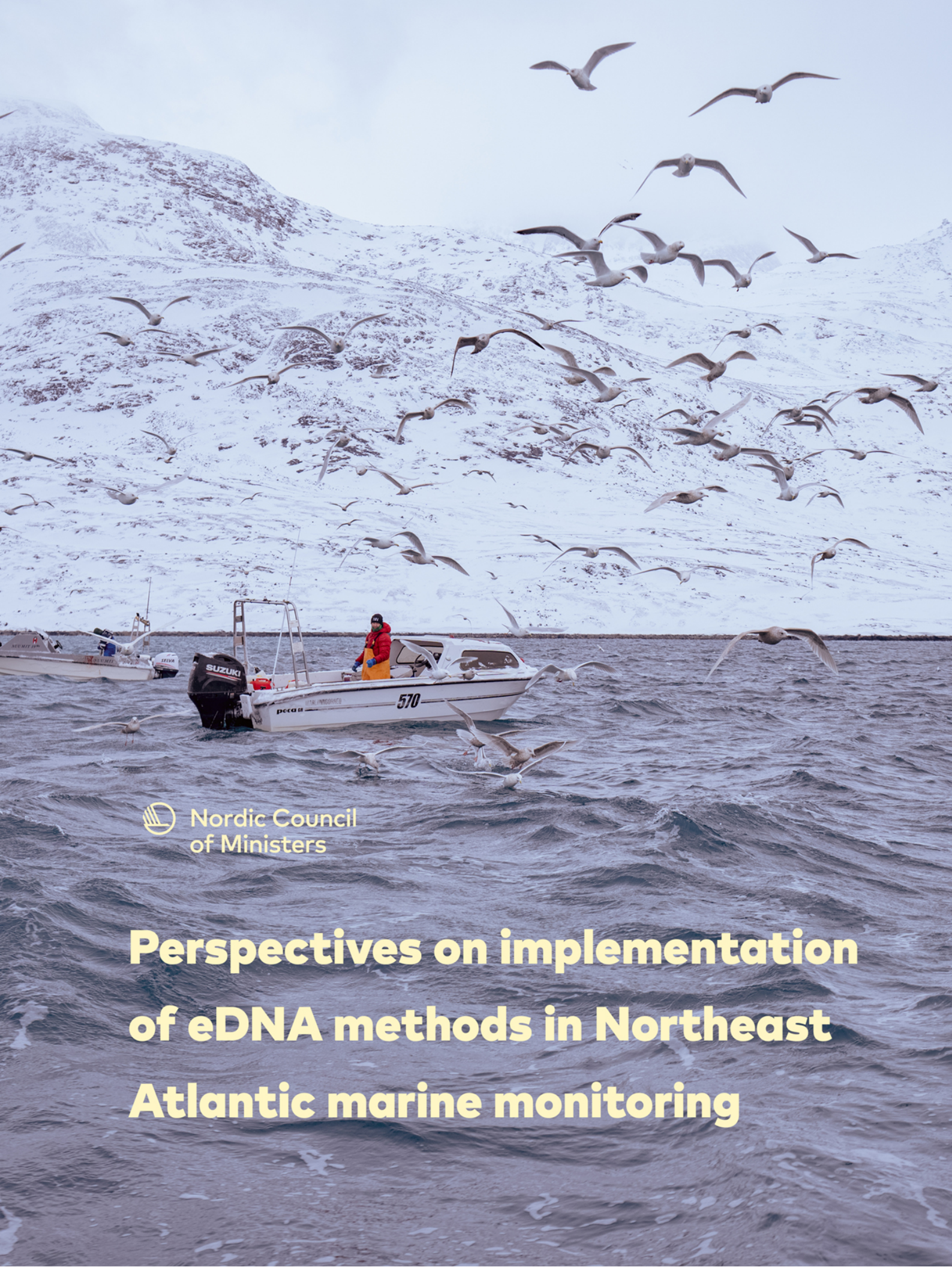

Nordic Council of Ministers

Perspectives on implementation of eDNA methods in Northeast Atlantic marine monitoring

Content

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Acknowledgements

Members of the UNIFIeD project group:

Project manager

The Faroe Islands:
Ása Jacobsen, Research scientist, PF Fiskaaling

Steering group

The Faroe Islands:
Amanda Vang, Head of Biotechnology Dept., PF Fiskaaling

Ian Salter, Research scientist, Faroe Marine Research Institute

Greenland:
Thomas Juul-Pedersen, Senior scientist and Education coordinator, Greenland Climate Research Centre

Iceland:
Sæmundur Sveinsson, Research Group Leader, Matís

Christophe Pampoulie, Research Director, Marine and Freshwater Research Institute

Norway:
Owen Wangensteen, Research scientist, UiT the Arctic University of Norway

Consulting researchers

Norway:
Kim Præbel, Professor and Group Leader, UiT the Arctic University of Norway

The Faroe Islands:
Svein-Ole Mikalsen, Professor, University of the Faroe Islands

Anni Djurhuus, Assistant Professor, University of the Faroe Islands

Iceland:
Snæbjørn Pálsson, Professor, University of Iceland

Davíð Gíslason, Project Manager, Matís

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1 Preface

This report is the result of a collaboration between researchers from eight different research institution in Greenland, Iceland, the Faroe Islands and Norway, all working with the application of environmental DNA (eDNA) in marine research and monitoring.

Fiskaaling, Aquaculture Research Station of the Faroes, took the initiative to propose this project because we are including eDNA analysis in our coastal research and monitoring and felt our work could benefit from establishing a consensus with researchers in other Northeast Atlantic countries on fundamental issues related to the standardization and implementation of eDNA methods.

The implementation of eDNA methods in marine monitoring has also proved challenging, in part due to researchers and stakeholders lacking a common understanding about how eDNA can be used and and needing better insight into the various challenges and ambitions of both parties.

Researchers in Greenland, Iceland, Norway and other institutions in the Faroe Islands shared these challenges and all contributed to the project by discussing main issues to address, constructing surveys and providing input for both surveys and a summary workshop. Therefore, the project plan and outcome relied on the knowledge and experience of all project participants.

The aim of the UNIFIeD (Unifying Nordic Initiatives and Fostering Involvement on eDNA) project was to assess the status of implementation of eDNA based methods in marine research and monitoring in the Northeast Atlantic. This included: identifying main challenges and ambitions on this issue for the various countries, providing a basis for enhanced communication between researchers and stakeholders, as well as increasing the possibility of collaboration between researchers across the Nordic region with a better understanding of each countries strenghts, challenges and ambitions. We estimate this knowledge to be a prerequisite for standarization and implementation of eDNA methods in an internationally coordinated scheme.

The report describes results from surveys investigating the current status of the implementation of eDNA methods to marine monitoring. Respondents included stakeholders from Iceland, the Faroe Islands and Norway as well as researchers in the field from most Nordic Countries. In addition, the discussions and consensus from the following workshop is presented.

2 Executive summary

The UNIFIeD (Unifying Nordic Initiatives and Fostering Involvement on eDNA) project's overall goal was to help solve the problem of lacking harmonization and implementation of eDNA methods in marine monitoring in the Northeast Atlantic by promoting a discussion among relevant stakeholders and researchers about the challenges and ambitions on this issue, based on data on current status.

The increasing call for harmonisation and standardisation in the use of eDNA methods has revealed a fundamental need for creating an overview of the current status in eDNA application to marine research and monitoring, especially in the smaller countries and remote areas.

For the smaller communities there has been the added challenge of lacking the relevant national scientific and political schemes in marine monitoring programs and struggling to get enough regional application from larger international consortiums. This increases the inconsistent nature of eDNA application in these countries and hampers the possibility of collaboration and comparison of approaches, analyses and results.

The lack of continuity in the application of eDNA methods in marine monitoring, leaves implementation in long-term monitoring and initiation of new initiatives in a difficult position.

In the UNIFIeD project the current status in eDNA application to marine research and monitoring has been illustrated through input from relevant stakeholders and researchers in the field by responses to targeted surveys. At a subsequent workshop, researchers and stakeholders discussed how each Northeast Atlantic country's differences, strengths and challenges would impact collaboration on standardization and implementation of eDNA methods in marine monitoring in this region. One of the subjects discussed was how to clarify and enhance communication between researchers and stakeholders on this issue.

The thematic scope of this project was restricted to monitoring of the marine environment in the Northeast Atlantic. The surveys were targeted to stakeholders in Greenland, Iceland, the Faroe Islands and Norway and researchers in all Nordic countries.

Key findings:

Perception of eDNA methods

- The survey detected a discrepancy between some stakeholders and researchers in the perception of eDNA methods.
- Contrary to researchers, some stakeholders estimated that directly comparable results between eDNA methods and more traditional methods were required before implementation. This highlights the need for clear communication between the research community and stakeholders about the application of eDNA.

Standardization challenges

- Researchers estimated that the main challenges for standardization of eDNA methods were
    - Financial issues
    - That eDNA protocols are still in a developmental stage
    - Deciding which international protocol to align to.
- Due to
    - Many choices in technical design
    - Lack of standard institutional protocols to adhere to
    - Project or practicality based decisions
    - Limited funding for projects of longer duration

Implementation

- Only few stakeholders estimated that eDNA methods were at the implementation stage
- The most advanced area was Biodiversity/Climate change
- The second Marine Management
- The least advanced area was Impact Assessments
- Researchers and stakeholders agreed that the main challenges for implementation of eDNA methods are (in random order)
    - lack of continuity in funding
    - lack of standardization and validation of eDNA methods
    - lack of national biomonitoring programs

Regional differences

- Norwegian stakeholders demonstrated an intent to proceed with the process of implementation of eDNA methods
- Iceland and the Faroe Islands had more mixed answers from the stakeholders and fewer signs of implementation.
- None of the stakeholders approached in Greenland responded to the survey.
- Overall, the stakeholders in Norway estimated various issues in implementation of eDNA methods to be less problematic than their counterparts in Iceland and the Faroe Islands.

Note

The results from the surveys are based on a limited number of responses and should thus only be treated as a basis for further communication about this subject between researchers and stakeholder within and between countries in the Nordic region.

3 Introduction

3.1 Marine monitoring in the Northeast Atlantic

Marine monitoring in the Northeast Atlantic is divided and substructured under several different international, national and institutional schemes as well as being performed with different objectives in mind. Three focus areas cover the major themes of marine research and monitoring in the Northeast Atlantic.

1. Management of marine resources includes monitoring and regular stock assessments of economically important species, mainly commercial fish stocks and shellfish, and analyses of the food web sustaining them. Capture statistics have been carried out for many decades in the Northeast Atlantic for marine resource management purposes. The monitoring efforts are supported by the adherence to a range of international agreements and conventions such as FAO, NAFO, NEAFC, etc. that the various countries are committed to. Marine Research Institutes perform the monitoring usually following a pre-planned schedule feeding year and decade long time series, which can support the administrative decisions for marine resource management.
2. Impact assessment is a more fragmented or specialised segment mainly carried out or supervised by environmental agencies. These assessments usually relate to various industrial enterprises such as aquaculture or other human activities including construction and tourism. The involved industries also sometimes perform such impact assessments to live up to various international standards.
3. The current focus on climate change and the pressure it enforces on the marine environment has also increased the awareness and importance of monitoring and maintaining biodiversity. Although, or perhaps because, these issues are overarching and universal, monitoring is often less structured or exclusively managed by any specific institution. It currently has high political focus and research and monitoring within this area is increasing.

3.2 Application of environmental DNA

Environmental DNA is a highly sensitive and efficient method for establishing the presence of species of any certain taxonomic group and for determining compositions of these communities (Pawlowski *et al.* 2021; Pascher *et al.* 2022). Therefore it can identify relatively rare species as well as circumvent potential difficulties in morphological identification of certain species.

In addition, eDNA is often a more non-intrusive and efficient sampling method (Ratcliffe *et al.* 2020). Therefore, eDNA sampling can potentially provide a higher sampling frequency, smaller grid size, and/or cover a larger geographical area. Sometimes, it can also be used in areas where other forms of sampling can be challenging. These characteristics of eDNA methods can support the vital efforts to produce time series estimated to be of vital importance for detecting changes in the marine ecosystems (Benway *et al.* 2019; Bianchi *et al.* 2022).

Because marine monitoring has been performed using other methods that can be time consuming and challenging, the notion that eDNA methods might be able to partially replace or complement some of these methods for a more efficient monitoring has interested both researchers and stakeholders (Hinz *et al.* 2022; Ramirez-Amaro *et al.* 2022). This has also resulted in the need of comparing eDNA methods to these more traditional methods (Closec *et al.* 2019; Fediajevaite *et al.* 2021; Keck *et al.* 2022).

In this process, misunderstandings have sometimes developed about the potential and application of eDNA. Essentially, it has resulted in a tendancy for some stakeholders to expect that eDNA methods could serve as a replacement for some of the traditional and more expensive methods, instead of evaluating how the methods complement each other and can together provide better monitoring, which many recommended (Ruppert *et al.* 2019*;* Rogers *et al.* 2022; Rourke *et al.* 2022; Suarez-Bregua *et al.* 2022).

Although eDNA methods often perform well in comparison with traditional surveys (Chavez *et al.* 2021; Fediajevaita *et al.* 2021; Keck *et al.* 2022), researchers also struggle to provide the clear-cut results that stakeholders need in order to justify investing in eDNA-based reasearch and monitoring. Improved communication is a key issue for stakeholders to gain the understanding that eDNA methods, as all other methods, have strenghts and challenges that need to be taken into consideration.

Therefore, the challenge for all parties is to get a common understanding of the great potential of eDNA methods and apply these to marine monitoring in a fruitful manner, without complicating the process of implementation with unrealistic expectations and requirements.

3.3 Global and large-scale initiatives

In marine resource management, the methods commonly used are based on observations, capture, or bulk sampling for morphological classification and enumeration. These traditional methods are and have been essential for providing data for knowledge-based management of the marine environment. However, these methods have some limitations such as the constraint on sample frequency and geographical area covered due to the nature of the sampling method and/or the time consuming process of morpho-taxonomic identification and enumeration. The application of eDNA can address these and other issues and can provide a valuable complementary approach.

The potential in the application of eDNA to supplement and improve environmental monitoring has despite standardization challenges inspired many large national and international institutions to start the process of implementation. As an example, the American Agency NOAA (National Oceanic and Atmospheric Administration), renowned for its scientific work and services in climate monitoring and fisheries management amongst other things, has recognised the potential in eDNA methods to supplement other more traditional methods used to produce valuable survey data. Amongst other applications, the agency recommends commencing implementation of eDNA methods to support Ecosystem-Based Fisheries management (UNIG 2020)

The current loss of Biodiversity has also inspired UNESCO to launch a global eDNA project to study vulnerability of species to climate change at marine World Heritage sites, recognized for their unique biodiversity, outstanding ecosystems, or for representing major stages in Earth's history (https://www.unesco.org/en/edna-expeditions). The aim is to measure the vulnerability of marine biodiversity to climate change and the impacts of that change on the distribution and migration patterns of marine life across marine World Heritage sites. This knowledge will help understand global trends and inform ongoing efforts to protect marine ecosystems and ensure future generations continue to enjoy the services they provide.

The Ocean Biomolecular Observing Network (OBON) also uses eDNA methods to greatly enhance coastal and open ocean biodiversity observations (https://www.obon-ocean.org/). The program utilizes biomolecular technologies to monitor, research and understand life in the sea at every trophic level and scale, how life varies in response to climate and anthropogenic impacts, including fisheries, and how these changes impact society.

Various other initiatives have also been made to instigate more coordinated efforts in implementation of eDNA methods. As an example, the European network

DNAqua-Net (COST Action CA15219) presented a suggested roadmap for successful implementation of DNA-based biomonitoring for freshwater ecosystems (Blancher et al. 2022). Although focused on freshwater monitoring, it provides valuable reflections of relevance for the marine environment. Their paper gives an "overview of the forum discussions and common European views that emerged from them, while reflecting the diversity of situations in different countries". They recognized that implementing DNA-based methods in biomonitoring will require significant technical and organisational changes and that their adoption will require coordinated actions at national and international levels.

Another initiative is the European Marine Omics Biodiversity Observation Network (EMO-BON), which focuses on marine monitoring and including eDNA methods in its approach (https://www.embrc.eu/emo-bon). EMO BON aims to establish a coordinated, long-term, marine biodiversity observatory. By bringing together individual biological observation stations, EMBRC provides the context and opportunity for partner institutions to participate in EMO BON and build a modern biodiversity observation framework for Europe. Currently, EMO BON includes 16 marine stations, located from the Arctic to the Red Sea.

3.4 Nordic initiatives

Nordic institutions and researchers are involved in many international initiatives applying eDNA methods to marine research and monitoring and in the recent years, there has been a steady increase in the application of eDNA methods to marine research in the Northeast Atlantic (Salter *et al.* 2019; Turon *et al.* 2022; Pampoulie *et al.* 2023). However, there has been little consistency in the methodology as different projects follow different protocols for sampling, storage, data management etc. that are estimated to be most appropriate at the time.

This is causing difficulties in conforming to a gold standard when initiating eDNA based monitoring of the marine environment since there are no standard guidelines to follow for the application of eDNA methods. It is also hampering fruitful collaboration between the Nordic countries in the process of implementation eDNA methods in marine monitoring.

Box 3.4.1

Nordic Marine Phytoplankton Group (NOMP)

The Nordic Marine Phytoplankton Group (NOMP) consists of persons in Denmark, Iceland, Norway, Sweden and the Faroe Islands working with research and environmental monitoring related to phytoplankton, algal blooms and harmful algae. One aim of the group is to improve quality and intercomparability of results by using consistent names of organisms and algal groups. Other aims include collaboration through yearly meetings to share recent results, intercomparisons of methods, including molecular methods, and application of the Nordic microalgae website http://nordicmicroalgae.org , which is operated by the Swedish Meterological and Hydrological Institute and is a service part of the Swedish Biodiversity Data Infrastructure (SBDI), funded by SMHI and the Swedish Research Council.

Coordinator of the NOMP Group is Bengt Karlson at SMHI

Box 3.4.2

Extracts from Norway's integrated occean management plans 2019–2020

"There is a need to further develop several of the indicators used for environmental monitoring.."

"A closer focus on species composition is needed, including species at lower trophic levels, and more time series are needed on population size and habitat use."

"There are also gaps in the monitoring of benthic communities, alien species, threatened species and pollution. Monitoring of pressures and impacts associated with human activity needs to be further developed. We also need to improve our understanding of which changes are caused by pressures from human activity in the management plan areas or adjoining coastal waters and land, and which are related to climate change and other large-scale processes or to natural processes and variability in the oceans."

"Better and more cost-effective methods also need to be developed for use in mapping and monitoring Norwegian waters."

Norwegian Ministry of Climate and Environment – report to the Storting

However, there are some national and international initiatives in the Nordic region that are good examples of how researchers and stakeholders, directly or indirectly, work towards the standardisation and implementation of eDNA methods in marine monitoring. A few current examples illustrating the efforts of researchers and stakeholders are described in the information boxes.

The NOMP Group described in Box 3.4.1 includes researchers working with eDNA methods and is, through the induced knowledge sharing and cooperation, instrumental in the promotion of standardization and implementation of eDNA methods. It is also a good example of how interactions between an international scientific network and national scientific infrastructure can be benefical for both parties.

In Norway's integrated ocean management plans 2019–2020, presented in Box 3.4.2, there are indications that stakeholders are positive towards the development and implementation of new methods to enhance the insight gained from marine monitoring. Although eDNA is not mentioned by name, it is a good fit for the ideas presented in the document. As such, it is a good demonstration of the discussions and decisions required by stakeholders when forming political schemes that can facilitate the implementation process of new methods such as eDNA.

Box 3.4.3

Roadmap for implementing environmental DNA (eDNA) and other molecular molecular methods in Finland

In Finland, molecular monitoring methods have been tested and piloted by all major institutions responsible for environmental monitoring, and the methods are already applied routinely in the monitoring of individual game species. Other areas remain less developed, and national efforts and expertice are scattered across different organizations. In marine monitoring some pilot projects have been started but the field is fragmented with no national coordination.

The Finnish Environment Institute (SYKE) developed a Roadmap that:

- Proposed an action plan for promoting coordinated implementation of eDNA methods
- Launched a national discussion and provided actionable recommendations
- Was tied to national environmental monitoring strategy framework and implementation program, National biodiversity strategy and action plan for 2035

Vision and Action Plan for 2022–2025 commissioned by the Finnish Ministry of the Environment

In the work performed by SYKE and described in Box 3.4.3, many of the primary areas identified for needed development and proposed actions require increased national and international coordination and communication across sectors and the involvement of both researchers and stakeholders.

3.5 The UNIFIeD project

The focus of UNIFIeD (Unifying Nordic Initiatives and Fostering Involvement on eDNA) was to estimate strengths, challenges and ambitions of the participating countries, Greenland, Iceland, the Faroe Islands and Norway, for standardization and implementation of eDNA methods in marine monitoring in the Northeast Atlantic. The project targeted these issues from the perspectives of both researchers in the field and stakeholders who can facilitate the implementation process. The aim of UNIFIeD was to lay a foundation for collaboration between researchers and stakeholders as well as between the Nordic countries countries based on this knowledge.

Following discussions between the UNIFIeD participants at the kick-off meeting it was decided to make the planned survey in two versions, one for researchers in the field and one for stakeholders. Using this approach, the **survey for researchers** could investigate details about fundamental issues such as sampling, storage and archiving for potential international alignment and probe into details about current status in project setup and challenges in standardization and implementation. without alienating stakeholders with irrelevant questions.

Likewise, the **survey for stakeholders** could be designed to investigate the interest and aspect of those public entities responsible for establishing the needed support and framework for implementation of eDNA methods in marine monitoring. This insight from stakeholders was estimated to be highly valuable as an important aspect of the UNIFIeD project is to determine the possibility of involvement of all participating countries in a unified process of standardization and alignment for Northeast Atlantic marine monitoring.

At the kick-off meeting it was also agreed to focus the surveys to centre around three essential areas of application. This would make the questions and answers more tangible in terms of potential implementation. The three focus areas cover the major themes of marine research and monitoring in the Northeast Atlantic:

1) Biodiversity/Climate Change,
2) Marine Resource Management,
3) Impact assessment

The survey for researchers was distributed to researchers in the Nordic countries working with eDNA in the marine environment in the Northeast Atlantic, while the stakeholder survey was limited to respondents from the UNIFIeD countries only.

The survey results were compiled in a report, which was sent to the project participants and was the basis for a following workshop. The report was organized in four sections reflecting the theme of the presentations that were on the agenda for the UNIFIeD online workshop scheduled for December 2022. The workshop was organized as an online event with the four presentations held by various UNIFIeD project participants, each presentation followed by discussions among all workshop attendees. All relevant stakeholders and researchers initially contacted in relation to the UNIFIeD survey were invited.

The project report including the survey results was distributed to all parties as soon as it was published.

4 Survey results and discussion

4.1 Background of stakeholders and researchers

The results from the surveys are based on 27 responses in total. Twelve responses were received to the Stakeholder survey and 15 responses to the Researcher survey. Stakeholders were represented by people in management, advisory or civil servant positions at various relevant ministries, environment agencies and research institutions in Iceland, the Faroe Islands and Norway, including representatives of relevant International Organisations (Table 1). Although no written responses were from Greenland, we communicated with the Arctic Hub to ensure best possible inclusion of the views of Greenlandic stakeholders.

Responding researchers represented various research institutions in Iceland, the Faroe Islands, Norway, Sweden, and Finland (Table 1). Although no responses were from researchers representing Greenland, some of the other respondent perform relevant work in Greenland.

Table 1. Stakeholders, international organizations and research institutions represented*

*Other relevant international organisation than those listed might be represented.

Icelandic Institute of Natural History	Iceland
Marine and Freshwater Research Institute	Iceland
Matís	Iceland
University of Iceland	Iceland
Faroese Environment Agency	The Faroe Islands
Faroe Islands National Museum	The Faroe Islands
Faroe Marine Research Institute	The Faroe Islands
Fiskaaling, Aquaculture Research Station of the Faroes	The Faroe Islands
University of the Faroe Islands	The Faroe Islands
Institute of Marine Research	Norway
Ministry of Climate and Environment	Norway
NINA, Norwegian Institute for Marine Research	Norway
Norwegian Environment Agency	Norway
NORCE Norwegian Research Center	Norway
UiT, The Arctic University of Norway	Norway
SMHI, Swedish Meteorological and Hydrological Institute	Sweden
SYKE, Finnish Environment Institute	Finland
CAFF, Coastal Steering Group	International
CAFF, Marine Steering Group	International
OSPAR Commission	International

The stakeholders were overall engaged in the defined focus areas in marine issues (Fig 1). Biodiversity / Climate change was the focus area of highest interest, while the lowest number of stakeholders were engaged in the field of Marine Resource Management. The climate change and loss of biodiversity are higly urgent issues and of current political focus. In addition, Biodiversity / Climate Change is often an essential component of the other two focus areas. Therefore, those focusing on Marine Resource Management and Impact Assessment might, in one way or other, also need to have the Biodiversity / Climate Change aspect in mind, while it is not necessarily so the other way around.

Q: What is your level of engagement in these areas of marine issues?

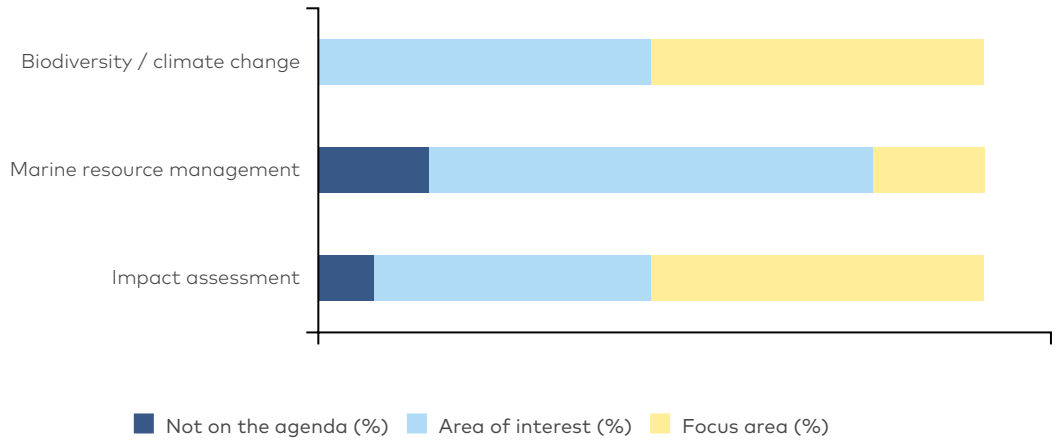


Figure 1. Level of engagement (Stakeholders)

Overall, the stakeholders seemed to have fairly good insight into the concept of eDNA and two thirds were positive towards implementation of eDNA in marine monitoring while one third was neutral or undecided (Fig 2). It is noteworthy that no one leaned towards not recommending eDNA for implementation into marine monitoring.

What is your level of awareness of environmental DNA (eDNA) and your approach to it's implementation in marine monitoring?

No knowledge	0%	16,7%	58,3%	25,0%	0%	In-depth knowledge
Do not recommend	0%	0%	33,3%	66,7%	0%	Actively encourage implementation

Figure 2. Level of awareness and approach to implementation (Stakeholders)

The high level of engagement demonstrated by the stakeholders is likely skewed by the fact that mainly people engaged in this issue answer on behalf of their institution. The survey was distributed to many more than those who answered, which might be an indication of a more balanced picture with some stakeholders not being as engaged as those who responded.

From the Researcher Survey it was possible to see what those applying eDNA methods in research and monitoring in the Northeast Atlantic are focusing on. Overall, the research respondents cover a wide area of research fields, habitats and taxonomic groups. Their project roles were mainly as researchers and project managegers while a few provided technical or adminstrative support.

When asked what the aim of their research was, most mentioned Biodiversity / Climate change (86.7%). Well over half of the respondents indicated Marine Reseource Mangement as relevant (66.7%) while fewest worked with impact assessment (46.7%).

All researchers working with Biodiversity indicated that they work with conservation and/or establishing baselines. Over fifty percent answered that they were also focusing on Climate change while almost 40% focused on invasive species. Only one respondent focused on endangered species in particular.

The research performed by the respondents working with biodiversity and climate change is mainly concentrated around the open ocean or coastal waters, but other environments such as the seabed and kelp forests are also being investigated (Fig 3). Their research covered a wide array of larger taxonomic groups such as bacteria, viruses, phytoplankton, zooplankton, invertebrates, fish and marine mammals.

Q: What environment or habitat are you focusing on?

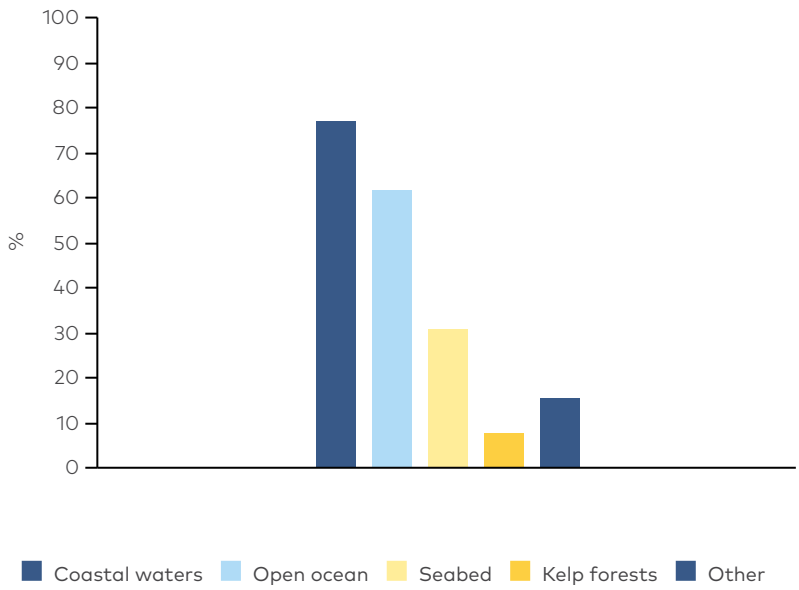


Figure 3. Marine habitats investigated in Biodiversity/Climate Change studies (Researchers)

Researchers working with marine resource management focused on both pelagic and demersal fish, invertebrates, phytoplankton and zooplankton. When asked about their research focus, 80% responded that they worked with ecosystem dynamics while 40% worked with stock assessments of economically important species (Fig 4). A few focused more specifically on invasive species, species at risk or food availability.

Q: What best fits the focus of your research?

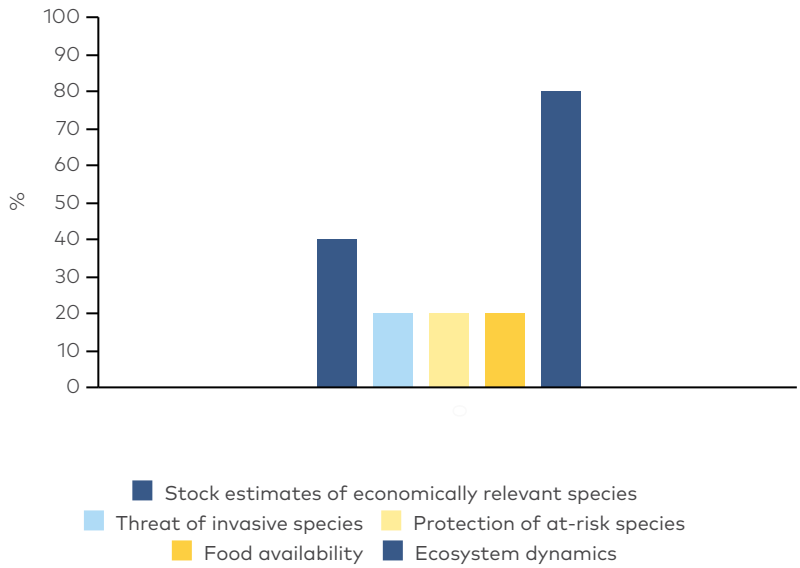


Figure 4. Research focus in Marine Resource Management (Researchers)

Researchers working with impact assessment mainly focused on the coastal environment (71.4%) and the seabed (42.9%). Kelp forests and the open ocean were mentioned only a few times. Focus of the impact assessments was mainly the aquaculture industry and human activity/traffic while the oil drilling industry was mentioned once.

As within the area of Biodiversity/climate change and marine resource management although fewer in number the researchers working with Impact assessments investigated a wide array of taxonomic groups (Fig 5).

Q: Which larger taxonomic group are you working with?

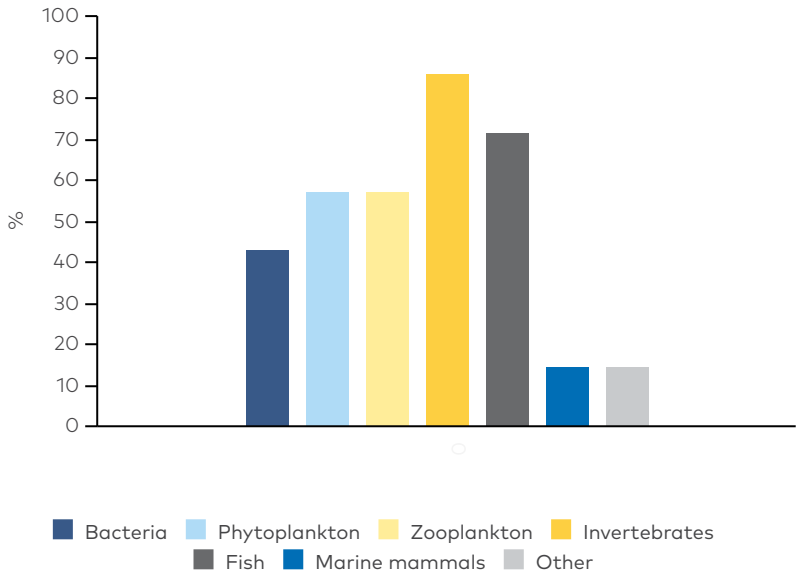


Figure 5. Taxonomic groups covered in Impact Assessment studies (Researchers)

In order to get an understanding of potential differences in implementation challenges in the selected focus areas, researchers were asked to state which technical approaches they used in the application of eDNA, as different approaches might entail different requirements before implementation.

The technical approaches that could be selected included targeted approaches and metabarcoding (Fig 6a–c). The targeted approaches, where only one specific taxon at a time is investigated, were also divided into analyses of presence/absence determination, abundance estimation, and/or population genetics.

The results showed that the targeted approaches were most frequently used for abundance estimates, as more than half of the researchers in every focus area used this approach in their studies.

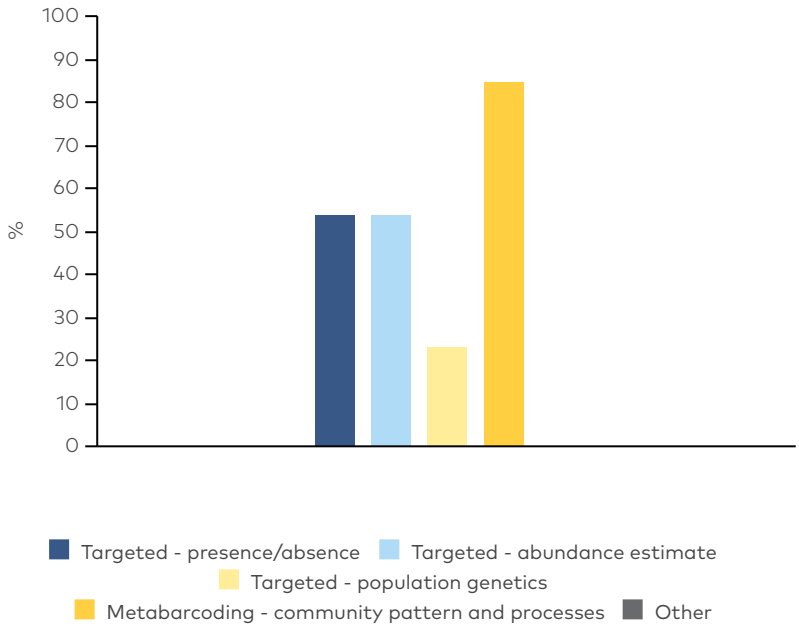
In Biodiversity/Climate Change studies targeted approaches were equally often applied to presence/absence determination. In Marine Resource Management presence/absence determination was used by 50% of the researchers and in Impact Assessment studies less than thirty percent of the researchers used presence/absence determination. Population studies were performed by between 15–30% of all the researchers.

Metabarcoding, where entire communities of any selected higher taxonomic groups are investigated for analysis of community structure and dynamics, was in all focus areas used more frequently than the targeted approaches. Metabarcoding was used used by 70–100% of the researchers, the lowest percentage being in Marine Resource Mangagement and the highest in Impact Assessment.

Figure 6a–c. Technical approaches used in application of eDNA (Reserchers)

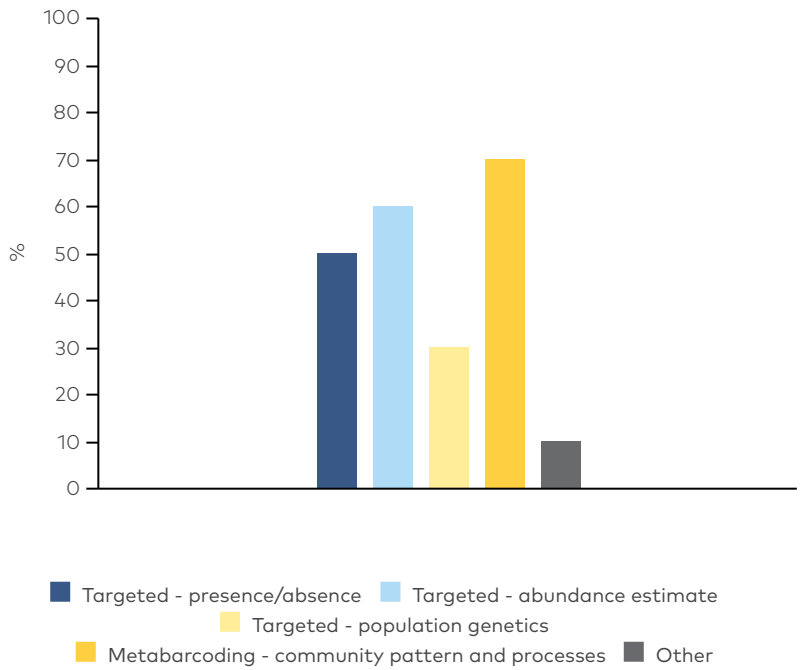
Q: What approach do you use?

a. Biodiversity/Climate change



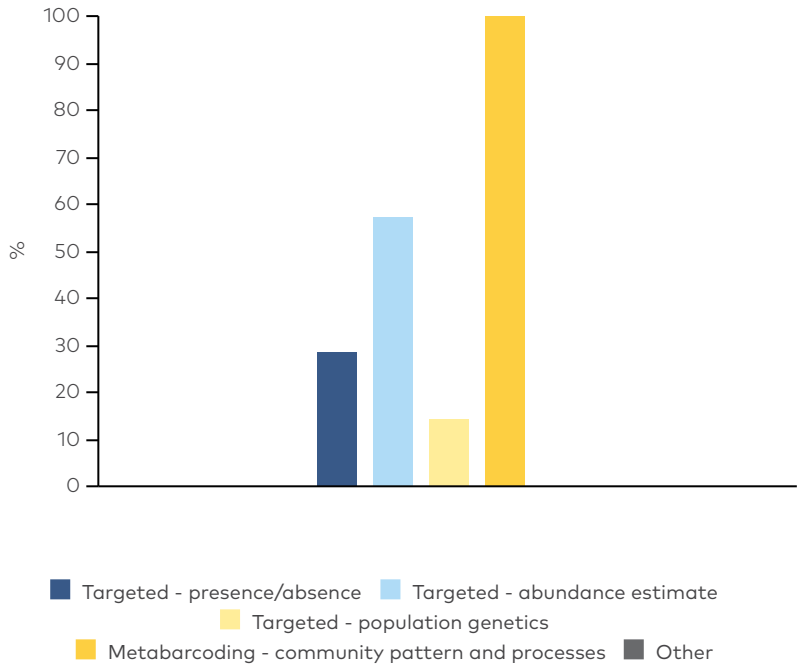
Q: What approach do you use?

b. Marine Resource Management



Q: What approach do you use?

c. Impact Assessment



4.2 Perception of eDNA as a method for marine monitoring

In many instances eDNA is known by reputation rather than by experience. This means that researchers and especially stakeholders are susceptible to sometimes expecting unrealistic or unsubstanciated things of the application of eDNA. The potentially detrimental effect of this situation is that stakeholders either dismiss the application of eDNA or set very specific requirements for its implementation.

In order to promote a correct implementation of eDNA methods to marine monitoring it is necessary to identify these discrepencies in the understanding of what the strengths, weaknesses and potentials of eDNA methods are. What could and should we apply it to at the present, what should we not use eDNA methods for and what are promising future perspectives that we need to research.

One important aspect is discerning when eDNA methods can replace more time consuming and expensive traditional methods and when they provide a complementary or independent approach that improves monitoring.

Researchers were asked if they perform studies comparing eDNA methods with more traditional methods and what the results were. Eighty percent of the researchers perform such studies and compare various types of estimates such as diversity, abundance, biomass, community structure and presence/absence. Researchers were also asked to state if their comparisons showed concordance, discordance or varying results within the different types of measurements (Fig 7).

Q: What do you compare between eDNA and other methods in your work and what are the results?

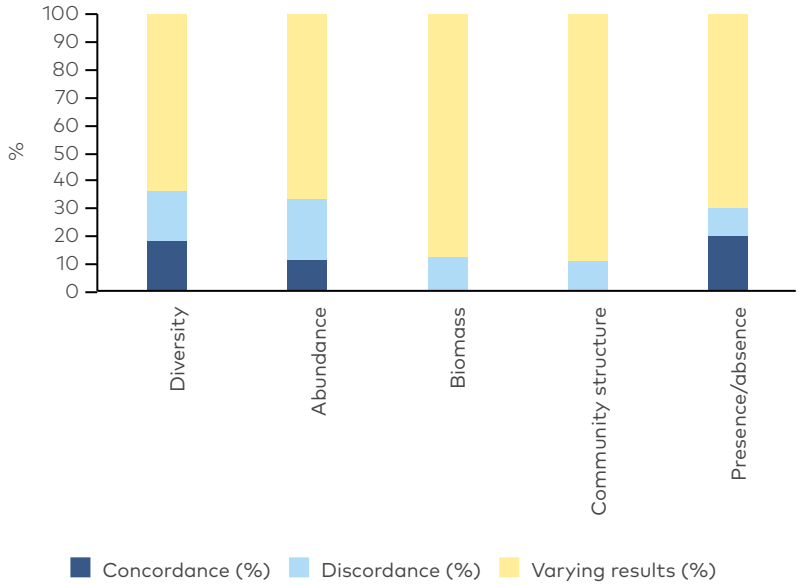


Figure 7. Results from comparisons of eDNA with traditional methods (Researchers)

According to the researchers, the comparisons of eDNA with more traditional methods often showed varying results. Some reported direct concordance in their comparisons and some discordance. In addition, several comments were made about their "comparative work" being at an early stage, making it premature to decide how comparative the different methods are.

Keeping this in mind, both researchers and stakeholders were also asked about their view on the comparison of eDNA methods with more traditional methods, because this could say something about what they expect of the application of eDNA methods.

Overall, the researchers did not think the eDNA methods necessarily need to be directly comparable with traditional methods before implementation, but that it mostly depends on the application (Fig 8).

Q: Do you think eDNA methods need to be directly comparable with other traditional methods before implementation?

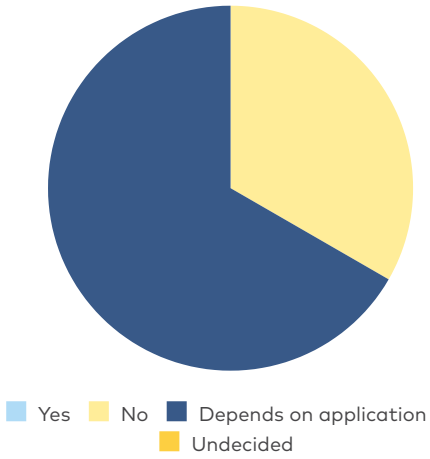


Figure 8. Comparison of eDNA with traditional methods (Researchers)

This is in contrast to the stakeholders' view. When asked if they think eDNA methods need to demonstrate directly comparable results with more traditional methods before implementation, only 8.3% answered "No" and over 40% answer "Yes", while another good 40% answered that it depends on the application (Fig 9).

However, when comparing the answers from stakeholders in Norway with those from Iceland and the Faroe Islands, there also seemed to be a regional difference. In Iceland and the Faroe islands more than 70 percent of the stakeholders responded that eDNA methods need to demonstrate directly comparable results with more traditional methods before implementation. In comparison, no one in Norway had this view and their responses were quite similar to the responses from the researchers.

Q: Do you think eDNA methods need to demonstrate directly comparable results with more traditional methods before implementation?

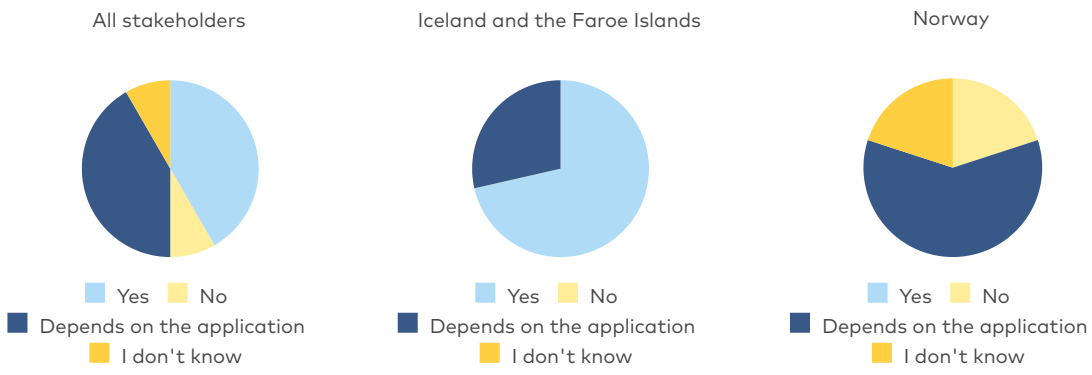


Figure 9. Comparison of eDNA with traditional methods (Stakeholders)

The varying results in comparative studies and reasons and implications of the discrepancies in the perception of eDNA as a replacing or complementary method need to be included in discussions across regional and professional boundaries. This could promote a common understanding of the potential role of eDNA methods in marine monitoring and the process needed to reach the implementation stage.

Stakeholders were also asked to state their view on some of the common statements made about the application of eDNA methods (Fig 10). Most agreed that eDNA methods are arelatively non-invasive method, that it has good potential in detection of rare or invasive species and that it can be used to address issues in marine monitoring of international focus. However, all did not agree that it can easily increase spatial and temporal sampling and even more disagreed that it can provide more effective monitoring (Fig 10).

Q: Do you agree with these statements about the benefits of implementation of eDNA?

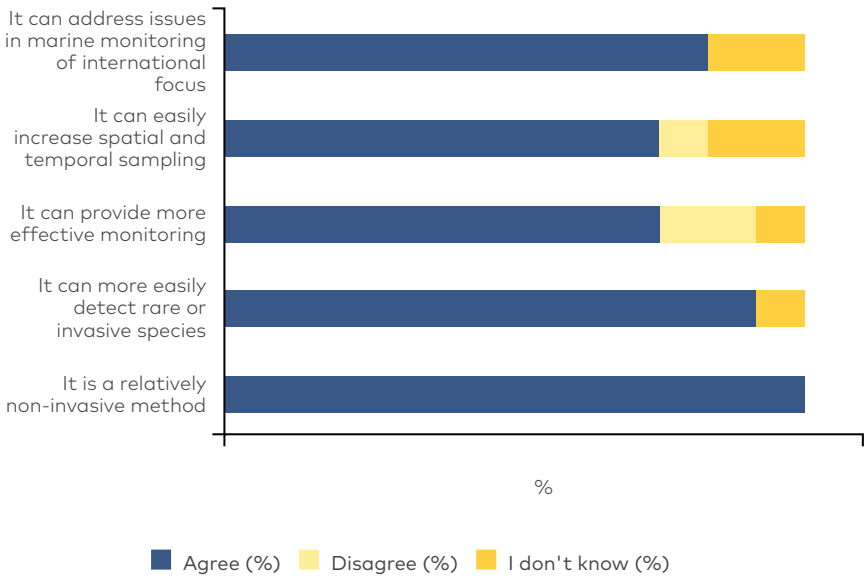


Figure 10. Stakeholders' view on common statements about eDNA

The concept of eDNA methods providing a more effective monitoring relies on a range of factors, including which role these methods will have. Therefore, it is valuable to see that this might be a concern for stakeholders. There is of course a difference in monitoring expenses whether eDNA can replace other more expensive and time consuming methods or if eDNA methods are added as a complementary approach for improved monitoring.

4.3 Standardization status and challenges

Currently, both researchers and stakeholders have a lot of focus on the standardization process of eDNA protocols as this is a fundamental requirement for national and international collaboration. In addition, minimizing the risk of putting time, effort and funding in producing data that might become obsolete within a short timeframe due to the application of out-dated protocols is important for all parties.

Because eDNA methods are still relatively new and continuously evolve, the standardization process at regional scale or beyond is difficult. There are different views on what the main challenges are for standardization/alignment of eDNA protocols. However, the issues researchers felt were most challenging (Fig 11), when responding to the UNIFIeD survey, were:

1. Financial issues
2. That eDNA protocols are still in a developmental stage
3. How to select a protocol to align to

Q: What are the main challenges for standardization/alignment of eDNA protocols?

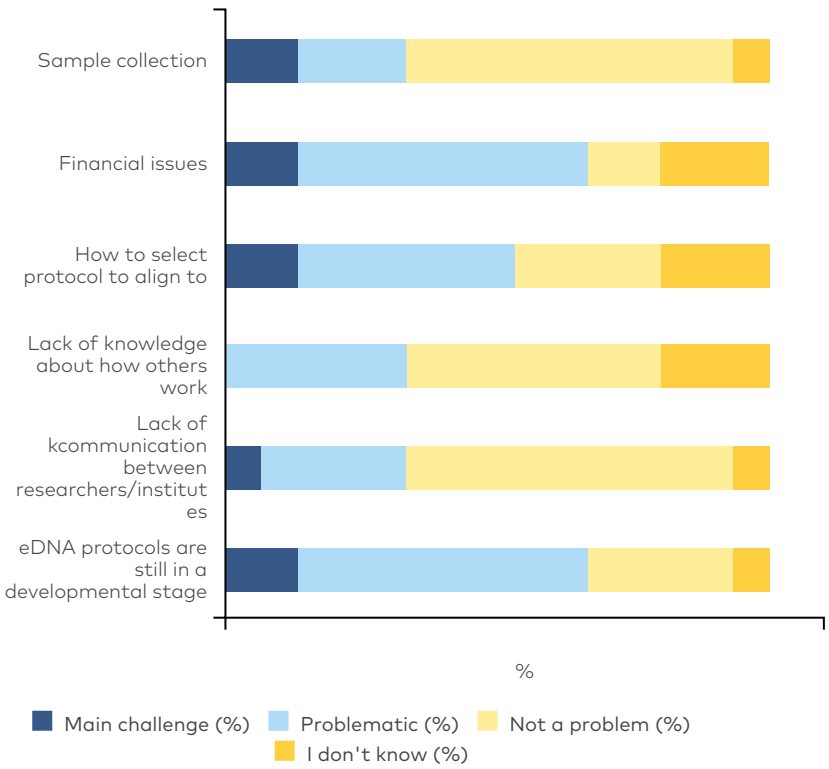


Figure 11. Challenges for standardization (Researchers)

In the survey, reseachers stated that their funded projects including eDNA were predominantly short term projects of duration between ½ to 2 years, while funding for projects of longer duration (3–5 years) was a third less frequent. Funding for long term monitoring programs including eDNA was selected a few times, mainly as funding from international research funds.

Most researchers answered that their funding came from national or international research funds or was funded in-house. Some mentioned industry funding while national policy based funding was only mentioned once.

Some of the questions in the survey explored the technical issues about the researchers' eDNA protocols and the answers relate to the complexity of standardizing eDNA protocols.

All but one worked with sea samples, while 60% of the respondents worked with sediment or bulk samples. Almost thirty percent of the researchers stated that they always used the same protocol when working with sea samples. In comparison, this value was 11.1% for the sediment and bulk samples (Fig 12).

Q: Which sample types do you work with and do you use a standard protocol for each sample type?

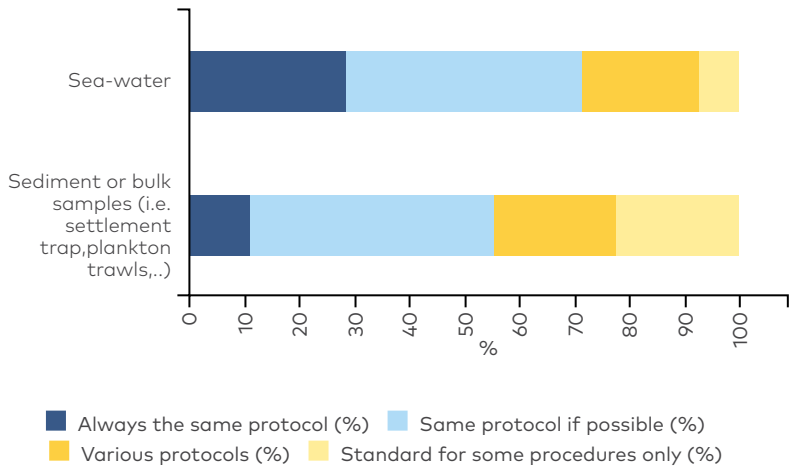


Figure 12. Sample types and protocols (Researchers)

For both sample types, most answered that they used the same method if possible. Although the majority of the researchers did not consistently use the same protocol, it is also clear that most attempt to adhere to a standardized protocol if possible. The option "various protocols" was on average only 21.8% while all other options aspire to some level of standardization.

Due to the multitude of possibilities in sediment and bulk sampling, the survey was not expanded in that area. However, sea water sampling protocols were

investigated further and Niskin bottles and pumps were described as the most commonly used methods for sampling, while deployed automatic sampling was mentioned only once.

Equally many researchers used open and closed/sterivex filtering systems, and some used both methods for filtering (Fig 13). The most commonly used mesh size was ~0.2 um, but some used larger mesh sizes and some used several for size fractioning samples.

Q: What is your typical filtering method?

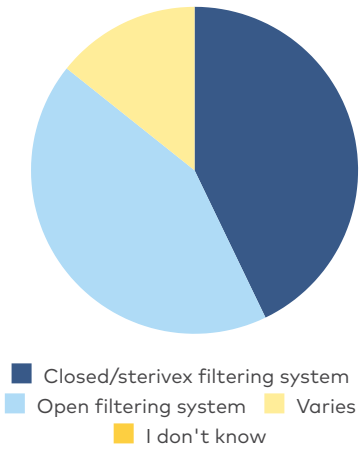


Figure 13. Sea water sampling methods (Researchers)

Concerning storage of filters, samples and DNA, the overall responses showed little consistency in the methods used (Fig 14). The filters were stored in -18 and -80 degrees Celsius, in ethanol and some responded that it varied depending on practicality or duration. The storage methods used for sediment and bulk samples were more restricted as they were consistently frozen, although both freezing temperatures were used. Respondents also stored DNA in various ways. Overall, freezing filters, samples and DNA was the most applied method for storage.

Q: How do you store the filters, samples and extracted DNA?

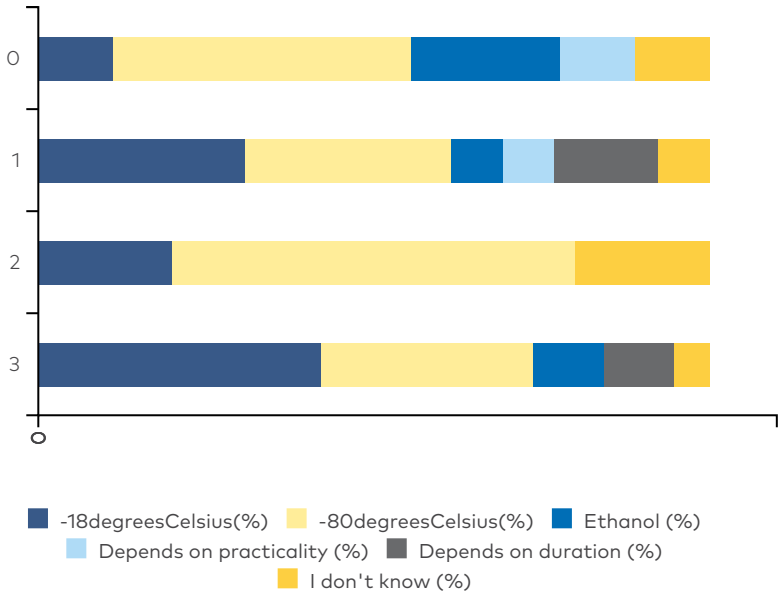


Figure 14. Storage of filters, samples and DNA (Researchers)

Researchers were also asked if and how they archive their filters, samples and DNA. The results showed that more than 40% of the filters, samples and DNA are not archived in any formal system (Fig 15). Between 40% and 50% of the researchers used an in-house Biobank system, while only about 7% archived their filter, samples and DNA in an open access Biobank system.

Q: How do you archive the filters, samples and extracted DNA?

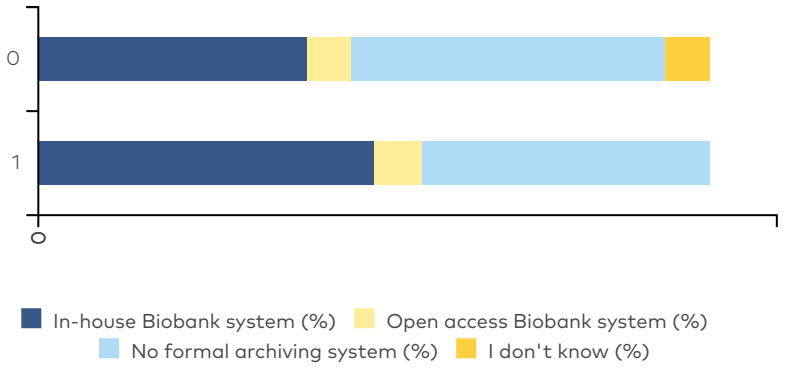


Figure 15. Archiving of filters, samples and DNA (Researchers)

A factor that complicates the issue of adhering to a standardized protocol is that the decision about which protocol to use is very seldom standard for any particular institution or department but relies on the individual researchers forming the projects (Fig 16).

Q: Who typically decides which protocol to use?

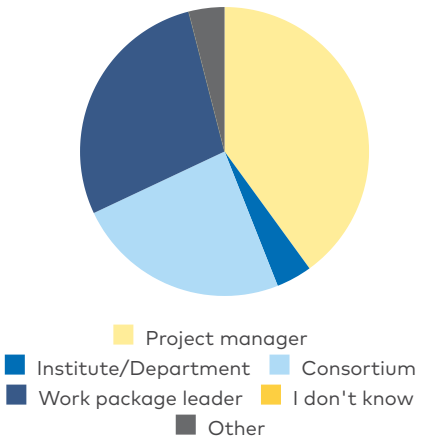


Figure 16. Decision of which protocol to use (Researchers)

According to the responses in the survey, this decision is often taken by the various project consortiums, the relevant work package leader or the project manager. This gives the leading researchers in the various projects a large responsibility in terms of standardization and alignment on a larger regional scale.

When asked what the choice of ptotocol typically is based on, 80% of the researchers stated that the choice is project or purpose based (Fig 17). In addition, over 25% said that the decision is based on practicality or opportunity.

Q: What is the choice of protocol based on?

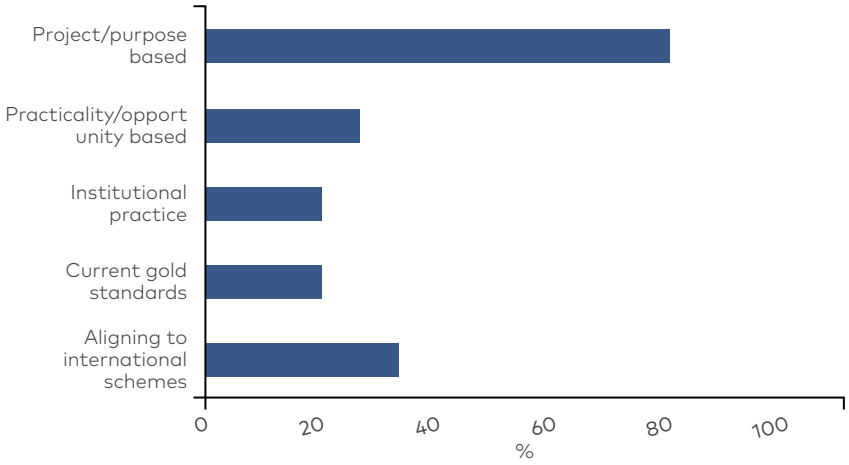


Figure 17. Basis for choice of ptotocol (Researchers)

However, there are attempts to align to current gold standards and/or international schemes as a third of the respondents stated that the choice of protocol is also based on an alignment to international schemes. Ocean Best Practices https://www.oceanbestpractices.org/ was recommended as a source for finding protocols to align to. In addition to specific protocols in various scientific papers, the web page also contains the OBON Community practices repository https://repository.oceanbestpractices.org/handle/11329/1804 , which includes more general and inclusive guidelines for working with eDNA such as the EU COST Action DNAqua-Net report (Bruce *et al.* 2021).

4.4 Implementation

In order to get an insight into the process of implementation of eDNA methods in marine monitoring in the respective countries, stakeholders were asked to state their view on at what stage the implementation was for the three focus areas in their respective countries.

From their responses, there seemed to be very few instances of eDNA methods presently being implemented (Fig 18). However, in the field of Biodiversity / Climate change 83.3% of the respondents stated that eDNA methods were currently being tested or planned for trial in this field.

Q: Overall, at what stage is implementation of eDNA methods in these fields in your country?

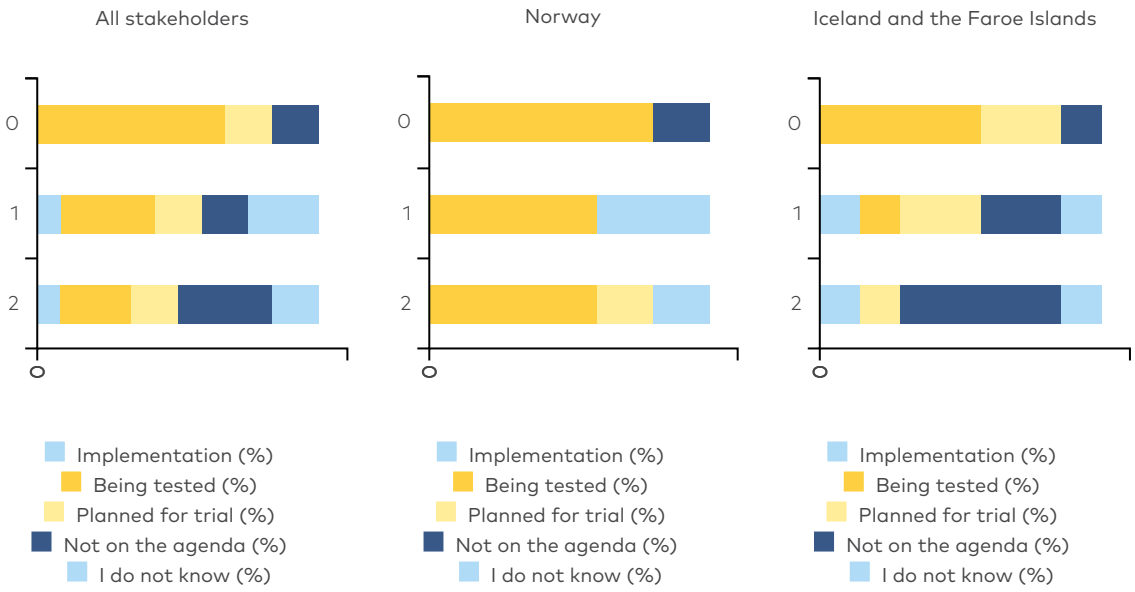


Figure 18. Stage of implementation of eDNA methods (Stakeholders)

For Marine Resource Management 25% of the stakeholders did not know what the current status was and 16.7% stated that eDNA methods were not on the agenda. However, almost 60% of the respondents knew of eDNA methods being implemented, tested or planned for trial. For Impact assessment, a third of the stakeholders stated that implementation was not on the agenda and an additional 16.7% did not know the status. Therefore, Impact assessment was the focus area that seemed to have the least progress in implementation of eDNA methods.

Another feature to be noticed is that there were several more responses from the Faroe Islands and Iceland, who had similar responses to this question, stating that the implementation of eDNA methods was not on the agenda than from Norway (Fig 18). Also, there were many different answers to the same questions, especially

for Iceland and the Faroe Islands, indicating that there are no national strategies or common understanding of the process of implementation. In Norway there seemed to be a slightly better consensus, and some Norwegian stakeholders commented that they intend to "highlight the need for standardization of methods and coordination of guidelines from the scientific community". In addition, they wish to address the infrastructure for the storage of data and reference materiale, samples, etc.

In comparison, in Finland there has been a coordinated work led by the Finnish Environment Institute (SYKE) to prepare a national strategy for the implementation of eDNA methods in monitoring (Norros *et al.* 2022) as well as guidelines for using eDNA in marine phytoplankton monitoring in Finland (Jerney *et al.* 2022). In Sweden, the LIFE-DNAquatic project, funded by the Swedish Environmental Protection Agency's Research Grant in collaboration with the Swedish Agency for Marine and Water Management, was initiated to establish guidelines for using eDNA methods for monitoring of aquatic environments in Sweden. This work resulted in two reports (Hellstrøm *et al.* 2021a, 2021b) that Swedish researchers in the field can adhere to.

These and other similar Nordic initiatives could provide an opportunity for others to draw inspiration from and consider in terms of international alignment in the process of forming their own eDNA strategies and guidelines.

In the UNIFIeD survey, stakeholders identified budget restrictions and lack of validation of eDNA methods as the main challenges for implementation (Fig 19). In the Faroe Islands and Iceland, where the responses again were similar, the lack of biomonitoring programs and low priority in the administration were also considered a major challenge. Overall, the stakeholders in Norway estimated the situations to be less problematic than the stakeholders in Iceland and the Faroe Islands as a third of the responses from Norway were "not a problem" while only 14.3% of the stakeholders in the Faroe Islands and Iceland chose that statement.

Q: What are the main challenges for implementation of eDNA methods in marine monitoring?

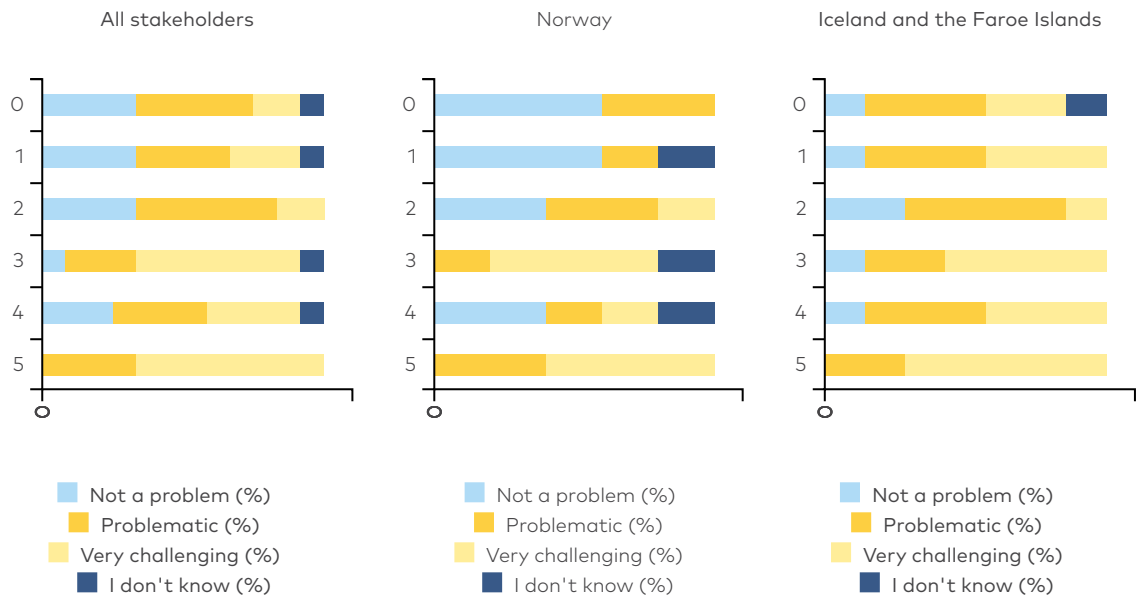


Figure 19. Main challenges for implementation (Stakeholders)

The researchers stated that the lack of national biomonitoring programs and lack of continuity in funding were the main challenges for the implementation process.

The higest ranking challenges as estimated by the researchers were:

1. Lack of national biomonitoring program
2. Lack of continuity in funding
3. Lack of validation of eDNA methods
4. Lack of standardization of eDNA methods

It seems that researchers and stakeholders do not disagree on what the major challenges are, although there are minor differences in ranking of the main issues. For researchers, biomonitoring programs and more long-term funding provide valuable opportunities for testing and validating eDNA methods, which the researchers also state is a challenge. Some relevant monitoring programs mentioned by the researchers are listed in Table 2.

Table 2. Monitoring programs mentioned by the researchers

ICES stock assessments
Marine Biodiversity Observation Network
Faroese Marine Ecosystem Observing Program (FAMEOS)
ØKOFERSK
EMO-BON, UNESCO eDNA expeditions

Stakeholders estimated that validating the eDNA methods was the most needed constructive measure (Fig 20) for the implementation process. Since the collective responses in the UNIFIeD survey seemed to illustrate that the validation and implementation of eDNA methods in marine monitoring requires a coordinated effort from researchers and stakeholders, a joint discussion between researchers and stakeholders on how to proceed might be a way forward.

Q: Please rank the following constructive measures according to what you think is most needed/wanted

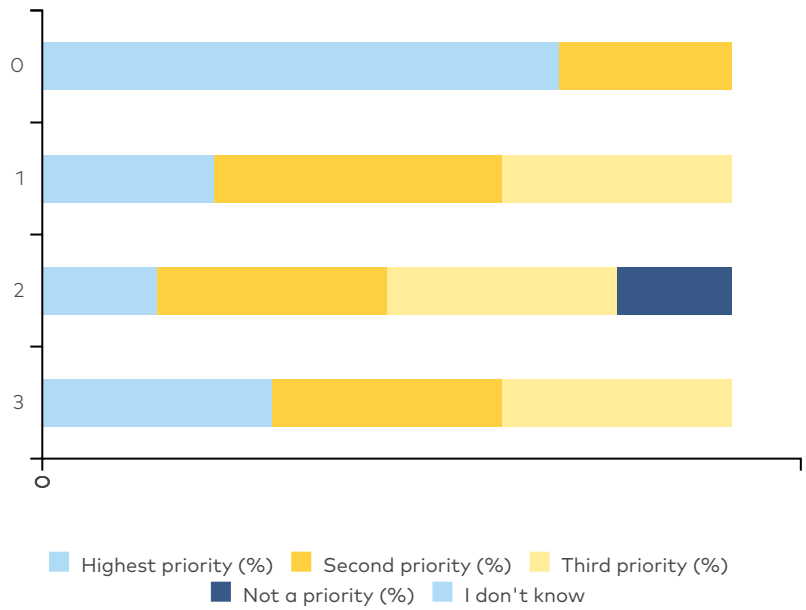


Figure 20. Constructive measures (Stakeholders)

5 Perspectives from the UNIFIeD workshop

A summarizing UNIFIeD workshop was held for project participants, survey contributers and other interested stakeholders. After the UNIFIeD project was introduced, results from the surveys were presented followed by thematic discussions.

Results related to the perception of eDNA were presented by Ian Salter from the Faroe Marine Research Institute. In the following discussion, workshop participants discussed various challenges they had experienced when stakeholders have had unrealistic expectations and requirements to the application of eDNA methods. This issue seemed to cause frustrations and solving this discrepency between researchers and stakeholders was recognised as having high priority for the implementation of eDNA methods in marine monitoring.

It was also acknowledged, that researchers have a responsibility of being clear about strenghts and weaknesses of eDNA methods towards stakeholders in order to promote a common understanding about the potential application of eDNA methods. Any misunderstandings could hamper the explotitation of this great potential of applying eDNA methods to marine monitoring.

At the same time, researchers have experienced difficulties relating these messages. However, the UNIFIeD project report including the survey results was mentioned by some as a potential tool and basis for engaging with stakeholder in constructive discussions and advancing a joint understanding between various partners.

The standardization challenges described in the survey results were presented by Christophe Pampoulie from the Marine and Freshwater Institute in Iceland. The related discussion demonstrated that obstacles for adhering to gold standard protocols were often practical and logistical issues such as cold storage during transport of samples and the frequent shifts between projects of short duration with varying oppertunities and settings. The will to follow recommended guidelines was not lacking, the issue was rather the lack of Institutional or National guidelines, leading to the dependency on consortium, project, and work package management.

In addition, the possibilities that biomonitoring programs and continuity in funding provide for the effort of establishing standardized protocols for eDNA methods were estimated to be highly valuable. Researchers with limited access to national scientific and political schemes supporting such activities are at a disadvantage and as a consequence the region lags behind in the effort to establish national

guidelines and taking part in international harmonization of eDNA methods.

Therefore, scientific and political schemes focusing on biomonitoring programs and continuity in funding were identified as key issues where stakeholders can add support for the advancement in standardization of eDNA methods.

The workshop participants also discussed the possibility of enhanced collaboration between the Nordic countries, combining our individual strengths in joint projects to tackle these hurdles and together strenghten the Northeast Atlantic marine research and monitoring efforts by the application of eDNA methods.

The discussion of establishing national guidelines for eDNA methods led to the question of how specific or inclusive such guidelines should be. The inclusive argument was that in order to promote standardization and collaboration beyond their own country, researchers should consider the feasability of researchers from other institutions and countries being able to follow these guidelines. Such guidelines should take into account that not all have the same equipment, surroundings, etc. On the other hand, some level of specificity needs to be witheld for results to be comparable.

Most agreed, that under any circumstances, such a process needs to be open for other parties, if the established guidelines are to be applicable on a larger scale.

Results concerning the implementation process were presented by Ása Jacobsen from the Aquaculture Research Station of the Faroes / PF Fiskaaling. Workshop participants agreed that the implementation process is complicated even though there are interested stakeholders. Consensus seemed to be that researchers should do their part in communicating with stakeholders and continuing working on standardization, but that the implementation requires a coordinated effort from various stakeholders in collaboration with the research community.

Several of the workshop attendees also stated an intent to increase research collaboration within the Nordic region on this issue and to use the knowledge gained from the UNIFIeD project to engage with relevant stakeholders about future efforts.

6 Concluding remarks and recommendations

The UNIFIeD project has produced an initial insight into the views of researchers and stakeholders in the Nordic region about the standardization and implementation of eDNA in marine monitoring. Although the number of participating researchers and stakeholders has been limited, it has facilitated increased international knowledge sharing and communication between key parties on this issue.

Since the UNIFIeD survey results are based on a limited number of responses, the report does not represent the views of all researchers and stakeholders in the Nordic region. However, it provides an excellent basis for enhanced communication between research communities and stakeholders about the potential and applicability of eDNA methods. It touches on important issues to discuss about the challenges of standardization and how stakeholders can facilitate an improvement in this area in collaboration with the research community.

For researchers in this field, a total alignment in all technical aspects may not be feasible. However, it is not required as long as the collaboration is based on an awareness of strengths and challenges in each country or region and is not hampered by misunderstandings and lack of insight into the circumstances of others. Future projects could benefit from building on this awareness, perform alignments when possible, and initiate collaboration on standardization and implementation based on this commen understanding.

An open and inclusive approach in the research community to standardization of eDNA methods and their application to marine monitoring is essential for international alignment and collaboration. Therefore, it would be highly valuable if research groups working on standardization of eDNA protocols would keep in mind the possibility of others to align to the developed protocol. An inclusive collaborative approach aught to be benficial for all parties, both researcher and stakeholders, and strengthen the position of the entire Nordic region in the application of eDNA methods in marine monitoring.

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Appendices

Appendix 1. Stakeholder survey questions

Q1. In which country is your place of employment? ***Greenland, Iceland, The Faroe Islands, Norway***

Q2. What is your place of work? ***Environmental Agency, Ministry, Advisory body, Research Council, Research Institution, Other (please specify)***

Q3. What best describes your job function? ***Management, Advisory role, Drafting policies, Coordinator, Civil servant***

Q4. What is your level of engagement in these areas of marine issues? ***Not on the agenda, Area of interest, Focus area***

- Biodiversity / Climate change
- Marine reseource management
- Impact assessment

Q5. What is your level of awareness of environmental DNA (eDNA) and your approach to it's implementation in marine monitoring? ***1–5***

- No knowledge | In-depth knowledge
- Do not recommend | Actively encourage implementation

Q6. Overall, at what stage is implementation of eDNA methods in these fields in your country? ***Implementation, Being tested, Planned for trial, Not on the agenda, I don't know***

- Biodiversity / Climate change
- Marine reseource management
- Impact assessment

Q7. What are the main challenges for implementation of eDNA methods in marine monitoring? ***Not a problem, Problematic, Very challenging, I don't know***

- Lack of insight in the Administration
- Low priority in the Administration
- Lack of information from the scientific community
- Lack of validation of the method
- Lack of monitoring programs to adhere to
- Budget restrictions

Q8. Do you agree with these statements about the benefits of implementation of eDNA? ***Agree, Disagree, I don't know***

- It can address issues in marine monitoring of international focus
- It can easily increase spatial and temporal sampling
- It can provide more effective monitoring
- It can more easily detect rare or invasive species
- It is a relatively non-invasive method

Q9. Do you think eDNA methods need to demonstrate directly comparable results with more traditional methods before implementation? ***Yes, No, Depends on the application, I don't know***

Q10. Please rank the following contructive measures according to what you think is most needed/wanted ***Highest priority, Second priority, Third priority, Not a priority, I don't know***

- Validation of eDNA methods
- Investigate short and long-term investment for cost-efficient eDNA monitoring
- Improve awareness within the Administration
- Demonstrate the need for eDNA methods

Appendix 2. Researcher survey questions

Q1. What type of job position do you have? ***Researcher, Technician (lab, field,..), Manager/coordinator (lab, ship, base,..)***

Q2. In which country or countries is your place of employment and where do you work/perform your sampling? ***Place of employment, Place of sampling/working***

- Greenland
- Iceland
- The Faroe Islands
- Norway
- Denmark
- UK
- Germany
- Sweden
- Finland

Q3. What kind of function does your workplace have? ***Research Institute, Environmental Agency, Private service company, Private R&D, Industry, University, Other (please specify)***

Q4. What is the aim of your marine research or what is your work most relevant for? ***Biodiversity / climate change, Marine resource management, Impact assessment***

Skip logic from Q4 "Biodiversity / climate change"

Q5. What is your focus? ***Climate change, Invasive species, Endangered species, Biodiversity baselines / comparisons. Other (please specify)***

Q6. What environment or habitat are you focusing on? ***Coastal water, Open ocean, Seabed, Kelp forests, Other (please specify)***

Q7. Which larger taxonomic group are you working with? ***Bacteria, Phytoplankton, Zooplankton, Invertebrates, Fish, Marine mammals, Other (please specify)***

Q8. What approach do you use? ***Targeted – presence/absence, Targeted – abundance estimate, Targeted – population genetics, Metabarcoding – community pattern and processes, Other (please specify)***

Q9. What type of studies are you working on? ***Within the Institute, National collaboration, International collaboration***

- Pilot studies
- Technical development
- Research projects
- Implementation process
- Established Biomonitoring program

Skip logic from Q9 "Established Biomonitoring program"

Q10. Optional. Please provide reference(s) for the biomonitoring program(s) you work with

Skip logic from Q4 "Marine resource management"

Q11. Which marine resources/organisms are you working with? ***Pelagic fish, Demersal fish, Invertebrates, Other (please specify)***

Q12. What best fits the focus of your research? ***Stock estimates of economically relevant species, Threat of invasive species, Protection of at-risk species, Food availability, Ecosystem dynamics***

Q13. What approach do you use? ***Targeted – presence/absence, Targeted – abundance estimate, Targeted – population genetics, Metabarcoding – community pattern and processes, Other (please specify)***

Q14. What type of studies are you working on? ***Within the Institute, National collaboration, International collaboration***

- Pilot studies
- Technical development
- Research projects
- Implementation process
- Established resource management

Skip logic from Q14 "Established resource management"

Q15. Optional. Please provide reference/link to the established management program

Skip logic from Q4 "Impact assessment"

Q16. What environment or habitat are you focusing on? ***Seabed, Kelp forest, Coastal, Open ocean, Other (please specify)***

Q17. Which circumstances are you investigating for potential impact? ***Aquaculture industry, Oil drilling industry, Resource harvesting industry, Other pollution, Human activity/traffic, Other (please specify)***

Q18. Which larger taxonomic group are you working with? ***Bacteria, Phytoplankton, Zooplankton, Invertebrates, Fish, Marine mammals, Other (please specify)***

Q19. What approach do you use? ***Targeted – presence/absence, Targeted – abundance estimate, Targeted – population genetics, Metabarcoding – community pattern and processes, Other (please specify)***

Q20. What type of studies are you working on? ***Within the Institute, National collaboration, International collaboration***

- Pilot studies
- Technical development
- Research projects
- Implementation process
- Established monitoring

Skip logic from Q20 "Established monitoring"

Q21. Optional. Please provide reference(s) for the established monitoring work

Q22. What are your typical project roles? ***Administrative support, Technical support, Project management, Researcher***

Q23. How are your projects funded? ***½–2 years, 3–5 years, Long term monitoring***

- In-house funding
- National research funds
- International research funds
- Industry funding
- National policy based

Q24. Which sample types do you work with and do you use a standard protocol for each sample type? ***Always the same protocol, Same protocol if possible, Various protocols, Standard for some procedures only***

- Sea-water
- Sediment or bulk samples (i.e. settlement plates, plankton trawls,..)

Q25. Who typically decides which protocols to use? ***Project manager, Institute/Department, Consortium, Work package leader, I don't know, Other (please specify)***

Q26. What is the choice of protocol based on? ***Project/purpose based, Practicality/opportunity based, Institutional practice, Current gold standards, Aligning to international schemes, I don't know***

Q27. Optional. Which international sampling protocols do you align to and/or recommend?

Skip logic from Q24 "Sea-water"

Q28. How do you typically collect your samples? ***Deployed Automatic samplers, Automatic samplers on ships, pumps, Niskin bottles / water samplers, I don't know, Other (please specify)***

Q29. What is your typical filtering method? ***Closed/Sterivex filtering system, Open filtering system, Varies, I don't know***

Q30. What mesh size(s) do your filters typically have? ***~0.2µm, ~0.4 µm, ~0.7 µm, 10–20 µm, > 20 µm***

- Whole sample
- Size fractioned sample

Q31. How do you store the filters, samples and extracted DNA? ***-18 ºC, -80 ºC, Ethanol, Depends on practicality, Depends on duration, I don't know***

- Sterivex filter
- Other filters
- Sediment/bulk samples
- DNA

Q32. How do you archive the filters, samples and extracted DNA? ***In-house Bioank system, Open access Biobank system, No formal archiving system, I don't know***

- Filters / samples
- DNA

Q33. Do you have access to the DNA for other projects/purposes at a later time if needed? ***Yes, Yes - by request, I don't know, No, For some projects only***

Q34. Do you sometimes compare eDNA methods with more traditional methods in your work? ***Yes, No***

Skip logic from Q34 "Yes"

Q35. How explicit are your comparisons)? ***Sampling as similar in time and space as possible for eDNA and other method(s), Relatively independent sampling strategies but with same objective, A mixture of both depending on project***

Q36. What do you compare between eDNA and other methods in your work and what are the results? ***Concordance, Discordance, Varying results***

- Diversity
- Abundance
- Biomass
- Community structure
- Presence/absence

Q37. Do you think eDNA methods need to be directly comparable with other traditional method(s) before implementation? ***Yes, No, Undecided, Depends on application***

Q38. What are the main challenges for standardization/alignment of eDNA protocols (not including downstream analysis)? ***Main challenge, Problematic, Not a problem, I don't know***

- Sample collection
- Financial issues
- How to select protocol to align to
- Lack of knowledge about how others work
- Lack of communication between researchers/Institutes
- eDNA protocols are still in a developmental stage

Q39. What are the main challenges for implementation of eDNA methods in established biomonitoring programs? ***Main challenge, Problematic, Not a problem, I don't know***

- Policy issues
- Lack of continuity in funding
- Lack of Institutional support
- Lack of National biomonitoring programs

- Lack of standardization of eDNA methods
- Lack of validation of eDNA methods
- Lab infrastructure
- Local know-how

About this publication

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A basis for researchers and stakeholders to discuss challenges and ambitions

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Ása Jacobsen, Amanda Vang, Ian Salter, Thomas Juul-Pedersen, Sæmundur Sveinsson, Christophe Pampoulie, Owen Wangensteen, Kim Præbel, Svein-ole Mikalsen, Anni Djurhuus, Snæbjørn Pálsson, Davíð Gíslason

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The Nordic Council of Ministers
Nordens Hus
Ved Stranden 18
DK-1061 Copenhagen
pub@norden.org

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