 2020. Changes in sea-ice protist diversity with declining sea ice in the Arctic Ocean from the 1980s to 2010s. Frontiers Marine Science, https://doi.org/10.3389/fmars.2020.00243
9. **Kortsch, S., Primicerio, R**., Fossheim, M., Dolgov, A., **Aschan, M**. 2015. Climate change alters the structure of arctic marine food webs due to poleward shifts of boreal generalists. Proceedings of the Royal Society of London B: Biological Sciences 282(1814): 2015.1546. Ecography 41: 1–14, 2018 doi: 10.1111/ecog.03443
10. **Reigstad M.,** Carroll J., Slagstad, D., Ellingsen, I., **Wassmann, P**. 2011. Intra-regional comparison of productivity, carbon flux and ecosystem composition within the northern Barents Sea. Progress in Oceanography 90(1-4), 33-46. https://doi.org/10.1016/j.pocean.2011.02.005
11. **Wassmann, P., Reigstad, M.** 2011. Future Arctic Ocean seasonal ice zones and implications for pelagic-benthic coupling. Oceanography 24, 220-231. https://doi.org/10.5670/oceanog.2011.74

4. Details of the impact (please see reccomdation letters at the end of section 5.)
Combined field observations from several research projects over the past decades within the seasonal ice zone (SIZ) described and quantified lower trophic levels (sea ice biota, phytoplankton, zooplankton, and benthos) in terms of their a) biodiversity and community composition, b) production, c) trophic interactions, d) contributions to vertical flux. Derived from that, the cited research generated knowledge on the coupling mechanisms between sea ice, the pelagic and benthic habitats. The field observations have been extended with numerical physical-biological coupled model-based studies upscaling results in time and space (in collaboration with SINTEF), and conceptual models illustrating functional relationships of seasonal dynamics in productivity and carbon flow and future scenarios for the Seasonal Ice Zone. This underpinnning research provides considerable contributions to basic description of the themes sea ice biota, productivity and carbon flow in ecosystems in the SIZ, and food web structures. The impact, measured as citations in referenced material (SVO report section 5), is strongest in the areas of sea ice biota and plankton compartments as well as primary production and regulation.

Technological developments facilitated detection and exploration of biological activities during the Polar night and monitoring of biological activities under the drifting sea ice. Arctic marine

winter ecology has based on this emerged as a more sensitive period than assumed representing a knowledge gap in international Arctic marine synthesis reports, like the Arctic Council initiated SWIPA report (chapter 5). A stronger impact to future management is expected.

The BFE research in the seasonal ice zone provides management-relevant information on role of sea ice for ice-associated and planktonic species and functional groups in the seasonal ice zone via habitat and food sources (and their availability), life cycles (e.g., life stages using sea ice), and unique or enriching biodiversity (e.g., ice-typical or endemic species). The information was disseminated in form of scientific publications, data sets, scientific and public presentations, and through networks and outputs of the CBMP of the Arctic Council. From this information, classifications of sensitivity of these ecosystem components in the seasonal ice zone were done based on criteria established for 'Ecologically and Biologically Significant marine areas' (EBSAs) for Norwegian ice-covered waters (Barents Sea including Svalbard) in the SVO reports. BFE also lead the Arctos research network (https://arctos.uit.no) including management institutions on national level like the Norwegian Polar Institute and the Institute of Marine Research that has facilitatated knowledge transfer.

The referenced work included collaborations with SINTEF researchers who maintain the SINMOD model, where BFE (Wassmann, Reigstad) has contributed to extend and develop the ecosystem model with relevant processes, functional groups, research questions and data to evaluate model performance. For time series resulting from the Institute of Marine Research management surveys, BFE has provided expertise on multivariate statistics (Primicerio) and educational research capacity (Kortsch) to increase the scientific utilisation and outcome of the existing data. The polar night ecology discoveries (Berge, Daase) have emerged in close collaboration with the technology community at NTNU (Trondheim), UNIS ecologists (Svalbard), and the oceanography and technology group at Scottish Association of Marine Science (SAMS). SVO reports were largely written by IMR and NPI staff; BFE-researchers contributed to writing of the SVO 2021 report (Bluhm, Gradinger, Hop) via a contract with the Norwegian Environmental Agency. Biodiversity knowledge and syntheses fed into and were done under the State of the Arctic Marine Biodiversity Report (2017) that is compiled by Arctic Council member state representatives (of whom a BFE researcher, Bluhm, is one) and disseminated via the Conservation of Arctic Fauna and Flora office and website.

The beneficiary of the SIZ information generated by BFE and collaborators is eventually the Norwegian government with its ministries and agencies (such as the Norwegian Environmental Agency) that make decisions on what kind of activities may be conducted in 'Very valuable and vulnerable areas'. In addition, the primary advice-giving institutions covering ice-covered seas in Norway (Norwegian Polar Institute, Institute of Marine Research) have hired a substantial number of BFE-educated researchers that have contributed to producing management-relevant information and reports that underlie management plans of marine areas. The Norwegian Polar Institute and the Institute of Marine Research currently have over a dozen and over fifty employees, respectively, that received their Master of Science or PhD education at BFE institutes or their pre-cursors, rendering BFE a substantial impact towards management.

Norwegian knowledge and ecosystem based management of resources and activities in marine regions is based upon Management plans for larger marine regions. The region comprise large ecosystems that needs to be managed as one region due to migrations of fish, marine mammals as well as the connectivity within the region caused by advection by the North Atlantic Current. Activities like fisheries, petroleum activities, maritime traffic, and potential future activities like ocean wind installations is managed to minimize the impact and risk of harm to communities, habitats or ecosystems. The management plan is evidence based, and ecosystem knowledge is part of that evidence. The impact of the research from BFE lies in providing complementary knowledge to the management institutions on lower trophic level ecosystem components, productivity and multivariate analysis of ecosystem data to a) value the sea ice zone as a habitat and part of a larger ecosystem to evaluate consequence of a changing climate, and b) ensure science based decisions on what activities

can be carried out in the seasonal ice zone today and with a reduced sea ice extent, and c) to guide candidate regions for future potential Marine Protected Areas.

As evidence of impact extent, SVO reports, and BFE-research in seasonal ice zones within them, provide a basis to the states' committments to the UN Biodiversity Conference COP15 agreement, held in December 2022 in Montreal, to protect 30% of land and water areas. Identifying areas for protection requires a set of standardized indicators that in part are already applied in selection of SVO, EBSAs etc. The impact has primarily occurred via the SVO reports in 2018, 2019 and 2021 to contribute the thematic basis for the management plans of Norwegian marine areas.

5. Sources to corroborate the impact

1. AMAP, 2017. Snow, Water, Ice and Permafrost in the Arctic (SWIPA) 2017. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xiv + 269 pp https://www.amap.no/documents/doc/snow-water-ice-and-permafrost-in-the-arctic-swipa-2017/1610
2. **Bluhm, B.A.,** Hop, H., Melnikov, I.A., Poulin, M., Vihtakari, M., Collins, E. **Gradinger, R**., Juul-Pedersen, T., von Quillfeldt, C. 2017b. Sea-ice biota. S. 33-62 in CAFF State of the Arctic Marine Biodiversity Report. Conservation of Arctic Flora and Fauna International Secretariat, Akureyri, Iceland. 978-9935-431-63-9.
3. CAFF. 2017. State of the Arctic Marine Biodiversity: Key Findings and Advice for Monitoring. Conservation of Arctic Flora and Fauna International Secretariat, Akureyri, Iceland. ISBN: 978-9935-431-62-2 https://www.caff.is/assessment-series/431-state-of-the-arctic-marine-biodiversity-report-full-report
4. Eriksen, E., van der Meeren, G.I., Nilsen, B.M., von Quillfeldt, C., Johnsen, H. 2021 with contributors. Særlig verdifulle og sårbare områder (SVO) i norske havområder - Miljøverdi | Havforskningsinstituttet (hi.no) Rapport fra havforkningen 2021-26, ISSN 1893-4536 (Very valuable and vulnerable areas – environmental value)
5. Faglig forum for norske havområder. 2019. Særlig verdifulle og sårbare områder - Faggrunnlag for revisjon og oppdatering av forvaltningsplanene for norske havområder M-1303 2019, 304 s. (Very valuable and vulnerable areas. Basis for revision and update of management plan for Norwegian marine areas) https://www.miljodirektoratet.no/globalassets/publikasjoner/m1303/m1303.pdf

Vår dato / Vår referanse
Deres dato / Deres referanse

Side 1 av 1

EVALBIOVIT
The Research Council of Norway
Postboks 564
1327 Lysaker

Tromsø, 12.01.2023

EVALBIOVIT evaluation panel

I am writing this letter in support of my colleagues in the marine biological and fisheries sciences at the Faculty of Biosciences, Fisheries and Economics (BFE) at UiT – The Arctic University of Norway. I am the leader of the Barents Sea and Arctic Ocean program at the Institute of Marine Science and Head of Office in Tromsø, and have known, worked with, and published with several colleagues at the BFE faculty.

The research several BFE faculty members conduct in the partly seasonally ice-covered Barents Sea and Svalbard region is comprehensive, rigorous, and insightful, and contains useful contributions to management planning of those areas. The seasonal ice zone contains areas that are important to several branches of Norway's economy. BFE research results from the seasonal ice zone, within and beyond the Norwegian exclusive economic zone, is also relevant to a suite of national obligations and international agreements, and documents developed by various working groups (such as those in ICES and the Arctic Council) that Norway is part of. Most recently, IMR has been referencing BFE results on various ecosystem components from zooplankton to sea ice biota, benthos, and fish in the 2021 report on 'Very valuable and vulnerable areas in Norwegian marine areas – environmental value' (Eriksen et al. 2021).

In addition, I point out that a noteworthy number of IMR researchers in our Tromsø and Bergen staff combined have received their training in marine ecology and fisheries related topics at the BFE faculty and its pre-cursors. Vice versa, several of our staff contribute to teaching as well as Master and PhD education at the BFE faculty.

We look forward to continuing our fruitful collaborations with BFE colleagues.

Sincerely

Maria Fossheim
Programme Director
Barents Sea and Arctic Ocean
Institute of Marine Research

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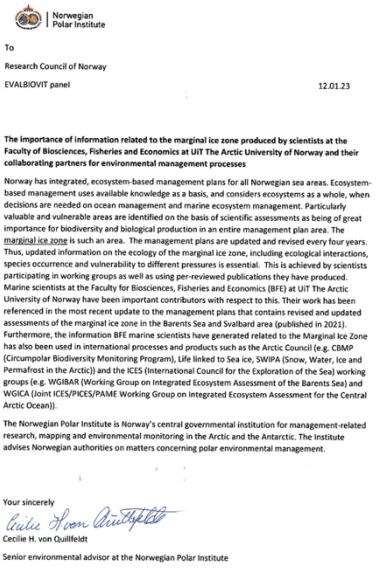

Norwegian Polar Institute

To
Research Council of Norway
EVALBIOVIT panel

12.01.23

The importance of information related to the marginal ice zone produced by scientists at the Faculty of Biosciences, Fisheries and Economics at UiT The Arctic University of Norway and their collaborating partners for environmental management processes

Norway has integrated, ecosystem-based management plans for all Norwegian sea areas. Ecosystem-based management uses available knowledge as a basis, and considers ecosystems as a whole, when decisions are needed on ocean management and marine ecosystem management. Particularly valuable and vulnerable areas are identified on the basis of scientific assessments as being of great importance for biodiversity and biological production in an entire management plan area. The marginal ice zone is such an area. The management plans are updated and revised every four years. Thus, updated information on the ecology of the marginal ice zone, including ecological interactions, species occurrence and vulnerability to different pressures is essential. This is achieved by scientists participating in working groups as well as using per-reviewed publications they have produced. Marine scientists at the Faculty for Biosciences, Fisheries and Economics (BFE) at UiT The Arctic University of Norway have been important contributors with respect to this. Their work has been referenced in the most recent update to the management plans that contains revised and updated assessments of the marginal ice zone in the Barents Sea and Svalbard area (published in 2021). Furthermore, the information BFE marine scientists have generated related to the Marginal Ice Zone has also been used in international processes and products such as the Arctic Council (e.g. CBMP (Circumpolar Biodiversity Monitoring Program), Life linked to Sea ice, SWIPA (Snow, Water, Ice and Permafrost in the Arctic)) and the ICES (International Council for the Exploration of the Sea) working groups (e.g. WGIBAR (Working Group on Integrated Ecosystem Assessment of the Barents Sea) and WGICA (Joint ICES/PICES/PAME Working Group on Integrated Ecosystem Assessment for the Central Arctic Ocean)).

The Norwegian Polar Institute is Norway's central governmental institution for management-related research, mapping and environmental monitoring in the Arctic and the Antarctic. The Institute advises Norwegian authorities on matters concerning polar environmental management.

Your sincerely

Cecilie H. von Quillfeldt

Senior environmental advisor at the Norwegian Polar Institute

[BFE] [Case no. 4]

Institution: UiT – the Arctic University of Norway		
Administrative unit: Faculty of Biosciences, Fisheries and Economics (BFE)		
Title of case study: COAT (**C**limate-ecological **O**bservatory for **A**rctic **T**undra)		
Period when the underpinning research was undertaken: 2011-2021		
Period when staff involved research were employed by the submitting institution:		
Names:	**Position:**	**Period:**
Rolf A. Ims	Professor	2011-2021
Nigel G. Yoccoz	Professor	2011-2021
John-André Henden	Researcher	2011-2021
Ole Petter L. Vindstad	Researcher	2016-2021
Dorothee Ehrich	Researcher	2011-2021
Eeva M. Soininen	Researcher	2015-2021
Jarad P. Mellard	Associate professor	2019-2021
Audun Stien	Professor	2020-2021
Malin Ek	PhD student	2016-2021
Eivind F. Kleiven	PhD student	2017-2021
Marita A. Strømeng	PhD student	2017-2021
Hanna Böhner	Research technician	2020-2021
Kari Anne Bråthen	Professor	2011-2021
Period when the impact occurred: 2016-2021		

1. Summary of the impact (indicative maximum 100 words)

Climate-ecological **O**bservatory for **A**rctic **T**undra (COAT) is a comprehensive, long-term ecological research program led by the submitting institution. COAT documents ecosystem state changes resulting from rapid climate change in the Norwegian Arctic with the ultimate aim to aid the society to act adequately. COAT's impacts are due to (1) hosting a national research infrastructure that generates open access ecosystem data, (2) providing frameworks for ecosystem state assessments and management strategies, and (3) conducting research that underpins and actively includes management interventions in collaboration with stakeholders to safeguard biodiversity and highly valued ecosystem services in the Norwegian Arctic.

2. Underpinning research (indicative maximum 500 words)

In context of the establishment of the High North Research Centre for Climate and the Environment (Fram Centre; https://framsenteret.no/) in 2011, the submitting unit (hereafter SU) was tasked by the Norwegian government to plan a long-term research program providing science-based underpinning for the society's ability to anticipate and act on the impacts of climate change on the nation's Arctic terrestrial ecosystems. A task force led by professor Ims, consisting of ecologists (including 12 SU members) and climatologists from the Fram Centre, developed the COAT Science Plan that was published in 2013 and summarized in [**R1**]. The science plan consists of innovative protocols that integrate **ecosystem-based monitoring**, **model-based attribution and forecasting** of climate change impacts on ecosystem functions (services) and structure (biodiversity) and provides a **framework for ecosystem state assessments** and **implementation of management/mitigation measures**. Following the completion of the science plan, COAT was included in RCN's "Norwegian Roadmap for Research Infrastructure" in 2016. Subsequently, substantial funding and effort have been devoted to develop and establish COAT Infrastructure (e.g. field logistics, instrument systems and open access data portal) to be finalized in 2023. Although the full capacity for COAT to accomplish its long-term ambitions thus lies in the future, the program has already produced research that exemplify its ultimate impact. The published research described below (**[R1]**-**[R6]**) have been led by members of the SU. More information about all aspects of the program is available from COAT's web pages; https://www.coat.no/en/

Attribution and forecasting are paramount for the society's ability to act adequately on climate change impacts. [**R2]** exemplifies how COAT's ecosystem-based monitoring yields data to parametrize models that serve the dual purpose of identifying drivers of ongoing change (attribution) and making forecasts. In this case, the relative (and potentially interacting) impacts of harvesting and other drivers are assessed to aid management of a highly valued game species. **[R3]** exemplifies how quasi-experimental study designs can be used to attribute dwindling Arctic biodiversity to manageable drivers, in this case generalist predators that are increasing in a warmer climate and impact ground-breeding tundra birds.

Impacts of ecological research is particularly evident when forming the basis for **ecosystem state assessments** and **management/mitigation actions**. COAT's overall approach [cf. **R1**] has formed a framework for ecosystem state assessments and management strategies in context of ecosystems subjected to rapid climate change (detailed in Section 4 below). Regarding mitigation actions, **[R4]** reports a food web-based analysis of a large-scale predator control action that was able to halt the decline of a red-listed game species (cf. **[R3]** for research underpinning this management action). Climate warming-induced outbreaks of tree- and shrub-defoliating insects (previously documented by COAT-related research; cf. https://www.coat.no/en/Tundra-forest-ecotone) is arguably one of the most large-scale and pervasive ecosystem impact of climate warming in Norway. [**R5]** assesses whether salvage logging of severely damaged trees can induce more rapid regeneration of forest stands and thus be a potential mitigation measure. This research shows that the efficacy of the mitigation action is conditional on other manageable drivers such as the abundance of reindeer.

Finally, **[R6]** targets the population dynamics of an invasive rodent species in high-Arctic Svalbard, employing statistical analysis of time series data to identify the roles of intrinsic regulation (density dependence) and climatic forcing, and mathematical modelling to investigate how climate warming is expected to affect the frequency and amplitude of population outbreaks. This study (funded by the Svalbard Environmental Protection Fund) is management relevant, because the rodents are host for the dangerous zoonotic parasite *Echinococcus multilocularis* (EM).

3. References to the research (indicative maximum of six references)
Authors in **bold letters** are/were members, guest researchers or students of the submitting unit.

[R1]: Ims, R.A. and **Yoccoz**, N.G. Ecosystem-based monitoring in the age of rapid climate change and new technologies. 2017. *Current Opinions in Sustainability Science* 29: 170-176. https://doi.org/10.1016/j.cosust.2018.01.003

[R2]: **Henden**, J.A., **Ims**, R.A., **Yoccoz**, N.G., Asbjørnsen, E.J., **Stien**, A., **Mellard**, J.P., Tveraa, T., **Marolla**, F. & Jepsen, J.U. End-user involvement to improve predictions and management of populations with complex dynamics and multiple drivers. 2020 *Ecological Applications,* 30**,** e02120. https://doi.org/10.1002/eap.2120

[R3]: Ims, R.A., **Henden**, J.A., **Thingne**s, A.V., **Garmo**, M.J., **Strømeng**, M.A. & Jepsen, J.U. Greening Arctic and bird nest predation risk across tundra ecotones. 2019. *Nature Climate Change* 9: 607-610. https://doi.org/10.1038/s41558-019-0514-9

[R4]: **Henden**, J.A., **Ehrich**, D., **Soininen**, E. & **Ims**, R.A. Accounting for food web dynamics when assessing the impact of mesopredator control on declining prey populations. 2021. *Journal of Applied Ecology* 58:104–113. https://doi.org/10.1111/1365-2664.13793

[R5]: **Vindstad**, O.P.L., Jepsen, J.U., **Klinghardt**, M., **Ek**, M. and **Ims**, R.A. Salvage logging of mountain birch after geometrid outbreaks: Ecological context determines management outcome. 2017. *Forest Ecology and Management* 405: 619-627. https://doi.org/10.1016/j.foreco.2017.09.027

[R6] Fauteux, D., **Stien**, A., **Yoccoz**, N.G., Fuglei, E. & **Ims**, R.A. 2021. Climate and density-dependent population dynamics: Lessons from a simple high-Arctic ecosystem. PNAS 118, https://doi.org/10.1073/pnas.2106635118

4. Details of the impact (indicative maximum 750 words)

Context of fundamental impact
Generation and analysis of ecosystem-based monitoring data are fundamental for assessing the state of ecosystems **[R1]**. Further, assessing ecosystem state, and identifying eventual state changes and their drivers, are necessary for devising mitigating actions and informing the public. These demands are set out both in Norway's Constitution (section 112) and Biodiversity Act (sections 4 and 10). Commissioned to develop and lead COAT by the Norwegian Government **[S1]**, the submitting unit (SU) has contributed fundamentally to fulfil these legislative demands for Norway's terrestrial Arctic.

COAT Infrastructure
A condition for COAT to become included in the "Norwegian Roadmap for Research Infrastructure" in 2016 is that data generated from the COAT infrastructure are openly accessible **[S2].** Thus, COAT infrastructure will broadly benefit other researchers and stakeholders that are in need of environmental data from the Norwegian Arctic. Certain components of COAT's sensor systems are located based on the wishes of stakeholders. For instance, the positioning of COAT's sub-arctic weather stations in Finnmark is based on reindeer herders' expressed needs for information on snow pack properties on their winter pastures **[S3]**.

In "COAT Tools" (a project funded by UiT and Tromsø Research Foundation in 2016) SU members together with physicists, computer scientists and statisticians at UiT have developed new technologies/methods to advance ecosystem monitoring in environmentally harsh and remote Alpine/Arctic locations. While such innovations are primarily aimed to further develop COAT infrastructure, they also have wider applications. For instance, SU members have been tasked by the Norwegian Environment Agency (NEA) to develop a new monitoring program of Alpine small mammals based on novel technology developed in COAT Tools **[S4]**.

Ecosystem State Assessment
COAT has provided frameworks for governmentally mandated ecosystem state assessments. In 2017, an expert committee appointed by the Norwegian government identified COAT as the only terrestrial monitoring program in Norway that is fully ecosystem-based **[S5]**. Subsequently, COAT was in 2019 tasked by the Norwegian Environment Agency (NEA) - first to develop a technical protocol for a Panel-based Assessment of Ecosystem Condition (PAEC) **[S6],** next in 2020 - to apply PAEC based on COAT data for a comprehensive state assessment of Norway's Arctic terrestrial ecosystems **[S7]**, and finally in 2021 to evaluate how PAEC/COAT more generally can be used to set ecosystem-based management objectives **[S8]**. The latter report **[S8]** highlights the challenges of setting specific management objectives for ecosystems subjected to climate warming-forced transient dynamics with uncertain endpoints. Policy makers and management authorities (e.g. NEA) are advised to adopt a more open and adaptive management strategy regularly informed by ecosystem-based observatories like COAT that are able to identify trajectories of change, their underlying drivers and make assessments of the efficacy of potential management actions. Time will show how these commissioned inputs from COAT to Norwegian environmental authorities will impact their policies regarding coping with the climate crisis in the Arctic. COAT has also contributed to several circumpolar assessments hosted by the Arctic Council (cf. https://www.coat.no/en/Assessments)

Stakeholder-involved research and management/mitigation actions
COAT actively involves stakeholders in context of locally important ecosystem services/disservices. **[R2]** demonstrates how stakeholders (landowner, hunters and NGOs) contributed to building a population dynamical model of a highly valued game species that both

identified change drivers and provided forecasts prior to the hunting season. Such model forecasts - annually updated with monitoring data, have since 2021 informed the landowner's decisions regarding harvesting quotas. The value of such model forecasts has been corroborated both regionally and internationally **[S9]**.

[R4] and **[R5]** exemplify the impact of COAT in terms of implementing and assessing actions to mitigate climate change-induced threats to biodiversity and ecosystem services. The regional landowner (FeFo) and forest management authority were tightly involved in planning and executing **[R5]**. Based on **[R5]** and associated research COAT obtained in 2019 funding from RCN to continue this stakeholder-involved research (Project no.: 301922 – KLIMAFORSK, PI: Vindstad) - now aimed to develop a model that forecasts insect pest outbreaks - so that forest managers may act timely (e.g., by salvage logging).

Another action-oriented, stakeholder-involved COAT module **[S10]** was financed by the Svalbard Environmental Protection Fund in 2018. This funding allowed the COAT Tools technology for monitoring rodent populations (cf. **S[4]**) to be implemented in Svalbard with the aim of alerting the community about the risk of transmission of the zoonotic EM-parasite to humans. According to the underpinning COAT research **[R6],** the transmission risk can be expected to increase in warming climate. COAT collaborates with the Governor of Svalbard in engaging local people in a "community-based action" to eradicate the host of the parasite (the invasive rodent) near human settlements, whenever a rodent population outbreak is emerging **[S10]**.

General outreach/dissemination
COAT provides services to teachers and schoolchildren in northern Norway (through the module "COAT School"; cf. https://www.coat.no/en/Education/COAT-School). Overall COAT is annually mentioned in a large number of notices in media (n=76 in 2021; https://www.coat.no/en/Publications/COAT-in-media).

5. Sources to corroborate the impact (indicative maximum of ten references)

[S1]: COAT Science Plan commissioned by the Norwegian Government (Ministry of Research and Education) and published after peer review in the Fram Centre's report series. The mandate and the process of developing the science plan (including how local stakeholders were involved) is corroborated in the foreword of the report (page 5): https://www.coat.no/Portals/coat/SciencePlanCOAT_web.pdf?ver=2017-10-26-130928-570&timestamp=1517476755257

[S2]: Terms for access to COAT Infrastructure, including its data portal: https://www.coat.no/en/Research/Infrastructure

[S3]: Importance of COAT sensor stations in Varanger (Finnmark) for Sámi reindeer herding is corroborated by an article (in Norwegian) in the Sámi newspaper *Ságat*: https://paper.opoint.com/?id_site=202443&id_article=68910&code=138

[S4]: Plan (summary in English) for a monitoring system of Alpine small mammals commissioned by the Norwegian Environment Agency in 2021 (lead author is COAT Tools PhD student *E. F. Kleiven*): https://brage.nina.no/nina-xmlui/handle/11250/3026446 based on COAT Tools developed technology: https://cdnsciencepub.com/doi/full/10.1139/as-2021-0006

[S5]: A report of an expert committee commissioned by the Norwegian government to give recommendation on technical systems for assessing of ecosystem state. In that report COAT is highlighted as the only fully ecosystem-based terrestrial monitoring program in Norway (pages 9 [English summary] and 178): https://www.regjeringen.no/contentassets/7c4be071791f439b83fa035c03cdfc82/fagsystem-for-fastsetting-av-god-okologisk-tilstand_2017.pdf

This report paved the way for COAT to be commissioned by the Norwegian Environment Agency to first develop and thereafter apply Panel-based Assessment of Ecosystem (PAEC) (cf. **[R6]-S[8]**).

[S6]: PAEC technical protocol for assessing ecosystem state: https://brage.nina.no/nina-xmlui/handle/11250/2720073

[S7]: PAEC-based assessment of Norwegian Arctic terrestrial ecosystem: https://framsenteret.no/wp-content/uploads/2021/05/rapportserie-153-web.pdf

[S8]: Report recommending how PAEC and COAT can be applied to set ecosystem-based management goals: https://framsenteret.no/wp-content/uploads/2021/05/npi-kortrapport-056.pdf

[S9]: Newsletter from the European Commission's "Science for the Environment Section" corroborating the international importance of this case of stakeholder relevant/involved research: https://ec.europa.eu/newsroom/env/items/690605/.
and a newspaper article (in Norwegian) providing statements from regional stakeholders (landowners and hunters) about the impact of COAT model forecast for game population management: https://redir.opoint.com/?key=qbBE4EyWDsqrVeAHfAbT

[S10]: A Norwegian Broadcasting (NRK) e-paper (in Norwegian) documenting the community-based action against an invasive rodent and its zoonotic parasite in Svalbard: https://www.nrk.no/tromsogfinnmark/ber-om-hjelp-til-a-fange-smittefarlig-mus-1.14298795
and COAT news outlet (in English) about this action: https://www.coat.no/Portals/coat/FIles/CaseStories/CCS_Community-based%20actions%20against%20an%20invasive%20rodent%20and%20its%20zoonotic%20parasite%20in%20Longyearbyen.pdf

[BFE] [no. 5]

Institution: UiT The Arctic University of Norway
Administrative unit: Facultry of Biosciences, Fisheries and Economics (BFE)
Title of case study: Enhancing knowledge and establishing tools for quality and food safety of *Vaccinium* berries
Period when the underpinning research was undertaken: 2012 - 2021
Period when staff involved in the underpinning research were employed by the submitting institution: Prof Laura Jaakola 2012 - present Assoc. Prof Katja Karppinen 2015 - present Researcher Dr. Anna Avetisyan 2016 - 2021 PhD student Amos Samkumar 2017 - 2022
Period when the impact occurred: 2020 -

1. Summary of the impact (indicative maximum 100 words)

The Arctic berries research group has in collaboration with international and national partners produced new knowledge and tools for the use of berry industry, breeding programs and for the quality and safety assurance of wild and cultivated *Vaccinium* berries. The novel knowledge of the regulation of anthocyanins, the key health compounds in these berries, will impact on production of new berry cultivars, cultivation practises, and improve berries nutritional value. Our sequencing data, the first ever on genome of Arctic bilberry, together with developed methods for authentication, has impact on the quality control and food safety of these commercially important berry crops.

2. Underpinning research (indicative maximum 500 words)

Food security is a global concern, which according to United Nations (UN) is defined as that all people, at all times, have access to sufficient, safe and nutritious food. **The UN's sustainable development goal 2: Zero hunger** sets globally agreed targets to end hunger, to achieve food security and improved nutrition, and to promote sustainable agriculture. Berries are among the food ingredients with the highest levels of antioxidants and other bioactive compounds beneficial to human health. **The genus *Vaccinium*** encompasses economically, nutritionally, and traditionally important cultivated and wild berries including blueberries, cranberries, bilberries and lingonberries, which are among the most important berry crops world-wide.

The Arctic berries research group at BFE´s Climate laboratory https://en.uit.no/forskning/forskningsgrupper/sub?p_document_id=341073&sub_id=342049) has produced new knowledge and tools for the use of berry industry, breeding programs and for the quality and safety assurance of the wild and cultivated *Vaccinium* berries. The selected reference articles show production of:

1) Novel knowledge on the regulation of anthocyanin production in berries (**R1, R2**). Berry development is a complicated process, which requires coordination of the various signalling routes at the different developmental stages. Our main research focus during 2012 – 2021 has revealed new knowledge on the key transcription factors coordinating anthocyanin biosynthesis in *Vaccinium* berries. This knowledge is now available for the breeding programs targeting to improve anthocyanin content in berries.
2) Possibility to modify anthocyanin production with selected light spectrum wavelengths (**R3**). This PhD study performed in 2017 – 2021 revealed that with red and blue wavelengths, the anthocyanin content of ripening bilberries can be several folds higher compared to control conditions. Through the transcriptomics and metabolite analyses novel light signalling routes were indicated, which deepens understanding on the light regulation of berry ripening. The results are directly applicable for berry production, for instance in tunnels with the possibility to use LED-lightning or selected nets affecting the light spectrum.

3) New knowledge on the effects of the predicted future temperature changes on the composition of berry wax layer affecting the quality (**R4**). This PhD study was performed in 2018 - 2019 both in field conditions through a latitudinal gradient, and under controlled conditions at the UiT's Climate laboratory, which offers excellent facility for the climate-controlled experiments.
4) First published annotated genome for bilberry (*V. myrtillus*), representing Arctic ecotype from Tromsø, serving for developing further DNA-based analyses and tools for breeding programs. This data gathered 2018 – 2019 together with international partners has a **Biocultural (BC) notice**, which is a visible notification of accompanying cultural rights and responsibilities that need further attention for sharing and use of the data (**R5**).
5) Authenticity of products and raw materials is a fundamental part of food security. We have developed through Nordic collaborative project (2019 -2021) knowledge (**S8**) and methods for authenticity analysis of *Vaccinium* berries for industrial use (**R6**).

Besides the selected six recent articles (see below), the research group has since 2012 published 31 scientific peer-reviewed publications focusing on nutritional composition, regulation of biosynthesis of the key compounds, and aspects affecting the quality of *Vaccinium* berries.

Prof. Laura Jaakola	2012 - present
Assoc. Prof Katja Karppinen	2015 - present
Researcher Dr. Anna Avetisyan	2016 - 2021
PhD fellow Amos Samkumar	2017 - 2022

3. References to the research (indicative maximum of six references)

R1 Karppinen K, Lafferty DJ, Albert NW, Mikkola N, McGhie T, Allan AC, Afzal BM, Häggman H, Espley RV, and **Jaakola L.** (2021) MYBA and MYBPA transcription factors co-regulate anthocyanin biosynthesis in blue-coloured berries. New Phytologist, 232, 1350-1367. https://doi.org/10.1111/nph.17669

R2 Lafferty DJ, Espley RV, Deng C, Günther CS, Plunkett B, Turner J, **Jaakola L, Karppinen K**, Allan AC, Albert NW. (2022) Hierarchical regulation of *MYBPA1* by anthocyanin- and proanthocyanidin-related MYB proteins is conserved in *Vaccinium* species. Journal of Experimental Botany, 73, 1344-1356. https://doi.org/10.1093/jxb/erab460

R3 Samkumar A, Jones D, **Karppinen K**, Dare AP, Sipari N, Espley RV, Martinussen I, **Jaakola L.** (2021) Red and blue light treatments of ripening bilberry fruits reveal differences in signalling through abscisic acid-regulated anthocyanin biosynthesis. Plant, Cell & Environment, 44 (10), 3227-3245. https://doi.org/10.1111/pce.14158

R4 Trivedi P, Klavins L, Hykkerud AL, Kviesis J, Elferts D, Martinussen I, Klavins M, **Karppinen K**, Häggman H, **Jaakola L.** (2022) Temperature has a major effect on the cuticular wax composition of bilberry (*Vaccinium myrtillus* L.) fruit. Frontiers in Plant Science, 13: 980427 https://doi.org/10.3389/fpls.2022.980427

R5 Wu C, Deng C, Hilario E, Albert NW, Lafferty D, Grierson ERP, Plunkett BJ, Elborough C, Saei A, Günther CS, Ireland H, Yocca A, Edger PP, **Jaakola L, Karppinen K**, Grande A, Kylli R, Lehtola VP, Allan AC, Espley RV, Chagné D. (2022) A chromosome-scale assembly of the bilberry genome identifies a complex locus controlling berry anthocyanin composition. Molecular Ecology Resources, 22, 345-360. https://doi.org/10.1111/1755-0998.13467

R6 Karppinen K, Avetisyan A, Hykkerud AL, **Jaakola L.** (2022) A dPCR method for quantitative authentication of wild lingonberry (*Vaccinium vitis-idaea*) versus cultivated American cranberry (*V. macrocarpon*). Foods, 11 (10), 1476. https://doi.org/10.3390/foods11101476

4. Details of the impact (indicative maximum 750 words)

The Arctic Berries Research group has been working at BFE/AMB since 2012. The group has produced, in collaboration with various international and national partners, considerable new

knowledges on the nutritional properties of *Vaccinium* berries, on the biosynthesis and regulation of these compounds, and developed methods on discrimination of berry products and raw materials for authentication.

1) Impact on the berry quality and the resilience under changing climate conditions

Vaccinium species encompass economically, nutritionally, and traditionally important cultivated and wild berries including blueberries, cranberries, bilberries and lingonberries, which are among the most important berry crops world-wide. Several studies have shown various health benefits and bio-functional activities of these berries when consumed as food ingredients or as compounds fractioned for pharmaceutical products, especially due to high content of the coloured anthocyanins (**S1**). Therefore, there has been a demand to gain a deeper knowledge on the regulation of the bio-functional compounds during berry ripening (**R1, R2**) and as response to environmental factors, such as light and temperature conditions (**R3, R4**). By studying the effect of light spectrum on anthocyanin production in bilberry, we were able to indicate novel light signalling routes deepening the understanding of light regulation in berry ripening. These published results are directly applicable for berry production, for instance in tunnels with the possibility for LED-lightning or selected nets/filters affecting the light spectrum.

For the needs of the berry industry and the consumers, it is also important to create knowledge on how much the value compounds will affect the food quality, among the species, cultivars or even in same cultivar or ecotype growing under different environmental conditions (**S2, S3, S4**). This is especially important for the wild berries with bioeconomic importance (**S5**). Studying the effect of environmental factors on the berry quality and performance is also important for understanding the resilience of these species under changing climatic conditions (**S6**). These aspects have been studied in the BFE´s Climate laboratory by the Arctic berries research group's through PhD projects, and in the ongoing NordPlant project financed by NordForsk (2018 - 2023), and in the WILDBERRIES project (2019 - 2023) financed by RCN, in both where the Arctic berries research group has partnered.

2) Impact on regulation of nutritional value of berries

Today it is imperative for any crop breeding program, to have access to genomic sequence data for targeted DNA-based improvement of the crops. The first sequenced genome of bilberry, representing an Arctic Norwegian ecotype, was published by the Arctic berries research group in collaboration with international partners (**R5**). This data is one of the first genome data which has a **Biocultural (BC) notice**, **being a visible notification for the accompanying cultural rights and responsibilities that need further attention for future sharing and use of the data** (**S7**). The availability of genome data has not only direct impacts on breeding programmes but also on providing access to deeper molecular level analyses of the biosynthesis and regulation of the key nutritional and health-beneficial compounds. Our genome data together with published knowledge of the key transcription factors regulating anthocyanin biosynthesis (**R1, R2**) gives possibilities for the breeding programmes to enhance anthocyanin content of berries. Genome sequence availability will also give possibilities for further development of precise DNA-based authentication methods for *Vaccinium* berries.

3) Impact on developing tools for authentication of the berry products and raw materials for industrial purposes

Berries and berry-based products are often subjected to fraudulent adulteration, either for economical gain or unintentionally, which is a great concern for the food safety. For this reason, different methods have been developed for detecting adulteration of the products and raw materials for the quality assurance (**S8**). As a part of the international **NovelBaltic research project** (2019 - 2021), funded by European regional developed fund through Interreg Baltic Sea Region Programme, various methods for the diversification of berry raw materials were developed and optimised together with partners from other Nordic and Baltic countries. In the project, the Arctic berries group developed a dPCR method for industrial use for quantitative diversification of lingonberries (*V. vitis-idaea*) and cultivated American cranberries (*V. macrocarpon*), which both as red *Vaccinium* berries are often mixed in the berry products (**R6**). With the developed method, it is possible to detect even 1% of the cranberry sample

intermixed with lingonberry sample. The method is applicable for other related species or products. In the NovelBaltic project, we also created an **online platform targeted for companies and other stakeholders for finding laboratory services for authentication of plant-based materials and products** (**S9**).

5. Sources to corroborate the impact (indicative maximum of ten references)

S1 Tundis R, Tenuta MC, Loizzo MR, Bonesi M, Finetti F, Trabalzini L, Deguin B. (2021). *Vaccinium* species (Ericaceae): From chemical composition to bio-functional activities. Applied Sciences 11, 5655. https://doi.org/10.3390/app11125655. This article describes the evidence of the bio-functional properties of the *Vaccinium* berries, which affect the nutritional properties and are targets of the breeding programs.

S2* Letter of support NIBIO showing the importance of the impact of the results of the Arctic berries research group for the Norwegian berry research and production.

S3* Letter of support from Bama; a commitment letter from a Norwegian industrial partner **for the WILDBERRIES** project proposal, showing impact of the research for the product development**.**

S4* Letter of support NorwegianBerries AS; a commitment letter from a Norwegian industrial partner for the **WILDBERRIES** project proposal, showing importance of studying the quality of berries for increasing value creation**.**

S5 Haapala A, Härkönen J, Leviäkangas P, Kess P, Häggman H, Arvola J, Stoor T, Ämmälä A, Karppinen K, Leppilampi M, Niinimäki J. (2015) Bioeconomy potential – focus on Northern Finland. International Journal of Sustainable Economy 7, 66-90. doi: 10.1504/IJSE.2015.066408. This article contributes to understanding the potential of bioeconomy on a regional context indicating possibilities for increased utilisation of wild berry resources in the Northern areas.

S6 Roitsch T, Himanen K, Chawade A, Jaakola L, Nehe A, Alexandersson E. (2022) Functional phenomics for improved climate resilience in Nordic agriculture. Journal of Experimental Botany 73, 5111-5127. https://doi.org/10.1093/jxb/erac246. This opinion article describes the future needs and possibilities of the functional phenomics for the improved climate resilience in sustainable agriculture and in the utilization of the wild berry crops in the Nordic countries.

S7 Anderson J & Hudson M. (2021) The Biocultural labels initiative: supporting indigenous rights in data derived from genetic resources. Biodiversity Information Science and Standard 4: e59230. https://doi.10.3897/biss.4.59230. This article describes the **concept of Biocultural labels** initiative aiming to encode cultural responsibilities into research data.

S8 Salo HM, Nguyen N, Alakärppä E, Klavins L, Hykkerud A.L., **Karppinen K, Jaakola L,** Klavins M, Häggman H. (2021) Authentication of berries and berry-based products. Comprehensive Reviews in Food Science and Food Safety, 5, 5197-5225. https://doi.10.1111/1541-4337.1281. This review article compiles information on the different methods for the authentication of berries and the berry-based products.

S9 NovelBaltic Platform: https://novelbaltic-platform.com/ NovelBaltic online platform was established during the NovelBaltic project to help companies to find laboratory services needed for the authentication of the raw materials or products they are working with.

*Appended below (page 5)

NIBIO
NORWEGIAN INSTITUTE OF BIOECONOMY RESEARCH

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nibio.no

Ent. nr: 988 983 837

Support letter for the impact of the Arctic berry research

NIBIO – Norwegian Institute of Bioeconomy Research – has had a good and close collaboration with the UiT's Climate laboratory at Holt through the joint research projects and students in addition to joint investments on the research infrastructure. NIBIO has also had active collaboration with the Arctic Berries Research group leading to several joint high quality research publications and method papers relevant for the needs of the berry industry.

The wild berry research in NIBIO is towards applied science with the aim of innovation and business development, especially in the Arctic areas. The collaboration with UiT's Arctic Berries Research group has given us the opportunity to combine the applied research with basic research being done by UiT. In addition, several of the students involved has been working for NIBIO in periods.

Tromsø 15.01.2023
Yours sincerely,

Dr. Inger Martinussen
Head of department Horticulture, NIBIO

Confirmation of project cooperation

I the undersigned Gunnar Sagstuen……,(name of signer) representative of NorwegianBerries AS……(company), partner in the project:

"Norwegian wild berries – increased predictability and value creation (Wildberries)",

confirm herewith to take part in the execution of the project.

As part of the project, we will actively contribute with input and suggestion of what is the important factors (challenges) to be able to reach the goal of the project. In WP1 ("Availability") we will contribute with collecting berries from plots fin Southern Norway and in WP3 ("Processing and innovation") we will contribute with knowledge and experience in processing and sales of wild berries and with production of prototypes for consumer testing, if requested.

We want to support and participate in this project because we need to better be able to predict the availability of wild berries. Furthermore, we want increased knowledge about sensory and health-related quality of lingonberry and lingonberry products in order to increase value creation of these berries.

Date and place
10.09.2018 Røyland gård……

Name and title
Gunnar Sagstuen CEO……

Signature ……

bama

BAMA Gruppen AS
Nedre Kalbakkvei 40
Postboks 263 Alnabru
0614 Oslo
Tlf: 22 88 05 00
Org.nr. NO 914 224 314 MVA

Confirmation of project cooperation

I the undersigned Jens Strøm, representative of the organisation/institution Bama A/S, partner in the project:

"Norwegian wild berries – increased predictability and value creation (Wildberries)",

confirm herewith to take part in the execution of the project.

We will contribute in the project with our experience, knowledge and interest in wild berries. Bama AS is particularly interested in the results of WP1; a prediction model for the yield of wild berries. Bama AS will also contribute with knowledge regarding processing and sale of berries in WP3 "Processing and innovative products". As part of the project, we will actively contribute with input and suggestion of what is the important factors (challenges) to be able to reach the goal of the project.

We want to support and participate in this project because we see wild berries as future possibilities as raw materials for new product development.

Oslo 6.09.2018

Jens Strøm
Research Director
Bama Gruppen AS

The Research Council of Norway

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