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Towards eDNA informed biodiversity studies – Comparing water derived molecular taxa with traditional survey methods

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A B S T R A C T

Managing natural resources in a sustainable manner requires understanding the complexity of ecosystems and the species that are associated with the different parts of the ecosystem. Much of this knowledge is derived from traditional sampling methods (e.g., different types of trawls). The analysis of environmental DNA (eDNA) can provide increased knowledge, complementary to the traditional methods. In the present pilot study, we sampled eDNA from two geographical areas, north and west of Svalbard (NWS) and in the southwestern Barents Sea (SWBS). The combination of trawling, visual identification of mammals and eDNA collection facilitated a robust analysis of fish and marine mammal diversity and species composition. Through 12S MiFish metabarcoding of the eDNA samples, we found that incorporating eDNA data provided an additional level of information on both the diversity of fish and marine mammals in the study areas. By adding eDNA data to the trawl data, we found that richness increased from 32 to 49 fish taxa. Significant differences in diversity and composition of the fish communities were detected by eDNA between the two study areas. Considering degradation and dilution factors it is postulated that the results represent resident species to the Barents Sea and that long -transported DNA from other areas are less likely.

1. Introduction

Conserving biodiversity is a critical factor in the management of the world's ecosystems (IPBES, 2019). To do so, we need efficient methods for mapping and monitoring biodiversity. In the marine realm, this is increasingly more important as ecosystems are shifting because of climate change and other anthropogenic drivers (Boyd et al., 2016; Andrews et al., 2019; Ingeman et al., 2019), and particularly in the Arctic, where climate change is accelerating due to polar amplification, giving rates of change up to three times higher than at lower latitude systems (Pörtner et al., 2019). The Barents Sea is one of the Arctic and sub-Arctic ecosystems where rapid climate change is ongoing (Lind et al., 2018). In this region extensive monitoring has documented substantial ecosystem changes associated with climate change (Eriksen et al., 2018), including rapid shifts in species geographic distribution, food web structure, and functional composition of local ecological communities (Fossheim et al., 2015; Kortsch et al., 2015; Frainer et al., 2017). As the Barents Sea lies at one of the two gateways to the Arctic Ocean, information about species inhabiting the Barents Sea may also provide information about species that have the potential to expand their distribution range into the Arctic Ocean.

Monitoring of the Barents Sea is probably the most extensive among the large marine ecosystem in the Arctic (Eriksen et al., 2018). However, it still relies on traditional sampling gear, such as trawls, optimized towards high catchability of commercial species (Eriksen et al., 2018), and zooplankton sampling nets with mesh size designed to capture smaller organisms (e.g., Skjoldal et al., 2019). Thus, complementary approaches are therefore needed to cover species that are not easily caught with these types of gear, or act as a supplement for traditional monitoring.

The analysis of extra-organismal environmental DNA (eDNA) from vertebrates, where eDNA is defined as "a pool of DNA isolated from environmental samples" (Pawlowski et al., 2020; Rodriguez-Ezpeleta et al., 2021), has shown an exponential use for monitoring biodiversity during the last decade (Hansen et al., 2018). Analysis of eDNA offers several advantages compared to traditional fisheries survey methods, the most obvious being that from one sample of eDNA, it is possible to study the taxonomic composition across widely different life styles and trophic levels (Taberlet, 2018). As the method is non-destructive, it does

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not have an impact on the ecosystem that is being monitored. The independence of taxonomic expertise, but reliance on curated DNA databases, is one of the major benefits of this approach over traditional morphological assessments.

As the eDNA approach has increased in popularity, so has the number of genetic markers developed for this type of analysis (Wang et al., 2021). The most widely used markers are derived from the mitochondrial genome, due to its higher abundance in the environment compared to nuclear DNA (Rees et al., 2014). Different fragments of the rRNA 12S gene are widely used for the analysis of vertebrate communities, including fish (e.g., Riaz et al., 2011; Miya et al., 2015; Valentini et al., 2016). Studies have shown that this gene outperforms other genomic regions in the precision of the taxonomic assignment of fish species due to a combination of variability and quality of the reference database (Miya et al., 2020; Polanco F. *et al.*, 2021; Xiong et al., 2022).

Focusing on the Barents Sea and Arctic waters north of Svalbard, the present pilot study assessed the vertebrate community structure, with emphasis on fish and marine mammals, using 12S metabarcoding of water derived eDNA. The fish communities revealed by 12S metabarcoding of eDNA were combined with trawl data (pelagic and/or bottom trawl) from the same locations to assess the level of species diversity extracted from each of the two methods and combined.

2. Material and methods

2.1. eDNA sampling

eDNA and trawl sampling were conducted on two cruises in May 2015 (TIBIA) and September 2016 (SI-ARCTIC), covering the areas north and west of Svalbard (NWS) and the southwestern Barents Sea (SWBS), close to Bear Island, respectively (Fig. 1). A total of four stations were sampled from the shelf break NW Svalbard (including Hinlopen Strait), while five stations were sampled in the southwestern Barents Sea (Fig. 1, Table 1). For eDNA, seawater was sampled from three depths (Table 1) at each station using Niskin bottles attached to a CTD. For SI ARCTIC, the depth at each station was sampled in triplicate (three bottles per depth). At each station both water negative and air negative samples were collected to monitor possible contamination. Filtration of the water samples was done using a peristaltic pump through a 0.22 μM polyethersulfone Sterivex filter (Merck Millipore, Massachusetts USA). After filtration, excess water was removed from the filter housing using a sterile syringe filled with air. The filters were individually placed in sterile zip-lock plastic bags and stored at −20 °C onboard the research vessel and transferred to −80 °C when they arrived in the lab until DNA extraction.

2.2. Trawl sampling

Demersal fish in the SW Barents Sea were sampled using a Campelen

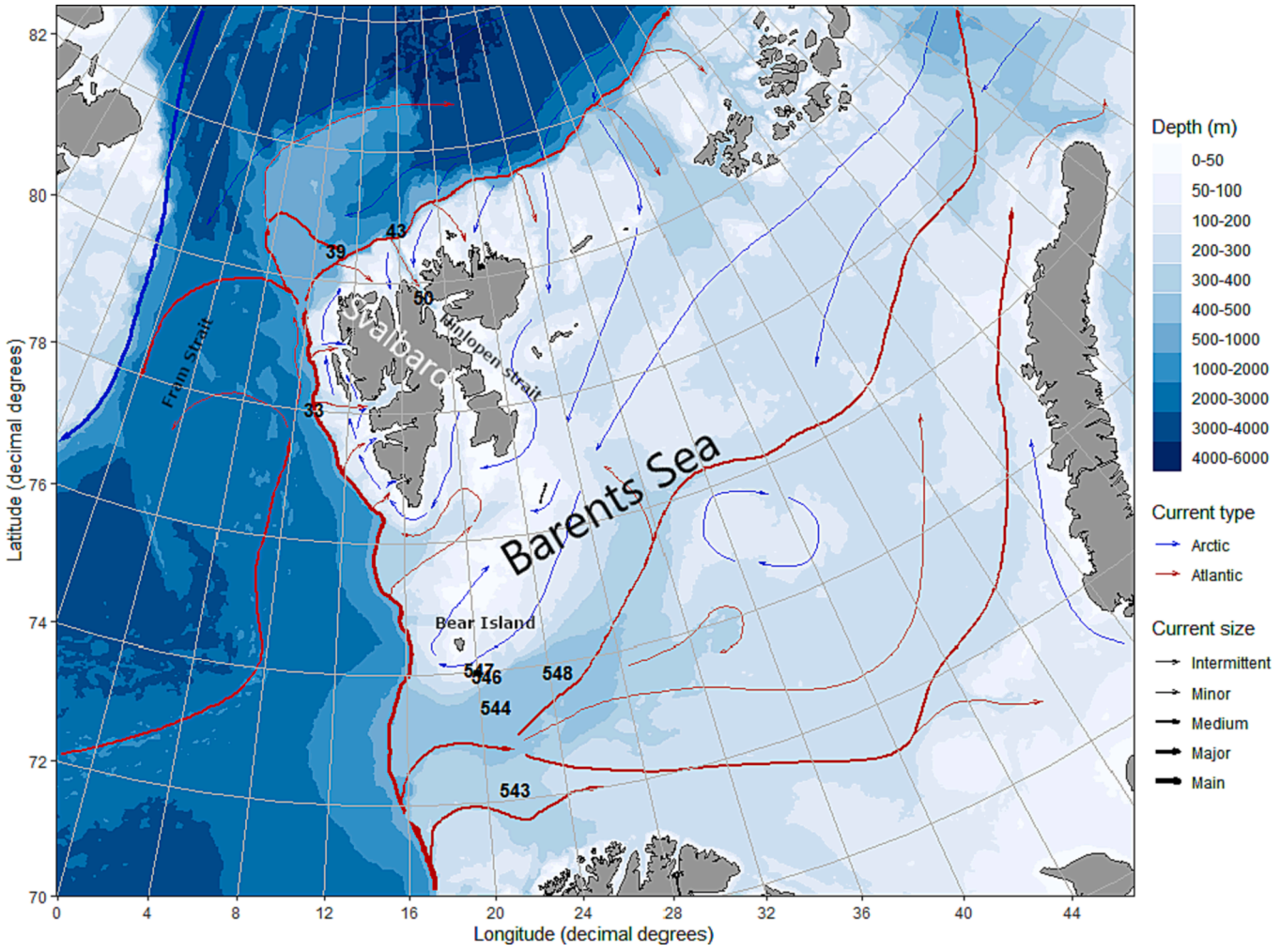

Fig. 1. Map showing the sampling locations. Stations with 3-digits are from the TIBIA cruise (southwestern Barents Sea), while station with 2-digits are from the SI-ARCTIC cruise (north and west of Svalbard).

Table 1
Overview of the sampled stations. Station number is related to CTD station number.

Station	Date	Latitude	Longitude	Depth (m)	Volume (ml)	Equipment
33	03.09.2016	78.03	9.46	50,350,420	1000	eDNA
39	05.09.2016	80.48	10.15	50,300,400	“	eDNA
43	07.09.2016	80.84	15.77	60,350,450	“	eDNA
50	09.09.2016	79.79	18.04	50,200,350	“	eDNA
543	29.05.2015	72.16	21.33	5,345	2000	eDNA
544	30.05.2015	73.46	20.76	5,30,478	“	eDNA
546	31.05.2015	73.95	20.44	5,30,243	“	eDNA
547	31.05.2015	74.06	20.08	5,30,148	“	eDNA
548	01.06.2015	73.86	24.37	5,30,447	“	eDNA
33	03.09.2016	78.03	9.40	77,350,437	NA	Trawl
39	05.09.2016	80.43	10.37	51,313,407	“	Trawl
43	07.09.2016	80.79	15.74	56,355,467	“	Trawl
50	09.09.2016	79.76	18.26	52,254,350	“	Trawl
543	29.05.2015	72.17	21.36	340	“	Trawl
544	30.05.2015	73.41	20.65	420	“	Trawl
546	31.05.2015	73.95	20.36	200	“	Trawl
547	31.05.2015	74.05	20.11	151	“	Trawl
548	01.06.2015	73.95	22.65	445	“	Trawl

trawl (Engås and Ona, 1990), a small demersal trawl originally designed for catching shrimps. Trawling time was 15 min.

For the NW Svalbard area, the pelagic Åkra trawl (Valdemarsen and Misund, 1995) was deployed with a mesh size of 8 mm in the cod-end used for sampling pelagic fish. This trawl was equipped with a multi-sampler, which is a device with three nets that can be opened and closed at predefined depths (de L. Wenneck et al., 2008). As a standard, these nets were deployed at (i) lower base of the deep scattering layer, (ii) high concentrations scatter in the deep scattering layer and (iii) at 50 m depth. Each layer was trawled for 30 min.

2.3. Marine mammals

On the SI ARCTIC cruise in the NW Svalbard area, visual observations of marine mammals were conducted by two experienced observers on the bridge of the R/V Helmer Hansen, covering approximately the front 90° sector (45° of each side). Species were recorded along the cruise transects when steaming between stations if visibility and weather conditions were acceptable. Species were also recorded when the ship was cruising along the ice edge zone and even when it was working its way through the ice. Observations were made during some of the station work, primarily when weather and visibility permitted.

2.4. DNA extraction and library preparation

DNA extractions were performed in a PCR free room dedicated to eDNA extraction using a modified protocol of the Qiagen blood and tissue kit as described in (Turon et al., 2022).

Prior to PCR amplification all equipment was sterilized using bleach and UV light and the PCR was prepared in a sterilized cabinet. We employed a two-step PCR approach, where the first PCR amplified the 12S mitochondrial gene (Miya et al., 2015) with an Illumina overhang adapter sequence which is used as a template for the second PCR. In the second PCR sequencing adapters and individual barcodes were attached. The first PCR was performed in a 12.5 μl reaction volume comprising 6 μl Qiagen Multiplex PCR kit, 0.25 μl of each primer (10 μM), 0.1 μl BSA (50 mg/ml), 3.4 μl of dH_2O and 2.5 μl of template DNA. The PCR profile was an initial denaturation of 95 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for 90 s and extension at 72 °C for 15 s, ending with a final extension at 72 °C for 5 min. The PCR products were then subsequently purified using AMPure XP beads (Beckman Coulter, USA) following the manufacturers protocol and using a final elution volume of 40 μl.

For the second PCR, 5 μl of the purified PCR products were used together with 25 μl Qiagen Multiplex Master Mix, 10 μl of dH_2O and 5 μl of each of the Nextera XT index primers N7XX and N5XX from the Nextera XT index kit (Illumina). The temperature profile for the second PCR involved an initial denaturation step of 95 °C for 15 min, 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. The PCR ended with a final extension step at 72 °C for 5 min. This second PCR produced a dual indexed PCR product with a unique index combination for each sample. The purification of the index PCR products followed the same approach as after the first PCR, but with an elution volume of 25 μl.

Before sequencing the PCR products, all samples were run on a 2 % agarose gel stained with SYBR Safe (Invitrogen) to check that PCR products were present and at the correct size (~170 bp). To estimate the concentration of PCR products with attached Illumina sequencing tags (i5/i7) we used a qPCR approach with the NEBNext Library Quant Kit for Illumina (New England Biolabs, USA) on a QuantStudio 6 Real-Time PCR cycler (ThermoFisher, USA). Subsequently, the libraries were normalized to 4 nM and pooled in equimolar ratios.

The pooled library was spiked with 1 % PhiX before sequenced on a MiSeq sequencing platform (Illumina) using the 600 cycles MiSeq reagent kit v3 (Illumina), following the instrument protocol.

2.5. Bioinformatics

Read quality of the sequences was checked with FASTQ before entering the OBITools (Boyer et al., 2016) bioinformatic pipeline. Forward and reverse reads were aligned with *illuminapairedend* and alignments with a quality <40 were discarded. Demultiplexing and removal of primer sequences was done with the *ngsfilter* command. Selection of amplicon sizes between 140 and 190 bp and removal of sequences with ambiguous bases was done with *obigrep* before applying *obiuniq* to dereplicate sequences. To search for and remove chimeras we used the *uchime-denovo* algorithm implemented in VSEARCH (Rognes et al., 2016) and amplicon clustering into molecular taxonomic units (MOTUs) was performed in SWARM (Mahe et al., 2014; Mahe et al., 2015) with *d* value of 3. Singletons were removed from the dataset with *obigrep* before taxonomic assignment.

2.6. Taxonomic assignment

The taxonomic assignment of the MOTUs to the lowest common ancestor (LCA) against a locally curated database was done using *ecotag*. This database contains filtered 12S reference sequences with taxonomy information retrieved from NCBI. After the taxonomic assignment identical MOTUs were collapsed at the species level.

Any sequences falling below the 97 % similarity threshold from *ecotag* were manually controlled through a BLAST search against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The

taxonomic assignments were subsequently checked according to the known species distribution provided by FISHBASE (https://www.fishbase.se/) and corrected if there was any disagreement between the study area and known distribution to the lowest possible taxonomic rank.

The LULU algorithm (Froslev et al., 2017) was applied to remove MOTUs identified as erroneous based on sequence identity and patterns of co-occurrence. Vsearch 2.15 (Rognes et al., 2016) was used to create a file matching all pairwise MOTUs to infer their similarity percentage. The LULU algorithm was run using the default settings.

Ensuring that only one representative from each taxon is represented in the final dataset, we included only the MOTU with the lowest taxonomic rank in cases where there were multiple MOTUs within a family. Low levels of sequence reads appeared in the negative controls (>1 %), discarding any need for correction of potentially false positives in the samples.

2.7. Statistics

All statistical analysis were done in R version 4.0.3 (http://www.R-project.org). The eDNA MOTU table from the bioinformatics was transformed to a binary presence/absence table using the *decostand* function in the package *vegan*. Thereafter species richness for each sample was calculated with the package *phyloseq* (McMurdie and Holmes, 2013). As the observed species richness was normally distributed (Shapiro-Wilk, W = 0.98, $p = 0.65$), differences in species richness among stations were explored using an Analysis of Variance (ANOVA) approach, followed by the Tukey HSD test to confirm pairwise differences. Differences in community composition between sampling stations, area and depth were performed using Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations through the *adonis* function in *vegan*. Non-metric multidimensional scaling (nMDS) was performed based on Jaccard distance matrices with the function metaMDS and 250 random starts in *vegan* (Oksanen et al., 2019). Both the PERMANOVA and nMDS were done using Jaccard distance matrices. Because the southwestern Barents Sea stations comprised demersal trawl data only, the pelagic trawl data for the NW Svalbard stations were omitted when comparative statistics between sampling methods were done.

To indicate the species driving the differences between geographical areas, we used the indicator species analysis (Dufrene and Legendre, 1997) using the *indval* function int the *labdsv* package with 999 permutations.

2.8. eDNA vs trawl data

For comparing eDNA data with trawl data, the trawl OTU table, containing number of fish caught per species, was also converted to a presence/absence table for downstream analysis with the *decostand* function in *vegan*. Subsequently this table was merged with the binary MOTU table from the eDNA analysis A one-way ANOVA was used to determine variability in the number of observed species per sampling method (i.e., eDNA vs trawl) using Jaccard instead of Bray-Curtis distances as recommended for binary data. For the southwestern Barents Sea stations only, bottom trawling was employed so we used only the eDNA samples obtained at the greatest depths (148–478 m) when comparing eDNA vs trawl data. For the NW Svalbard samples all eDNA and trawl samples were included. An nMDS plot was constructed based on Jaccard distances using the metaMDS function in *vegan* to visualize the differences between the two sampling methods. Venn diagrams were constructed to represent the degree of overlap in the taxa distribution between sampling methods for all samples combined and within each geographic area separately. The variability within each of the sampling methods was tested with equipment as a group factor in the *betadisper* function in *vegan*.

3. Results

The sequenced library yielded a total of 10 178 857 sequences after quality filtering. These were assigned to 875 MOTUs. Of these, 59 had more than 10 reads, and 37 could be assigned to fish taxa and eight to marine mammals (Table S1). The 14 remaining MOTUs, which were removed before downstream analysis, comprised terrestrial mammals and birds. Of the 37 fish MOTUs, 23 could be assigned to the species level within the 97 % identity criteria. All eight of the marine mammal MOTU's could be assigned to the species level, using the same criteria as above. In addition, MOTUs comprising taxa where their known distribution is outside the study area, typically Pacific species, which is typical for species without an Atlantic reference sequence, were discarded from the dataset. Six species were found in all samples, five of these which belongs to commercially important species: Atlantic cod (*Gadus morhua),* beaked redfish (*Sebastes mentella),* haddock (*Melanogrammus aeglefinus),* Greenland halibut (*Reinhardtius hippoglossoides)*, and herring (*Clupea harengus*).

3.1. Horizontal and vertical distribution of fish eDNA

The number of species detected by eDNA was variable across stations (Fig. 2). A total of 21 species were detected in both in the southwestern Barents Sea (range 6–13) and in NW Svalbard (range 9–16). Both typical demersal and pelagic species were observed. Significant differences in the observed number of species were found between Hinlopen Strait (St.50) versus two samples from the southwestern Barents Sea (St.50 vs St.544, $p = 0.01$, and St.50 vs St.548, $p = 0.01$).

Though there was an overlap in species composition between the two areas, significant differences were detected (PERMANOVA; $F = 4.20$; $p < 0.002$; Fig. 3). Also, no significant differences were found within each of the two geographical areas (NW Svalbard; $F = 1.37$, $p = 0.279$, southwestern Barents Sea; $F = 1.61$, $p = 0.094$). According to the species indicator analysis, the species giving a significant contribution to the observed differences were blue whiting (*Micromesistius poutassou*), gelatinous snailfish (*Liparis fabricii),* lemon sole (*Microstomus kitt*) and Greenland shark (*Somniosus microcephalus*). All these species were related to a higher frequency of detection in the NW Svalbard area.

Depth related differences in taxonomic composition were not significant in the eDNA data from either NW Svalbard or southwestern Barents Sea (Table 2). Thus, the species assemblages revealed by eDNA analysis appeared homogeneous across the vertical water column. Also, the CTD data (Fig. S1), showed a less stratified water column.

3.2. Trawl fish data

The overall number of fish species detected in the trawl data was 28. The variation within stations ranged from 1 to 14, which translated into an overall significant difference in the observed number of species among stations (ANOVA, $F = 7.21$, $p < 0.01$). Pairwise tests showed that the majority of the significant differences found was related to one station (St.547), which also showed the highest number of observed species (Fig. 2). Regarding the species composition, we found a significant difference between the two geographical areas (PERMANOVA, $F = 2.17$, $p = 0.01$), considering demersal trawl only. In contrast to the eDNA data, we found a significant difference in species composition between the depths sampled. However, these data were only based on NW Svalbard, as the southwestern Barents Sea sampling was done only employing the demersal trawl. Within each of NW Svalbard and southwestern Barents Sea, we did not find any significant difference in species composition (NW Svalbard; $F = 1.90$, $p = 0.11$, southwestern Barents Sea; $F = 0$, $p = 1.00$).

The species indicator analysis showed that the species polar cod (*Boreogadus saida*), spotted wolffish (*Anarhichas minor*) and American plaice (*Hippoglossoides platessoides*) gave a significant contribution to the community differences between NW Svalbard and SW Barents Sea.

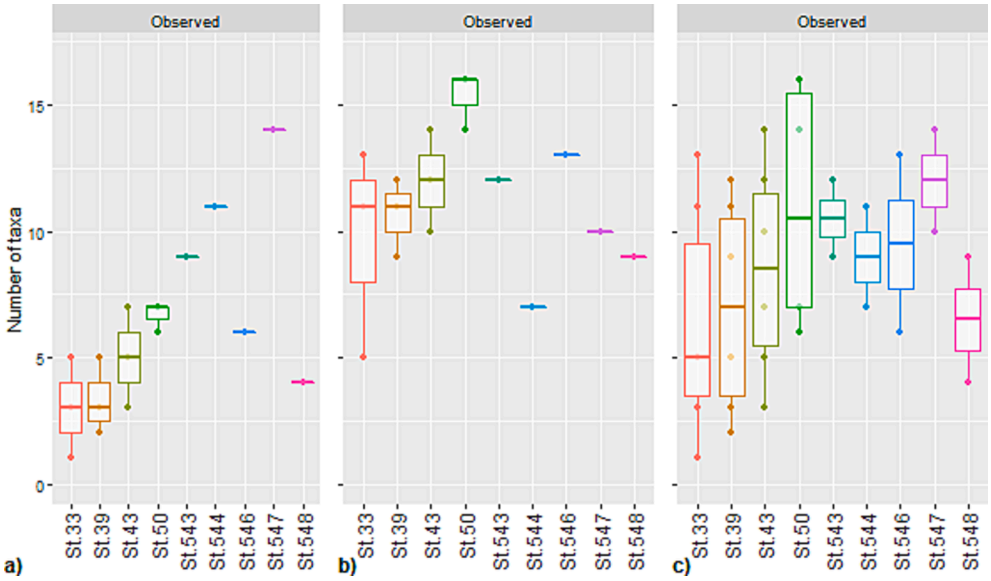

Fig. 2. Number of observed fish species per sampling station for (a) trawl, (b) eDNA and (c) combined eDNA and trawl.

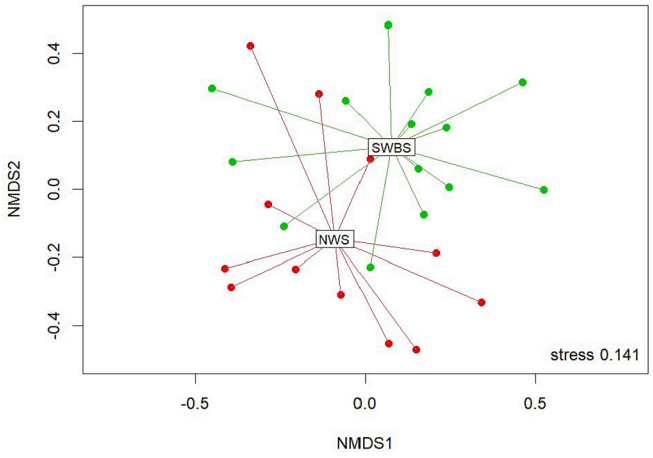

Fig. 3. Non-metric multidimensional scaling (nMDS) plot of the fish communities for the two sampling areas (southwestern Barents Sea (SWBS) and NW Svalbard (NWS) based on eDNA (presence/absence) data. Each point represents an eDNA sample.

Table 2
Results from the PERMANOVA tests. No test for trawl was done between depths in the southwestern Barents Sea as only bottom trawl was used. (NWS = Northwest Svalbard, SWBS = southwestern Barents Sea).

Areas				
	Df	SS	*F-statistics*	Pr(>F)
(eDNA)$areas	1	0.167	4.196	0.002 **
Depth				
	Df	SS	*F-statistics*	Pr(>F)
(glob)$equip	1	1.222	5.13	0.001 ***
(NWS_eDNA)$depth	2	0.038	0.434	0.826
(NWS_trawl)$depth	2	1.119	0.350	0.022 *
(SWBS_eDNA)$depth	2	0.044	0.502	0.856
Equipment				
	Df	SS	*F-statistics*	Pr(>F)
(glob)$equip	1	1.222	5.131	0.001***
(NWS)$equip	1	1.970	9.350	0.001***
(SWBS)$equip	1	0.800	3.389	0.007**

3.3. *eDNA vs trawl for fish*

Overall, the number of observed species per sampling method was significantly higher for eDNA than for trawl (ANOVA, $F = 21.15$, $p < 0.001$, Fig. 4 a), and significant geographical differences were also observed among stations within NW Svalbard ($p < 0.001$, Fig. 4 b), but not in the SW Barents Sea ($p = 0.86$, Fig. 4 b).

The comparative tests between eDNA and trawl revealed significant differences in species composition based on present/absence data, between the sampling methods, illustrated by the nMDS and confirmed by PERMANOVA (Fig. 5, Table 2).

For NW Svalbard both pelagic and demersal trawls were applied for sampling and the results were combined in this test. Significant differences between eDNA and trawl data were observed both overall and within each of the geographical areas by showing more taxa by eDNA compared to trawling (Table 2). The most profound difference was found on the shelf break of NW Svalbard (SI-ARCTIC: $F = 9.35$, $p < 0.001$). For the SW Barents Sea samples, a highly significant difference was also observed ($F = 3.39$, $p = 0.007$).

Also, a significant correlation was found between eDNA and trawl for the samples NW Svalbard (Pearson, $R = 0.76$, $p = 0.004$), but not for the samples from the southwestern Barents Sea ($R = 0.02$, $p = 0.98$). Interspecific variation within each of the two sampling methods was significantly higher across trawl samples than across eDNA samples (PERMDIST $p = 0.001$, Fig. 5, Fig. 6).

In contrast to the eDNA data, a significant difference was found in the taxonomic composition between depths NW Svalbard (PERMANOVA: $F = 0.35$, $p = 0.022$) from the trawl data.

3.4. *Marine mammals*

Visual observations were only performed on the survey in the NW Svalbard area. A total of 9 species of marine mammals, including three seal species, five whale, and one dolphin species was observed (Table S2, Fig. S2). Of these, one of the whale species could not be reliably assigned by the observers and was characterized as an unidentified whale species. The eDNA analysis, however, detected eight species of marine mammals, including three seal species and five whale species (Table S2).

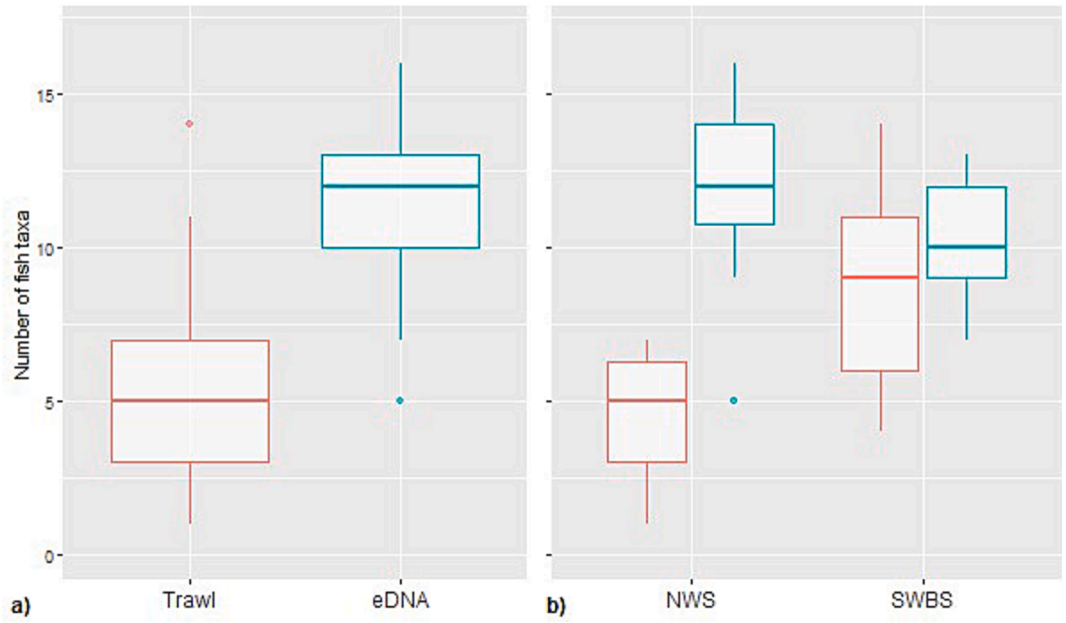

Fig. 4. Boxplot of the mean number of observed fish species for each sampling method (a) and per area/method (b). NWS: NW Svalbard, SWBS: SW Barents Sea (Trawl: red, eDNA: green).

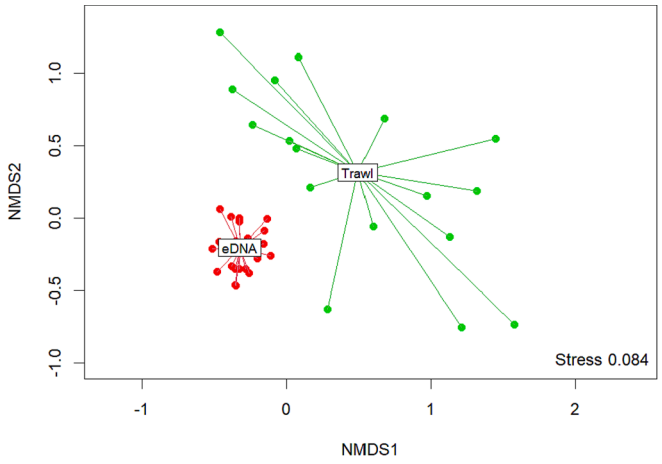

Fig. 5. Non-metric multidimensional scaling (nMDS) plot of fish communities based on presence/absence data. Illustrating the difference between sampling types. Each point represents an eDNA sample or a trawl haul.

Killer whale and ringed seal was not detected by eDNA despite being observed visually, but eDNA detected humpback whale, which was not observed visually in the same area. Of the eight species detected by eDNA in the area NW Svalbard, only four were detected in the SW Barents Sea. Due to the lack of visual observers in the SW Barents Sea survey, it was not possible to compare the performance of the two methods in that area.

4. Discussion

The diversity estimates derived from eDNA often supersedes the ones obtained by traditional sampling methods (e.g., trawl) or reveals a complementary pattern of biodiversity (Stoeckle et al., 2020; Zhou et al., 2022). In this study we find that analysis of eDNA provides additional information about the fish biodiversity in the two sampled areas. Thus, this demonstrates that eDNA is an important tool for biodiversity measures and could be used in addition to traditional tools such trawls and nets. This is shown by the significant increase in the number of discovered taxa found when adding eDNA results to the traditional trawl data (ANOVA, $F = 21.15$, $p < 0.001$). Substantial overlap in species detection was found between the two sampling methods (trawl and eDNA), but also that many species found with eDNA were not found by trawl and vice versa. Thus, integrating the data from several sampling sources, enables the detection of "hidden" biodiversity (e.g., Boussarie et al., 2018). For marine mammals, adding eDNA to the visual observation data gave one addition to the total number of species detected, although two species observed visually were not detected by eDNA.

Although eDNA data does not include life history information (e.g., sex, age, and size) of the individuals, it adds important information on the presence of fish and marine mammal species in Svalbard and the Barents Sea. To our knowledge this is the first study comprising eDNA data as far north as the entrance to the Arctic Ocean.

4.1. Horizontal distribution

The significant difference in species composition (β – diversity), both within and between the two geographical areas, suggests heterogeneity in both the species composition and relative strength of the eDNA signal for some of the species detected. Several factors influence the horizontal distribution of eDNA in the marine environment. Ocean currents can transport particles (i.e., eDNA) over large distances, thus homogenizing the eDNA signal between areas. However, both the dilution effect and degradation of the eDNA particles need to be considered. These two factors greatly influence how far away from the source an eDNA signal can be detected. As the water temperature ranges from 2 to 6 °C in the geographical areas of the present study, the degradation rate of the eDNA particles will be in the lower end of what has been reported so far by Andruszkiewicz Allan et al. (2021). These authors found that 90 % of the DNA was degraded within 1.6–5.2 days at 6 °C, depending on species. With an average speed of 20 cm/s for the West-Spitsbergen current (WSC) (Beszczynska-Moller et al., 2012) and 10 cm/s for the Svalbard branch of the WSC north of Spitsbergen (Renner et al., 2018), the eDNA particles can theoretically be transported between 45 and 90 km, using the slowest degradation rate (5.2 days). Here, the dilution effect has not been taken into account. This suggests that sampling design needs to be considered, especially if sampling sites are in proximity to each other. In this study we have circumvented this issue as we have combined stations

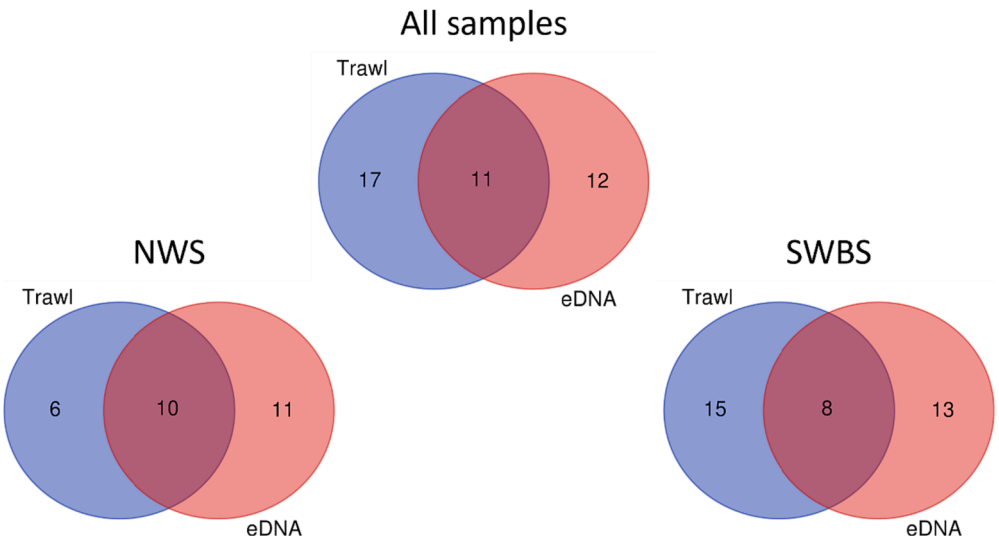

Fig. 6. Venn diagram for fish taxa comparing the results from eDNA and trawl data (NWS: NW Svalbard, SWBS: SW Barents Sea).

and focused on biodiversity in two distinct areas almost 500 km apart.

4.2. Vertical distribution

No evidence of vertical structuring of fish taxa was observed in either of the two areas based on the eDNA data. This contrasts the trawl data where such structuring was observed, although we only had trawl data from different depths available from the Svalbard area. Since the average sinking rate of a typical eDNA particle is slow (Allan et al., 2021), the particle will remain at the released depth layer for a certain period of time. Thus, if there is a vertical migration of fish, the eDNA signal will be retained at detectable levels throughout the vertical migration range, homogenizing the DNA trace from the individual across depths. Vertical migration patterns were found for some fish species by Gjosaeter et al. (2017), which showed evidence of diel vertical migration (DVM) of both fish and zooplankton north of Svalbard (Fig. 1). Thus, the lack of vertical signal in the eDNA is likely caused by DVM. As several studies using eDNA has revealed vertical stratification in species composition, analysis of eDNA has shown the power of detecting this (e.g., Canals et al., 2021; Guri et al., 2023).

4.3. Trawl vs eDNA

Other studies have contrasted the performance of trawl and eDNA to evaluate the correlation between these two sampling approaches. Our focus is on the complementary use of these two sampling methods. Our data shows a significant increase in the number of detected taxa when integrating eDNA sampling into the traditional trawl survey. This is especially obvious in the Svalbard samples where the eDNA data almost doubled the number of detected taxa, revealing "hidden" diversity (Boussarie et al., 2018). The three main factors differentiating the sampling in NW Svalbard and the southwestern Barents Sea were (i) the number of biological replicates per depth (2 vs. 3), (ii) the volume of water sampled for each depth (2 vs. 1 L), and (iii) the depth sampled. A careful interpretation of these results demonstrates the importance of sampling design, and that the number of biological replicates supersedes the volume of water sampled (Macher et al., 2021; Guri et al., 2023).

4.4. Marine mammals

The concordance between visual observations and eDNA methods reveals the effective application of 12S metabarcoding as a method of providing data on marine mammals spanning multiple trophic levels. We could even contribute with observations of marine mammals in the Barents Sea where we did not have any inspectors on board. For some of the marine mammal species in NW Svalbard, especially harp seals and hooded seals, more observations were done by eDNA than with visual observations. However, as little is known about how environmental factors affect the spatial and temporal persistence of eDNA in the study system, this could be explained by transported eDNA from other locations. Nonetheless, the analysis of eDNA demonstrates the possibility to serve as a complementary sampling tool for the study of marine mammals (Suarez-Bregua et al., 2022). We also note that eDNA would be an important tool when weather conditions limit the visual observation of marine mammals.

4.5. Future improvements

Several studies have advocated the use of multiple markers to cover a wider taxonomic range in eDNA analysis (e.g., Stat et al., 2017). The use of broad-scale metabarcoding primers, as 12Sin this study, might produce false negatives if other taxonomic groups are present in larger abundances in the eDNA sample (Miya et al., 2020) or if the primer – template mismatch result in a failed amplification. We found almost no elasmobranch species in our study which might be a result of mismatch with the primers applied (Miya et al., 2015). This could be improved by multiplexing several, more targeted primer sets in the same PCR reaction or combining multiple libraries, derived from different primer sets, as long as an appropriate sequencing depth is retained.

A well-curated reference database is the backbone of the analysis of eDNA. Recent studies have shown that applying a geographically pruned reference database could increase the precision in species assignment compared to a more general database (Valdivia-Carrillo et al., 2021). Thus, putting effort into curating the reference database will increase the taxonomical resolution of an eDNA study.

4.6. Concluding remarks

This study, although it was designed as a pilot study, clearly showed that sampling eDNA in conjunction with the traditional trawl surveys will be vital for the understanding of the biodiversity of fish species and marine mammals. This is demonstrated by the increased species richness detected when merging the data from eDNA and trawling in both sampling areas. Further work is needed to enhance the taxonomical resolution of eDNA datasets, with the emphasis of establishing well-curated local reference databases, to make this source of data even more useful. We advocate that a combined sampling effort is a good strategy for future monitoring of biodiversity in the Arctic including the Barents Sea.

CRediT authorship contribution statement

Jon-Ivar Westgaard: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Kim Præbel:** Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. **Per Arneberg:** Conceptualization, Writing – original draft, Writing – review & editing. **Brian P. Ulaski:** Investigation, Writing – original draft, Writing – review & editing. **Randi Ingvaldsen:** Formal analysis, Writing – original draft, Writing – review & editing. **Owen S. Wangensteen:** Methodology, Software. **Torild Johansen:** Funding acquisition, Project administration, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Contribution: JIW, PA, TJ+KP planned the project, BPU collected the TIBIA samples, JIW and KP did all the lab work, JIW did bioinformatic guided by OW, JIW did the statistical analysis and wrote the first draft of the manuscript. All authors contributed to the writing and accepted the submitted manuscript.

Appendix A. Supplementary material

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