



Nordic Council
of Ministers

Reliable environmental DNA detection of planktonic biodiversity

Minimum sample volume
for DNA metabarcoding



Contents

Authors and Affiliations	3
Summary	5
1. Introduction	6
2. Methods	8
3. Microscopic analysis	11
4. Results and Discussion	12
Future Perspectives	17
Acknowledgements	18
References	19
Appendix 1. eDNA sampling and filtration for testing different sample volumes	22
Appendix 2. DNA extraction and amplification protocols	25
Appendix 3. Harmful Algal bloom species detected from different samples	29
About this publication	31

This publication is also available online in a web-accessible version at:
<https://pub.norden.org/temanord2025-509>

Authors and Affiliations

Sirje Sildever	Department of Marine Systems, Tallinn University of Technology, Estonia (TalTech)
Bente Edvardsen	Department of Biosciences, University of Oslo, Norway (UiO)
Elianne Egge	Norwegian Institute for Water Research, Norway (NIVA)
Anette Engesmo	Norwegian Institute for Water Research, Norway (NIVA)
Sara Harðardóttir	Marine and Freshwater Research Institute, Iceland (MFRI)
Ása Jacobsen	Firum, The Faroe Islands (Firum)
Hans Jakobsen	Department of Ecoscience, Aarhus University (AU)
Iveta Jurgensone	Latvian Institute of Aquatic Ecology, Latvia, (LHEI)
Bengt Karlson	Swedish Meteorological and Hydrological Institute, Sweden (SMHI)
Anke Kremp	Leibniz-Institute for Baltic Sea Research Warnemünde, Germany (IOW)
Tiina Laamanen	Finnish Environment Institute, Finland (Syke)
Sirpa Lehtinen	Finnish Environment Institute, Finland (Syke)
Markus Majaneva	Norwegian Institute for Nature Research, Norway (NINA)
Kristian Meissner	Finnish Environment Institute, Finland (Syke)
Kenneth Mertens	Ifremer, COAST, F-29900 Concarneau, France (Ifremer)
Satoshi Nagai	Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Japan
Erna Lava Olsen	Firum, The Faroe Islands (Firum)
Ian Probert	Roscoff Culture Collection, France

Kristian Pärt	Department of Marine Systems, Tallinn University of Technology, Estonia (TalTech)
Nelli Rünk	Department of Marine Systems, Tallinn University of Technology, Estonia (TalTech)
Ingrid Sassenhagen	Leibniz-Institute for Baltic Sea Research Warnemünde, Germany (IOW)
Sanna Suikkanen	Finnish Environment Institute, Finland (Syke)
Aouregan Terre-Terrillon	Ifremer, COAST, F-29900 Concarneau, France (Ifremer)
Anders Torstensson	Swedish Meteorological and Hydrological Institute, Sweden (SMHI)
Kristiina Vuorio	Finnish Environment Institute, Finland (Syke)

Summary

Environmental DNA (eDNA)-based detection has become an attractive method for determining biodiversity as it can provide more detailed information compared to traditional morphology-based approaches. This is especially relevant for targeting overall biodiversity or screening for the presence of harmful algal bloom (HAB) species. However, despite the availability of several general guidelines, the workflow for marine environments has yet to be standardised. This lack of standardisation complicates the comparison between samples across different monitoring programmes and projects. This project focused on the first step of the workflow – determining and standardising the minimum reliable sample volume needed to detect phytoplankton diversity under various environmental and biological conditions. A study and a workshop were conducted to test different sample volumes (1000 mL, 500 mL, 200 mL, and 20 mL), using samples collected from the Baltic and North Seas and northwestern Atlantic Ocean under various environmental conditions. Across the studied samples, 200 mL was identified as a sufficient sample volume to detect various species across the different volumes tested. This result represents an important first step towards standardising the eDNA-based approach and incorporating it into routine monitoring. Furthermore, the results of this project are expected to contribute to reducing the time and resources required for monitoring.

1. Introduction

Environmental DNA (eDNA) is generally defined as DNA present in the environment, including the DNA inside single-celled organisms. High-throughput DNA-based community analysis methods and their resulting datasets have unveiled previously unimagined biodiversity (Sogin et al. 2006). However, questions remain on how to optimise the “depth of the investigation” through sampling schemes and strategies, particularly concerning the number of samples, the number of sequences analysed per sample, and the sample volume. The significant reduction in sequencing costs has eased constraints on the first two aspects and made DNA-based analyses more feasible for inclusion in marine monitoring programmes. This transition to incorporating eDNA-based analyses in monitoring efforts has been facilitated by the research community, which has developed laboratory protocols and adopted genetic markers with relative consistency. However, minimum reliable standards for various steps in the protocol, such as the sample volume, have not yet been established.

Current phytoplankton monitoring relies on standardised methods, such as those outlined in the HELCOM COMBINE manual (HELCOM 2017) and OSPAR CEMP guidelines (OSPAR 2016), to ensure comparability between samples and laboratories. Species identification in these methods is based on morphological differences observed using a light microscope. However, species that are small (<20 µm), exhibit variable morphology, or lack distinctive characteristics are challenging to identify reliably with this approach. Often, phytoplankton analysis also includes some consumers that are important for understanding energy transfer in the marine food webs. Those are primarily microzooplankton species, e.g. a ciliate species *Mesodinium rubrum*. However, similarly to the phytoplankton, there are the same difficulties in reliable species identification for microzooplankton. eDNA offers a potential solution by enabling identification of such species, as it is not dependent on the cell size or morphology. However, curated reference databases are needed to reliably identify the species based on DNA sequences.

Internationally agreed standards are needed to adopt eDNA-based methodologies in routine monitoring and facilitate comparison between samples. Currently, no such standards exist for detecting marine species and only best practices, guidelines, and protocols are available (Yarimizu et al. 2020; Salomasso et al. 2021a, 2021b; Andersson et al. 2022; Jerney et al. 2022, 2023; Andersson et al. 2024). This gap is due to the ongoing rapid development of methods and differences in infrastructures, budgets, and expertise across countries and institutes (Jacobsen et al. 2023).

The focus of this project is on the sample volume used for eDNA-based detection, as the volume of water filtered determines the sampling effort. In principle, filtering higher volumes of water (up to several litres), increases the likelihood of detecting rare species, including harmful algal bloom (HAB) species. However, the absence of established upper and lower limits for sample volume can lead to unnecessary time spent on filtration.

A prerequisite for establishing an eDNA-based monitoring standard is determining a sufficient sampling effort. To address this, an experiment was conducted in which eDNA metabarcoding was applied to samples of varying water volumes collected from different locations with varying biomass levels. By keeping all other variables constant, this approach allowed to determine the minimum reliable sample volume required for assessing phytoplankton (including microzooplankton) biodiversity and detecting HAB species. The findings of this study not only increase the cost-effectiveness of eDNA-based monitoring approaches but also ensure comparability between samples, contributing to the standardisation of eDNA methodologies.

2. Methods

2.1 Sampling and filtration

Samples were collected from 13 locations in the Baltic Sea, the North Sea, and the northwestern Atlantic Ocean (Fig. 1, Table 1). Sampling and filtration were conducted following the same protocol (Appendix 1). Briefly, about 5.5 L of seawater was collected from each location and used for both the minimum sample volume experiment and traditional morphology-based phytoplankton analysis (Fig. 2). The seawater was thoroughly mixed, and subsamples of varying volumes (1000 mL, 500 mL, 200 mL, 20 mL) were taken for filtration. Different sample volumes were individually filtered through 0.4 μ m pore size membrane filters (Whatman, product nr. 10417712) in triplicates. After filtration, the filters were stored at -20 °C to prevent DNA degradation. Samples intended for microscope analysis were preserved with acid Lugol's or formalin solution according to the routine monitoring protocols used by the participating institutes.

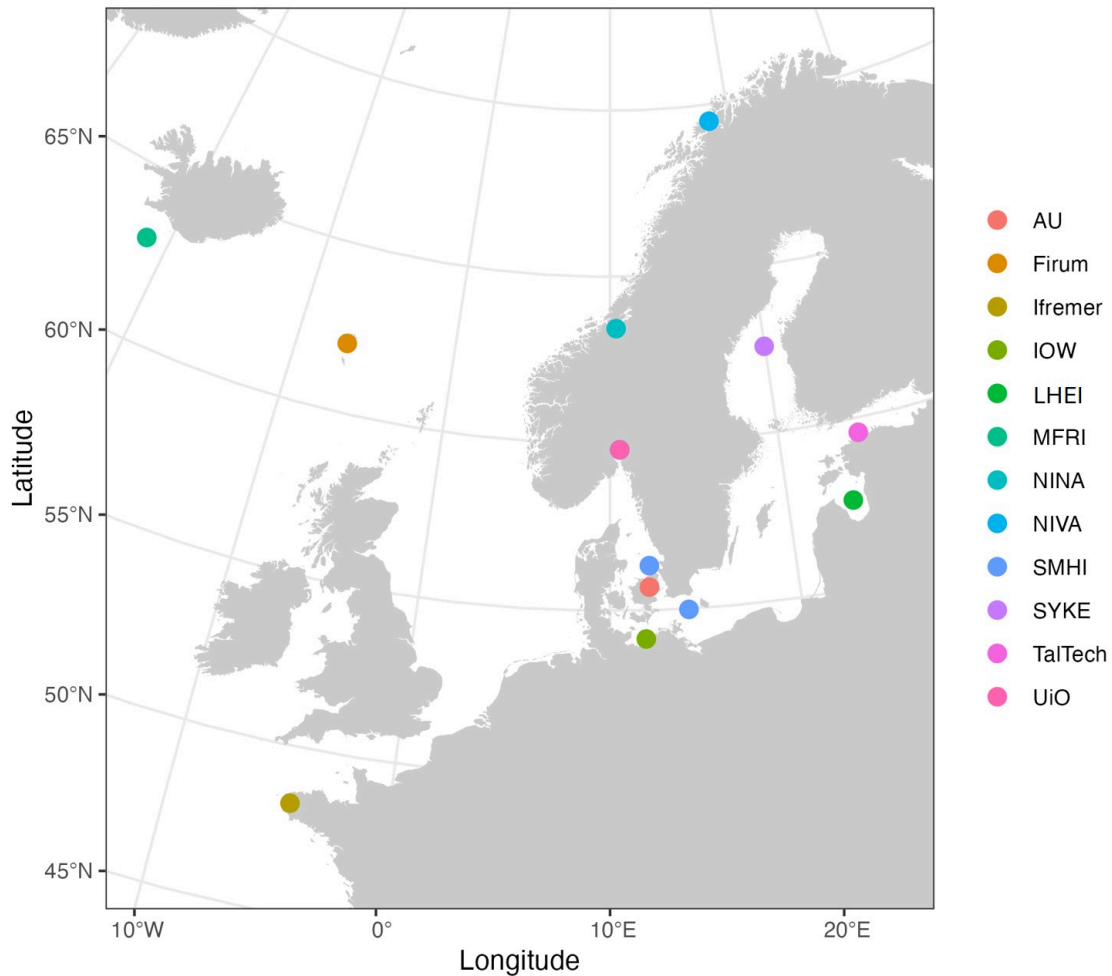


Figure 1. Sampling locations. The full names of the participating organizations are provided under Authors and Affiliations.

2.2 DNA extraction, amplification, and bioinformatics analyses

DNA was extracted from the filters and amplification of the DNA was done using the same protocol for all samples (Appendix 2). DNA was extracted using a commercial kit (Qiagen, product nr. 69506) and universal molecular markers targeting the V7-V9 variable region of the 18S ribosomal gene (Dzhembekova et al. 2018) were used. Success of the amplification was confirmed via gel electrophoresis. The samples were purified, amplified with index primers, and sequenced at the Genomics Core Facility, University of Tartu, Estonia. The resulting

sequences were analysed using an in-house bioinformatics pipeline (Nagai et al. 2022). Taxonomic identification of the sequences was performed using the NCBI database.

2.2 Data analysis

Sequences associated with fungi, macroalgae, and vertebrates were removed from the final dataset to focus exclusively on phytoplankton and microzooplankton species. To ensure reliable identification of species at the highest taxonomic level using the eDNA approach, only sequences with $\geq 99\%$ similarity to entries in the database were included in the final analysis. To compare differences between sample volumes, an analysis of variance (ANOVA) test was conducted based on average phytoplankton and microzooplankton abundance per volume. Harmful algal bloom species were determined based on the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Lundholm et al. 2024).

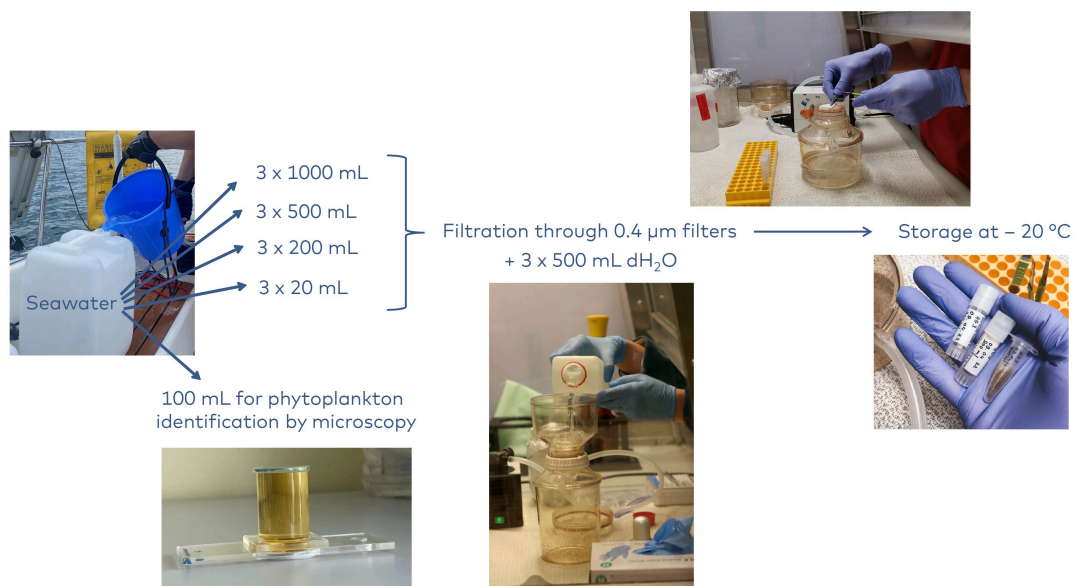


Figure 2. Overview of sampling and filtration. Photos by Sirje Sildever, Marlene Kaljumäe, and Kristian Pärt.

3. Microscopic analysis

The Utermöhl method (Utermöhl 1931; 1958; Edler & Elbrächter, 2010; HELCOM 2024) was used for the microscopic analysis of the samples. Organisms were identified based on their taxonomy and examples of identified phytoplankton species are shown in Fig. 3. From the 100 mL sample collected for phytoplankton analysis, 10-25 mL was sedimented for analysis using a special sedimentation chamber and a bottom counting slide (Fig. 2). The analysis was conducted based on the methods used in each institute for routine phytoplankton monitoring.

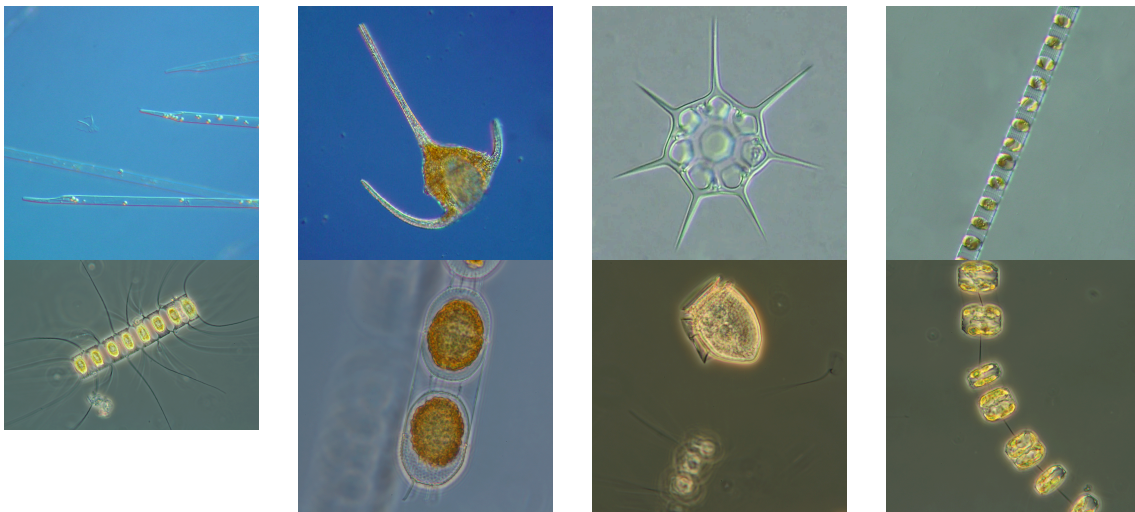


Figure 3. Examples of phytoplankton species observed in the samples using microscopy. The photos shown are generally from other samples collected as part of long-term monitoring programmes. The photos do not have the same scale.

Top row: left: *Proboscia alata* (a diatom), top middle left: *Tripos muelleri* (a dinoflagellate), top middle right: *Octactis speculum* (a silicoflagellate), top right: *Skeletonema marinoi* (a diatom). Photos by Bengt Karlson.

Bottom row: left: *Chaetoceros diadema*, bottom middle left: *Stephanopyxis turris* (both diatoms), bottom middle right: *Dinophysis acuta* (a dinoflagellate), bottom right: *Thalassiosira nordenskiöldii* (a diatom). Photos by Sara Harðardóttir.

4. Results and Discussion

The number of phytoplankton (including microzooplankton) species detected from the different sample volumes showed no trend in terms of sample volume (Table 1). In some instances, the highest number of species was detected from the smallest sample volumes (20 mL and 200 mL). However, the majority of detected species were consistently present across all sample volumes (Figure 4). This indicates that the sample was well-mixed before subsampling for different volumes and that all tested sample volumes are suitable for detecting most of the species present in the sample.

This finding is further supported by statistical analysis of the differences in the average number of species detected per sample volume (Table 2). For most samples, no statistically significant difference ($p > 0.05$) was observed between the average number of species detected across the sample volumes. Significant differences were identified only for the SMHI_Anholt E and SYKE samples. In the case of SMHI_Anholt E sample, further statistical testing revealed no significant differences between specific sample volumes. This suggests that variability across all sample volumes led to statistically significant result when testing all sample volumes together, but the differences between specific volumes individually were not large enough to show significance. Thus, this result was interpreted as having no meaningful differences between the sample volumes. In the case of the SYKE sample, a statistically significant difference was observed between the 20 and 500 mL sample volumes. This could potentially be attributed to how the sample was mixed prior to taking subsamples. Vigorous mixing of the sample may cause cells to burst, leading to the release of intracellular DNA. This could result in uneven detection of species across sample volumes and may also explain the detection of unique species in specific sample volumes.

Sample	Volume (mL)				Morph	Shared	HAB
	1000	500	200	20			
SMHI_Anholt E	80	88	97	92	52	7	8
SMHI_BY2	50	67	69	64	OW	OW	5
SYKE	91	95	79	80	48	8	4
MFRI	94	93	98	101	40	16	13
UiO	109	72	86	85	OW	OW	5
NIVA	ND	120	117	114	36	6	5
NINA	105	112	111	103	20	1	4
TalTech	136	138	135	128	24	5	2
IOW	99	OW	OW	OW	25	1	2
LHEI	87	89	91	80	56	9	4
AU	ND	ND	140	117	OW	OW	0
Firum	103	119	122	105	16	10	12
Ifremer	162	153	168	156	30	4	13

Table 1. Overview of average number of phytoplankton (incl. microzooplankton) species detected in different sample volumes and morphology-based detection (Morph). Shared: the number of species detected by eDNA and morphology, HAB: harmful algal bloom species detected based on eDNA, OW: ongoing work; ND: no data

When comparing the number of phyto- and microzooplankton species detected by eDNA and morphology, the species numbers identified by morphology were notably lower (Table 1). This difference can be attributed to the ability of eDNA to identify species that are either too small (<20 µm) or lack distinctive external characteristics required for species level identification. Additionally, the number of shared species between all sample volumes combined and morphology-based detection was relatively low, with a maximum of 16 shared species (Table 1).

However, morphology-based identification also detected some species that were not identified by eDNA. This discrepancy could be due to the absence of DNA sequences for those species in the public databases used for taxonomic assignment. This may also be influenced by the choice of molecular marker, as some markers are more effective at differentiating specific groups or genera. To address this limitation and capture a broader diversity, the use of several molecular markers has been recommended (Sildever et al. 2021). Moreover, discrepancies could arise from taxonomic inconsistencies, whereas the same species might be assigned different names in morphological and genetic databases. This highlights the need for improved integration and standardisation between morphological and molecular taxonomic frameworks.

Sample	Shapiro-Wilk test		Levene`s test			ANOVA			Tukey HSD
	W	p	F	Pr(>	df	F	Pr(>	df	
SMHI_Anholt E	0.90	0.16	1.42	0.31	8	5.03	0.03	8	NS
SMHI_BY2	0.97	0.88	0.68	0.59	8	1.87	0.21	8	
SYKE	0.96	0.77	0.30	0.83	8	4.81	0.03	8	20 vs 500 mL, S
MFRI	0.93	0.35	0.66	0.60	8	0.34	0.80	8	
UiO	0.92	0.27	0.97	0.45	8	1.24	0.36	8	
NIVA	0.98	0.98	2.01	0.22	6	0.75	0.51	6	
NINA	0.93	0.39	0.65	0.61	8	0.58	0.65	8	
TalTech	0.95	0.57	1.24	0.36	8	0.34	0.80	8	
LHEI	0.96	0.77	0.22	0.88	7	4.15	0.06	7	
Firum	0.94	0.49	1.28	0.35	8	1.81	0.22	8	
Ifremer	0.91	0.24	0.08	0.97	8	0.57	0.65	8	

Table 2. Statistical analysis of differences between the average number of species

detected by each sample volume. Samples from IOW and AU are not included due to work in progress. NS: not statistically significant ($p > 0.05$), S: statistically significant ($p < 0.05$).

The number of harmful algal bloom (HAB) species detected based on eDNA ranged from 2 to 13, depending on the sampling location (Table 1, Appendix 3). When comparing the HAB species detected across different sample volumes, a 200 mL sample volume reliably detected all HAB species present in the various sample volumes. In contrast, the detection of HAB species from the 20 mL sample was inconsistent. Based on these findings, it is recommended to use 200 mL as the reliable minimum sample volume for identifying phyto- and microzooplankton diversity as well as for detecting HAB species. Furthermore, the study also demonstrated that sample volumes between 200 mL to 1000 mL, filtered according to the described protocol, can be readily compared. This is similar to the findings reported by Latz et al. (2024), where different sample volumes (500mL, 200 mL, 100 mL, and 10 mL) were compared in terms of species richness, α - and β -diversity. They also found that from sample volume of 200 mL the diversity and richness reached a plateau and variation between replicates decreased from that sample volume.

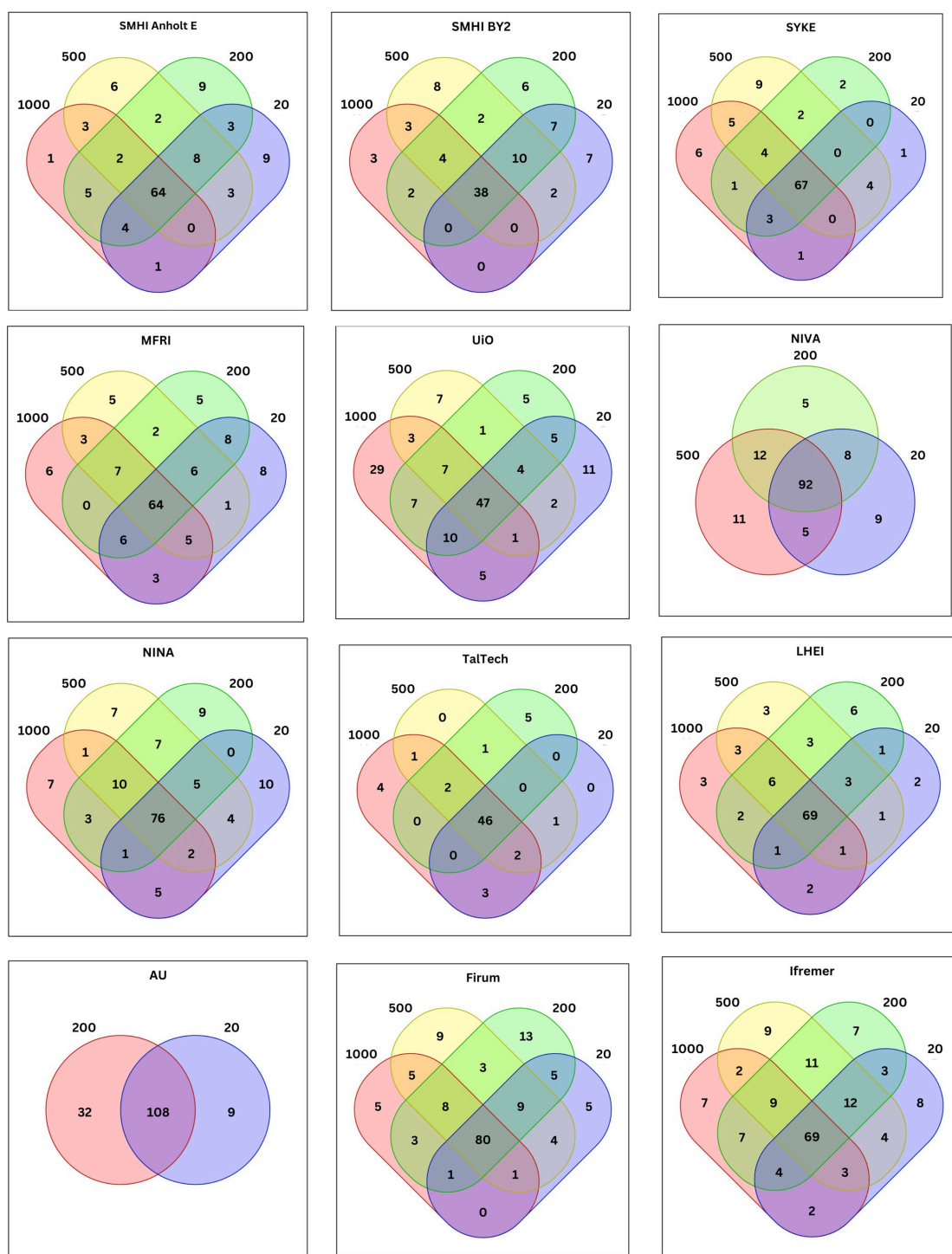


Figure 4. Venn diagrams for each sample displaying the overlap and differences in species detected between the different sample volumes (1000 mL, 500 mL, 200 mL, and 20 mL).

Future Perspectives

Detecting the minimum reliable sample volume is the first step toward standardizing the eDNA metabarcoding for detecting marine phytoplankton (including microzooplankton) species. Future work focusing on harmonizing the subsequent steps in the workflow is needed. For monitoring purposes, it would be beneficial to utilize a consistent molecular marker between projects and monitoring programs. Therefore, as a next step, comparison of different molecular markers could be conducted to identify the universal marker most suitable for detecting a wide range of phytoplankton species. Additionally, it is crucial to improve reference databases with taxonomically verified sequences from various phytoplankton species to ensure accurate taxonomic identification of species based on eDNA.

Acknowledgements

We would like to thank the student volunteers Marlene Kaljumäe, Pille Leesmäe, and Laura Johanna Kadak, for their help with organizing the workshop. We also appreciate Maria Cecilia Sarmiento Guerin and her team for providing the lab space and equipment for the workshop. Special thanks go to Philipp Braun for his assistance with collecting the water samples and conducting microscopic cell counts for IOW, Madeleine Nilsson for collecting and filtering the SMHI samples, Kristín J. Valsdóttir for microscopic counting of phytoplankton at the MFRI, Anne Doner for microscopic cell counts in Ifremer, Eiríkur Danielsen for microscopic cell counts and Elin Egholm for filtering the water samples at Firum, Maria Immonen for sample collection and filtering, Johanna Oja and Heidi Hällfors for the microscopical analysis of the Syke samples and Lubova Baraskova for collecting and filtering the LHEI samples.

This project was funded by the Nordic Council of Ministers (Project 240002).

References

Andersson A, Karlson B, Andersson AF, Brugel S, Latz M, Lycken J, Hedblom M, Torstensson A, Lindh M (2022). DNA extraction protocol for DNA-metabarcoding of marine phytoplankton using Zymobiomics DNA minprep kit (Zymo Research; D4300). Protocols.io. Retrieved from [dx.doi.org/10.17504/protocols.io.bucjnsun](https://doi.org/10.17504/protocols.io.bucjnsun)

Andersson A, Karlson B, Andersson AF, Torstensson A, Brugel S, Latz MAC, Jurdzinski KT, Hedblom M, Lycken J, Lindh MV (2024). DNA-streckkodning av marina växtplankton - ett nytt verktyg i miljöövervakningen. Rapport 7143. 75 pp. Naturvårdsverket. Retrieved from: <https://www.naturvardsverket.se/49e041/globalassets/media/publikationer-pdf/7100/978-91-620-7143-1.pdf>

Dzhembekova N, Moncheva S, Ivanova P, Slabakova N, Nagai S (2018). Biodiversity of phytoplankton cyst assemblages in surface sediments of the Black Sea based on metabarcoding. *Biotechnology and Biotechnological Equipment* 32(6), 1507–1513. DOI: <https://doi.org/10.1080/13102818.2018.1532816>

Edler L, Elbrächter M (2010). The Utermöhl method for quantitative phytoplankton analysis, In: Karlson, B., Cusack, C., Bresnan, E. (Eds.), *Microscopic and molecular methods for quantitative phytoplankton analysis*. UNESCO, Paris, pp. 13-20. Retrieved from: <https://unesdoc.unesco.org/ark:/48223/pf0000187824>

HELCOM (2017). Manual for Marine Monitoring in the COMBINE Programme of HELCOM. Retrieved from: <https://helcom.fi/wp-content/uploads/2020/02/Manual-for-Marine-Monitoring-in-the-COMBINE-Programme-of-HELCOM.pdf>

HELCOM (2024). HELCOM Guidelines on monitoring of phytoplankton species composition, abundance and biomass. Retrieved from: <https://helcom.fi/wp-content/uploads/2024/09/Guidelines-for-monitoring-of-phytoplankton-species-composition-abundance-and-biomass.pdf>

Jacobsen Á, Vang A, Salter I, Juul-Pedersen T, Sveinsson S, Pampoulie C, Wangensteen O, Præbel K, Mikalsen S-O, Djurhuus A, Pálsson S, Gíslason D (2023). Perspectives on implementation of eDNA methods in Northeast Atlantic marine monitoring: A basis for researchers and stakeholders to discuss challenges and ambitions. Copenhagen: Nordisk Ministerråd. DOI: <https://doi.org/10.6027/temanord2023-517> [F1]

Jerney J, Hällfors H, Oja J, Reunamo A, Suikkanen S, Lehtinen S (2022). Guidelines for using environmental DNA in Finnish marine phytoplankton monitoring.

- Improved biodiversity assessment through method complementation. *Reports of the Finnish Environment Institute* 40/2022. Retrieved from <https://helda.helsinki.fi/handle/10138/351131>
- Jerney J, Hällfors H, Jakobsen H, et al. (2023). DNA metabarcoding: Guidelines to monitor phytoplankton diversity and distribution in marine and brackish waters. Copenhagen: Nordisk Ministerråd. DOI: <https://doi.org/10.6027/temanord2023-505>
- Latz MAC, Andersson A, Brugel S et al. (2024). A comprehensive dataset on spatiotemporal variation of microbial plankton communities in the Baltic Sea. *Sci Data* 11, 18. DOI: <https://doi.org/10.1038/s41597-023-02825-5>
- Lundholm N, Churro C, Escalera L, Fraga S, Hoppenrath M, Iwataki, M, Larsen J, Mertens K, Moestrup Ø, Murray S, Tillmann U, Zingone A (2024). IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Retrieved from: <https://www.marinespecies.org/hab/>
- Nagai S, Sildever S, Nishi N, Tazawa S, Basti L, Kobayashi T, Ishino Y (2022) Comparing PCR-generated artifacts of different polymerases for improved accuracy of DNA metabarcoding. *Metabarcoding and Metagenomics* 6: e77704. DOI: <https://doi.org/10.3897/mbmg.6.77704>
- OSPAR, 2016. OSPAR Coordinated Environmental Monitoring Programme (CEMP). OSPAR Agreement 2016-01. Retrieved from: <https://www.ospar.org/documents?v=32943>
- Salmaso N, Riccioni G, Pindo M, Kurmayer R, Vasselon V, Domaizon I (2021a). Metabarcoding protocol – Analysis of protists using the 18S rRNA gene and a DADA2 pipeline (Version 1). *Zenodo*. DOI: <https://doi.org/10.5281/zenodo.5233527>
- Salmaso N, Riccioni G, Pindo M, Vasselon V, Domaizon I, Kurmayer R (2021b). Metabarcoding protocol – Analysis of Bacteria (including Cyanobacteria) using the 16S rRNA gene and a DADA2 pipeline (Version 1). *Zenodo*. DOI: <https://doi.org/10.5281/zenodo.5232772>
- Sildever S, Laas P, Kolesova N, Lips I, Lips U, Nagai S (2021). Plankton biodiversity and species co-occurrence based on environmental DNA – a multiple marker study. *Metabarcoding and Metagenomics*, 5, 175–197. DOI: <https://doi.org/10.3897/mbmg.5.72371>
- Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". *PNAS*, 103(32),12115-20. DOI: <https://doi.org/10.1073/pnas.0605127103>.
- Utermöhl H (1931). Neue Wege in der quantitativen Erfassung des Planktons (mit besonderer Berücksichtigung des Ultraplanktons). *Verh. int. Ver. theor. angew. Limnology* 5(2), 567-596.

Utermöhl H (1958). Zur Vervollkommen der quantitativen Phytoplankton-Methodik. Mitt. int. Ver. ther. angew. *Limnology* 9, 1-38.

Yarimizu K, Fujiyoshi S, Kawai M, Norambuena L, Cascales E-K, Rilling JI, Vilugrón J, Cameron H, Vergara K, Morón-López J, Acuña JJ, Gajardo G, Espinoza O, Guzmán L, Jorquera MA, Nagai S, Pizarro G, Riquelme C, Ueki S, Maruyama F (2020). Protocols for monitoring harmful algal blooms for sustainable aquaculture and coastal fisheries in Chile. *Int. J. Environ. Res. Public Health*, 17, 7642. DOI: <https://doi.org/10.3390/ijerph17207642>

Appendix 1. eDNA sampling and filtration for testing different sample volumes

Outline of the experiment:

Filtering varying sampling volumes of seawater through 0.4 μm filters, with each sample volume processed in triplicates.

Experimental volumes: 1000 mL x 3, 500 mL x 3, 200 mL x 3, 20 mL x 3 + negative control: 500 mL x 3 of distilled water (dH_2O).

Additionally, 100 mL of the same seawater is required for phytoplankton morphology-based analysis.

In total, about 5,500 mL of seawater and about 1,700 mL of dH_2O is needed.

Items provided:

- 0.4 μm filters (Whatman, product nr. 10417712): packaged separately for each experimental volume and negative control, four filters per pack (one filter added as a backup per volume);
- 1.5 mL tubes: three tubes per sample volume + negative control. Colors: white: 500 mL of dH_2O , green: 20 mL, blue: 200 mL, yellow 500 mL, red: 1000 mL;
- 50 mL tube: used for measuring 20 mL volume;
- Marker pen to label tubes.

Items needed for sampling and filtration from each participant (not provided):

- 70% ethanol for cleaning surfaces and for the alcohol burner (if used);
- Paper towels to clean surfaces;
- Lab gloves;
- 10% HCl or bleach solution for sterilizing the sampling flasks/jugs and carboy;

- Sampling flasks/jugs to collect the water;
- Carboy for pooling all the water needed and for mixing;
- Filtration system(s) and pump(s). The filtration system(s) should be autoclaved or acid-washed prior to use;
- Tweezers to handle filters. The tweezers should be sterilized before usage and between using another sample volume;
- Alcohol burner or Bunsen burner and matches to sterilize the tweezers between filtering samples;
- Graduated cylinder for measuring sample volume;
- Lugol's solution to fix phytoplankton samples;
- 100 mL glass bottle for phytoplankton sample storage;
- dH₂O for negative controls.

Sampling protocol

- Collect the required volume of seawater needed for the experiment (filtration + phytoplankton) from the same depth used for routine phytoplankton sampling (surface layer/ integrated sample of 0–10 m);
- Use clean sampling flasks/jugs to collect the seawater (acid-washed: 10% HCl or store-bought bleach solution) and well-rinsed: 10 x with tap water, 3 x with dH₂O).
- Pool all collected water in a clean carboy (cleaned as above) and mix well;
- Take 100 mL of the water and fix it with Lugol's solution;
- The sample water can be stored for a few hours at the ambient temperature if it is similar to the water temperature or in the fridge/climate room at 4 °C. Please take note of the time that the sample was stored before filtration.

Filtration protocol:

- Clean the surface used for filtration with ethanol;
- Thoroughly mix the seawater in the carboy before taking each sample volume and wear gloves when handling samples, filters, tweezers, and tubes;
- Begin with filtering the negative control: filter 500 mL of dH₂O through a 0.4 µm filter;
- The filtration time per filter should be within 60 min and the pressure should not exceed 200 mm Hg/ 267 mbar / 27 kPa. Note any deviations in time/pressure;
- Once the filtration is finished, fold the filter with sterile tweezers several times to fit into a 1.5 mL tube;

- Label tubes using the provided format (first two letters of the institute/university+A/B/C for each replicate and 1–4 for each volume starting from dH₂O, e.g. for TalTech dH₂O: TAA1, TAB1, TAC1 (please see the "Sample information sheet.xlsx" for example);
- We will know the sample volume based on the coloured sticker on the tube (please see under the items provided). If you wish you may write down the sample volume on the tube;
- Please store the filter immediately after inserting it into a 1.5 mL tube at -20 °C;
- Continue with replicates of the same sample by filtering each of those on a separate 0.4 µm filter as mentioned above;
- After the filtration of negative control in triplicates is finished, continue with the smallest sample volume (20 mL) and proceed to the next largest volume until all the volumes have been filtered in triplicates.

Phytoplankton analysis:

Please analyse the phytoplankton sample using the same approach as for routine monitoring in your country.

Appendix 2. DNA extraction and amplification protocols

BioStandard DNA extraction procedure using DNeasy Blood & tissue kit (Qiagen, product nr. 69506).

Modified based on the manufacturer`s protocol.

1. Place filters used to filter water samples into a 1.5 ml microcentrifuge tube. Add **180 µl** of **buffer ATL** to the sample. Ensure that the filters are completely covered by the buffer solution. Add glass bead and vortex for one minute.
2. Add **20 µl proteinase K** to the tube. Mix thoroughly by vortexing and incubate at 56°C on a hotplate until the sample is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysis is completed in 3 hrs.
3. After sample lysis add **4 µl RNase A** (at a concentration of 100 mg/ml) to ensure that only RNA-free genomic DNA will be acquired. Mix by vortexing and incubate for 2 min at room temperature (15–25 °C) before continuing.
4. Add **400 µl of buffer AL + ethanol**. Buffer AL + ethanol and sample should be mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.
5. Pipet the mixture from the previous step (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 13 200 rpm for 1 min. Discard flow-through and collection tube.
6. Place the DNeasy Mini spin column in a new 2 ml collection tube, add **500 µl Buffer AW1**, and centrifuge for 1 min at 132 000 rpm . Discard flow-through and collection tube.
7. Place the DNeasy Mini spin column in a new 2 ml collection tube, add **500 µl Buffer AW2**, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through since this will result in a carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000rpm).

8. Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube, and pipet **100 µl Buffer AE** directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at $\geq 6,000 \times g$ (8,000 rpm) to elute.
Repeat elution once as described in this step as it leads to increased overall DNA yield.

DNA amplification

1. Calculate volumes of PCR Master Mix based on the amount of extracted DNA samples. Add a little reserve (number of samples + three (depending on the number of samples)).
2. Mixing the PCR Master Mix into 1.5 mL Eppendorf tubes. Start with the highest volume ingredient (water) and continue with lower volumes. Lastly, add the HotFire polymerase. Polymerase should remain on ice as long as possible.
3. Add 23 µL of Master mix into each of the 200 µL tubes representing different samples, subsamples, and replicates.
4. In the last step add 2 µL of your extracted DNA to the PCR mixture. The total reaction volume is now 25 µL.
5. For negative control, one PCR sample without DNA must be tested for the components' purity. In the negative control tube, add 2 µL of dH₂O instead of 2 µL of DNA.

NB! MasterMix, PCR mix, or other mixtures containing polymerase enzyme should not be vortexed as it may break down polymerase resulting in an unsuccessful reaction.

PCR Mixture		
Ingredients	Quantity	
	1	x ...
HotFirepol	5 µL	... µL
Forward primer (10 µM; 18S-F1289-sn; Dzhembekova, et al. 2018)	1 µL	... µL
Reverse primer (10 µM; 18S-R1772-sn; Dzhembekova, et al. 2018)	1 µL	... µL
dH2O	16 µL	... µL
PCR mix volume	23 µL	... µL
DNA	2 µL	... µL
Combined volume (mix+DNA)	25 µL	... µL

6. Centrifuge and place PCR tubes into PCR machine and start suitable PCR program (See the table below).
 - 95 °C for 15 minutes – **polymerase activation** – high temperature activates DNA polymerase, which is not enzymatically active at lower temperatures. This stage also prevents extension of non-specifically bound primers and cleaves at a low-level temperature (during the preparation of the PCR reaction mixture) of the primer's dimers.
 - 95 °C for 20 seconds – **DNA denaturation** – this step breaks down the weak hydrogen bonds that hold DNA strands together as a double helix. The result is single-stranded DNA to which primers can bind.
 - 58 °C for 30 seconds – **Primer's binding** (annealing) – temperature lowering from 95 °C to 58 °C allows the primers to bind to the complementary sequence on the template DNA.
 - 72 °C for 40 seconds – **Elongation** – 72 °C is for DNA polymerase to function at optimum temperature. DNA polymerase extends primers by adding a primer to the ends of free deoxynucleotide triphosphates (dNTPs) in solution and using template DNAs as an example.
 - Repeat steps 2 to 4 29 times.
 - Finally, another five minutes at 72 °C to extend the incomplete PCR products.

	Step	Temperature (°C)	Duration	Nr of cycles
1.	First Activation	95	15 min	1
2.	Denaturation	95	20 sec	30
3.	Primer's binding	58	30 sec	
4.	Elongation	72	40 sec	
5.	Last elongation	72	5 min	1
6.	Sample preservation	8	∞	1

Appendix 3. Harmful Algal bloom species detected from different samples

Species/ sample	SMI- (Ant E)	SMI- (BY; E)	SYK	MFR	UiO	NIV/	NIN.	TaIT	IOW	LHE	AU	Firu	Ifren
<i>Dinophysis acuminata</i>	+	+	+	+	+	+		+		+		+	+
<i>Dinophysis acuta</i>	+			+	+								+
<i>Karlodinium veneficum</i>	+	+	+	+	+	+	+	+	+	+		+	+
<i>Karenia mikimotoi</i>				+						+			+
<i>Phaeocystis globosa</i>													+
<i>Phaeocystis pouchetii</i>						+						+	
<i>Phalacroma rotundatum</i>			+	+						+			+
<i>Protoceratium reticulatum</i>	+			+									
<i>Prymnesium polylepis</i>	+	+			+		+		+			+	+
<i>Pseudochatonella farcimen</i>	+	+		+		+	+					+	+
<i>Pseudochatonella verruculosa</i>	+	+		+			+					+	+

<i>Pseudo-nitzschia americana</i>		+					+	+
<i>Pseudo-nitzschia australis</i>		+					+	
<i>Pseudo-nitzschia delicatissima</i>	+		+	+	+		+	
<i>Pseudo-nitzschia fraudulenta</i>			+				+	+
<i>Pseudo-nitzschia pungens</i>							+	+
<i>Pseudo-nitzschia turgidula</i>		+	+				+	+

About this publication

Reliable environmental DNA detection of planktonic biodiversity: Minimum sample volume for DNA metabarcoding

Sirje Sildever, Bente Edvardsen, Elianne Egge, Anette Engesmo, Sara Harðardóttir, Ása Jacobsen, Hans Jakobsen, Iveta Jurgensone, Bengt Karlson, Anke Kremp, Tiina Laamanen, Sirpa Lehtinen, Markus Majaneva, Kristian Meissner, Kenneth Mertens, Satoshi Nagai, Erna Lava Olsen, Ian Probert, Kristian Pärt, Nelli Rünk, Ingrid Sassenhagen, Sanna Suikkanen, Aouregan Terre-Terrillon, Anders Torstensson, Kristiina Vuorio

TemaNord 2025:509

ISBN 978-92-893-8145-1 (PDF)

ISBN 978-92-893-8146-8 (ONLINE)

<http://dx.doi.org/10.6027/temanord2025-509>

© Nordic Council of Ministers 2025

Cover photo: Sirje Sildever, TalTech

Published: January 2025

Disclaimer

This publication was funded by the Nordic Council of Ministers. However, the content does not necessarily reflect the Nordic Council of Ministers' views, opinions, attitudes or recommendations.

Rights and permissions

This work is made available under the Creative Commons Attribution 4.0 International license (CC BY 4.0) <https://creativecommons.org/licenses/by/4.0>.

Translations: If you translate this work, please include the following disclaimer: This translation was not produced by the Nordic Council of Ministers and should not be construed as official. The Nordic Council of Ministers cannot be held responsible for the translation or any errors in it.

Adaptations: If you adapt this work, please include the following disclaimer along with the attribution: This is an adaptation of an original work by the Nordic Council of Ministers. Responsibility for the views and opinions expressed in the adaptation

rests solely with its author(s). The views and opinions in this adaptation have not been approved by the Nordic Council of Ministers.

Third-party content: The Nordic Council of Ministers does not necessarily own every single part of this work. The Nordic Council of Ministers cannot, therefore, guarantee that the reuse of third-party content does not infringe the copyright of the third party. If you wish to reuse any third-party content, you bear the risks associated with any such rights violations. You are responsible for determining whether there is a need to obtain permission for the use of third-party content, and if so, for obtaining the relevant permission from the copyright holder. Examples of third-party content may include, but are not limited to, tables, figures or images.

Photo rights (further permission required for reuse):

Any queries regarding rights and licences should be addressed to:
Nordic Council of Ministers/Publication Unit
Ved Stranden 18
DK-1061 Copenhagen
Denmark
pub@norden.org

Nordic co-operation

Nordic co-operation is one of the world's most extensive forms of regional collaboration, involving Denmark, Finland, Iceland, Norway, Sweden, and the Faroe Islands, Greenland and Åland.

Nordic co-operation has firm traditions in politics, economics and culture and plays an important role in European and international forums. The Nordic community strives for a strong Nordic Region in a strong Europe.

Nordic co-operation promotes regional interests and values in a global world. The values shared by the Nordic countries help make the region one of the most innovative and competitive in the world.

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

Read more Nordic publications on www.norden.org/publications