Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Thea Svendsen

The usability of eDNA to identify and quantify zooplankton communities

Master's thesis in Ocean Resources
Supervisor: Sanna Majaneva
Co-supervisor: Markus Majaneva, Anna Solvang Båtnes, Geir Johnsen

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Abstract

Our ocean is in the middle of a triple planetary crisis. Climate change, pollution, and biodiversity loss impacts its habitats and inhabitants. Ocean monitoring programs are valuable as they provide data which can help predict the impacts of both climate change and human activity. Yet, biodiversity monitoring has traditionally relied on morphological species identification, which is both time-consuming and costly. Molecular methods have, on the other hand, shown promising results in detecting the hidden species diversity. However, many studies emphasize that morphology is the only reliable method for quantification, and DNA-based methods cannot give reliable abundance data, but currently there is no consensus. Thus, the aim of this study was to test the applicability of DNA-based methods compared to traditional morphological species identification in biodiversity assessment for future monitoring programs, and test if molecular methods can be used in a quantitative matter. Zooplankton species are good indicators for changes in the environment, but species diversity assessments present challenges and thus main focus of this study. Water samples for environmental DNA (eDNA) and zooplankton net samples were collected at surface, 10-, 25- and 50-meters depth both night and day in spring 2022 from the Mausund Bank (63.8° – 64.2°N, 8.2° – 9.0° E), which is an area of significant importance for both biological diversity and production. QPCR of mock community of cultured *Calanus finmarchicus* and experimental water was used. In the samples collected at the Mausund Bank, a total of 180 taxa were detected using eDNA and only 29 taxa using morphological species identification, showing how DNA based methods are suitable for improving species identification and exposing the hidden diversity. On the other hand, quantification of mock communities showed no correlation between *C. finmarchicus* counts and eDNA reads, hence, emphasizing the difficulty in species quantification using molecular methods. DNA-based methods have a great potential for species identification and detection, and it is perhaps the most likely source of a new and innovating marine monitoring technique.

Sammendrag

Havet vårt står midt i en trippel planetarisk krise. Klimaendringer, forurensning og tap av biologisk mangfold påvirker både habitater og de som bor der. Havovervåkingsprogrammer er viktige siden de gir data som kan bidra til å forutsi virkningene av både klimaendringer og menneskelig aktivitet. Overvåkningen av biologisk mangfold har tradisjonelt vært avhengige av morfologisk artsidentifisering, som både er tidkrevende og kostbart. Molekylære metoder har derimot vist lovende resultater med tanke på å oppdage det skjulte artsmangfoldet. Mange studier understreker imidlertid at morfologiske artsidentifisering er den eneste pålitelige metoden for kvantifisering og at DNA-baserte metoder ikke kan gi kvantitative data, men det er foreløpig ingen konsensus. Målet med denne studien er derfor å teste anvendeligheten av DNA-baserte metoder sammenlignet med morfologisk artsidentifisering av biologisk mangfold for å se på muligheten for fremtidige overvåkningsprogrammer, og undersøke om DNA kan bli brukt til kvantifisering. Dyreplanktonarter er spesielt gode indikatorer på klimaforandringer, men identifisering av artsmangfoldet har en rekke utfordringer og dyreplankton er derfor hovedfokus i denne studien. Vannprøver for miljø-DNA og håvtrekk for dyreplanktonprøver ble samlet inn i overflaten, ved 10-, 25- og 50-meters dybde både natt og dag, våren 2022 fra Mausundbanken (63.8° – 64.2°N, 8.2° – 9.0° E), som er et område med stor betydning for både for biologisk mangfold og biologisk produksjon. QPCR ble brukt på en kjent konsentrasjon av *Calanus finmarchicus* og vannet de befant seg i. Fra prøvene samplet inn på Mausundbanken ble totalt 180 taksa identifisert ved bruk av miljø-DNA og 29 taksa ved bruk av morfologisk artsidentifisering. Dette viser hvordan DNA-baserte metoder kan forbedre artsidentifikasjon og hvordan metoden kan være med på å avsløre det skjulte artsmangfoldet. Ingen av de kvantifiserte prøvene viste korrelasjon mellom det kjente antallet *C. finmarchicus*-individer og miljø- DNA, og dette understreker altså hvor vanskelig kvantifisering ved bruk av molekylære metoder er. DNA-baserte metoder har et stort potensial innen artsidentifisering, og vil kanskje være den mest sannsynlige kilden til nye og innovative løsninger innen havovervåkning.

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List of Abbreviations

16S	16S Ribosomal RNA
18S	18S Ribosomal RNA
ASV	An Amplicon Sequence Variant
AUV	Autonomous Underwater Vehicle
AW	Atlantic Water
BOLD	The Barcode of Life Data System
COI	Cytochrome C Oxidase I
CTD	Conductivity, Temperature and Depth
DVM	Diel Vertical migration
eDNA	Environmental DNA
iBOL	International Barcode of Life
MPA	Marine Protected Area
mtDNA	Mitochondrial DNA
NAO	North Atlantic Oscillation
NCC	Norwegian Coastal Current
nDNA	Nuclear DNA
NorBOL	The Norwegian Barcode of Life
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
rRNA	Ribosomal RNA
SVM	Seasonal Vertical Migration

1 Introduction

1.1 Biodiversity of our oceans

Our oceans are facing a triple planetary crisis – climate change, pollution, and biodiversity loss – with potentially irreversible direct and indirect impacts to their habitats and inhabitants (Bindoff et al., 2019). Understanding and protecting marine biodiversity is crucial for tackling these challenges, as biodiversity and healthy, resilient ecosystems contribute essentially to climate regulation by the oceans (Baste et al., 2021; Pörtner et al., 2022). To understand the biodiversity, the number of existing species is one of the major biological questions today. These insights can provide knowledge about how much we do, or do not know about biodiversity (Appeltans et al., 2012), and information on how species are distributed, which species are facing some changes and importantly which species are endangered and about to disappear (Purvis & Hector, 2000). Biodiversity can be measured at many different levels, including genetic, species, community, and ecosystem, whereas species level is probably the most commonly used (Norse, 1993). It is calculated using traditional statistical indices such as the Shannon-Wiener index and species richness (Lamb et al., 2009; Nolan & Callahan, 2006; Wilson & Bossert, 1971). Species richness is the most common metric for estimating biodiversity, and studies predicting the magnitude of global species richness are many (Appeltans et al., 2012). As of April 2023, Catalogue of Life estimates that 2,3 million species in the world are taxonomically described, this probably includes 80% of the known species (Bánki et al., 2023), where estimation of species yet to be discovered range from 10 – 100 million species (Bouchet, 2006). Depending on the study, the estimated number of unknown species varies a lot. For example, for marine life everything between 295 000 (Costello et al., 2012) and 10 million (Grassle & Maciolek, 1992) non-described species have been predicted (Appeltans et al., 2012).

The ocean covers over 70% of the earth's surface (Whitty, 2006), and the marine biodiversity provides important ecosystem services for humans, including food, medicine, raw materials, protection from costal erosion, recycling of pollution and climate regulation (Norse, 1993; Sala & Knowlton, 2006). The increasing anthropogenic effects are heavily affecting marine systems and motivating a global discussion about expanding protection of the marine ecosystems and for example increasing the number of marine protected areas (MAPs; Bindoff et al., 2019; Sala et al., 2021). These areas are highly protected where destructive activities are banned, at least partly, so that the marine biodiversity can be restored (Grorud-Colvert et al., 2021). As of March 2023, about 8,2% of the world ocean was classified as MAPs, but only 2,9% of this is fully protected from human activity (Marine Conservation Institute, 2023). However, to reduce pressure on the species, habitats, and ecosystems the European Union (EU) requires, with the Biodiversity Strategy 2030, that all member countries must protect at least 30% of both land and sea areas by the year 2030. 10% of the 30% should be strictly protected (European Commission, 2021). United Nations (UN) have also released a Global Biodiversity Framework to guide countries worldwide how to protect 30% of the global sea and land areas by 2030 (UN, 2022). Similarly, the High Seas Treaty was agreed upon in March 2023 to protect 30% of the high seas – waters beyond national jurisdiction – which account for two-thirds of the world's

ocean, and of which today only 1% is governed by rules that restrict human activity in the interests of protecting biodiversity (Claudet et al., 2023). With these important and international agreements both UN and the EU aim to protect areas of highly important and valuable biodiversity (European Commission, 2021; UN, 2022). To fulfil the goals of the agreements, the individual countries are responsible for developing and managing their protected areas and conducting appropriate monitoring for these regions (European Commission, 2021). Yet, one of the key challenges related to fulfilling the goals of these treaties is how do we know which species are present and where, and which areas are important to protect?

There are three main approaches that MPA's are usually based on: (I) proactive, preservation of ocean or coastal areas, focusing on protecting areas with high diversity before something happens; (II) Interactive, where the reason to protect is to minimise conflict, e.g., between commercial and private fishermen; (III) Reactive, protect areas to restore overexploited areas (Agardy, 2000). The majority of MPA's designs follow the first strategy, and many prioritise preserving biological hotspots (Agardy, 2000; Costello et al., 2022). Biological hotspots are geographical areas with an extraordinary rich species diversity, distinct ecology, or areas with high endemism (taxon limited to a small geographical area (Morrone, 2008)). The latest one on the list have a high global value since species here are not usually found elsewhere (Costello et al., 2022). For analysing MPA's "success", Rossiter and Levine (2014) developed four outcomes based on common goals for MPA's:

1. Improved ecological conditions, population growth in targeted species and increasing biodiversity.
2. Legislation and rules for the area are complied with, either legally or by social pressure.
3. A positive view on the outcomes of the MPA from the local community.
4. No loss in income or livelihoods for local community, or there are alternatives that balance out the losses.

A necessary prerequisite and the key to evaluate MPA design and effectiveness is biodiversity assessments, research and monitoring. Monitoring of MPA's often starts with holistic baseline biodiversity assessment to establish a foundation of the species occurring in the area for long term monitoring. The baseline should also include monitoring of key ecological, socioeconomic, and cultural use of the area. The ecology data should provide an assessment of the key habitats, as well as provide information of habitats that have not been studied before, to evaluate and refine the condition for long term monitoring, and how to best possible conduct the monitoring plan. Long term monitoring of the ecosystem should provide information about the status and trends of the ecosystem. The programs should also be flexible, so new scientific improvements can be implemented, if necessary (California Department of Fish and Wildlife, 2017). Despite its importance, holistic baseline biodiversity assessments and long-term monitoring activities with regular occurrence are rarely carried out (Bianchi et al., 2022).

1.2 Biodiversity assessments and monitoring

Life cannot be expressed as a single number (Purvis & Hector, 2000), biodiversity is multidimensional, and therefore not a simple concept to measure (Naeem et al., 2016; Whittaker, 1972). A single measurement might not be sufficient to gain knowledge about structure, function, or the state of the ecosystem (Sala & Knowlton, 2006). In addition, water is not stationary and has no boundaries – current systems transport organisms both

in and out of different areas (Agardy, 2000; Sætre, 2007). Biodiversity indices such as species richness, evenness, species dominance, and Shannon-Wiener have traditionally been used as biodiversity metrics (Lamb et al., 2009; Nolan & Callahan, 2006). These indices can be relatively easy to use, calculate, and have historically been commonly used (Lamb et al., 2009). Species richness is the simplest and the most commonly used metric for biodiversity and it is defined as a number of species found in a specific area or sample (Appeltans et al., 2012; Lamb et al., 2009). Evenness describes how common or rare the species is in an area or sample and therefore it requires information on the abundance of each species relative to the other species (DeJong, 1975; Lamb et al., 2009; Nolan & Callahan, 2006). The Shannon-Weiner is a species diversity index, commonly used in ecology, which takes into consideration both the species richness and the evenness (Nolan & Callahan, 2006). Species dominance can be structured into a "dominance curve" where species detected are ranked after abundance or total biomass, in decreasing order (Clarke, 1990). In addition to these indices, biodiversity can be also classified into three interrelated aspects: (I) Alpha diversity measuring the diversity of species in a community at a local area, (II) beta diversity comparing the diversity of two different communities, often divided by a geographical barrier, and (III) gamma diversity, looking at a very large scale, comparing species diversity across many ecosystems (Babu, 2016; Walters & Martiny, 2020). All these biodiversity measures are, however, based on the knowledge of what species are present.

Monitoring can be defined as rigorous sampling of the environment (biological, physical and/or chemical) for a well-defined purpose and endpoint (McLusky & Elliott, 2004). Ocean monitoring programs can in some ways, be compared to weather forecasts. To manage the global ocean there is a need for observation systems that can provide accurate and timely information about both abiotic and biotic factors in the marine realm. This data can help predict impacts of both climate change and human activity (Estes Jr et al., 2021), and is highly important for ecosystem management (Borja & Elliott, 2013). Biology monitoring has traditionally been conducted using morphological species identification (Bates et al., 2007), requiring taxonomically skilled personnel, which is laborious and expensive. For example, pelagic ecosystems monitoring has been traditionally conducted with plankton nets and trawling and thereafter identifying and counting the specimens with microscope based on the different morphological characteristics (Arashkevich et al., 2015; Trenkel et al., 2019). Due to high costs of morphological identification-based biodiversity surveys and monitoring programs are often under cuts (Borja & Elliott, 2013). For example, the monitoring program of Trondheimsfjorden started in 1963 by Trondheim Biological Station, measuring salinity, temperature, and oxygen, as well as sampling phytoplankton and zooplankton community, but ended in 2014 due to lack of resources (Bakken, 2023). Despite high importance of long-term monitoring, most of our knowledge about environmental changes is based on scattered measurements gathered around the world since the 1700s. However, often, these datasets are not enough to state the changes in the environment, due to neither having enough temporal nor spatial coverage (Estes Jr et al., 2021). Therefore there is a high pressure that the monitoring methods must be improved since "*Improved understanding requires improved observations – we cannot understand or manage what we don't measure*" (Estes Jr et al., 2021, p. 36). Today, increasing number of biological assessments and monitoring programs lean on new innovative methods, with a goal of making it more efficient, cost effective, having higher temporal and spatial cover and being less labour intensive at the same time (Allotta et al., 2017; Dallolio et al., 2019; Yamahara et al., 2019)

Real time monitoring is important for detecting changes in the environment, and for better management. Data will not be sufficient if it takes a year to process and identify the species in the plankton net samples, and the management decision making needs to happen quickly (Borja & Elliott, 2013; Bourlat et al., 2013). Today, technology for sampling and monitoring evolves rapidly, with main focus on two specific areas (Danovaro et al., 2016): (I) Molecular approach for better assessment of biodiversity (Bourlat et al., 2013), and (II) Autonomous collection of *in situ* data, with a wide temporal and spatial scales (She et al., 2016). Observation tools like autonomous underwater vehicle (AUV), satellite, and air crafts, can perform remote sensing of large areas, and form a shift from the traditional stationary sampling from research vessels (Estes Jr et al., 2021; Fossum et al., 2012). However, buoy-based sampling, which are usually stationary or drifting, is a suitable monitoring system to observe the ocean due to both opportunities to monitor large timeseries and attach array of different sensors (e.g., CTD, weather, pH, plankton imaging, among other; Albaladejo et al., 2012; SNITEF, n.d.). For example, Ocean Lab utilize buoys in Trondheimsfjorden which are monitoring the fjord continuously (SINTEF, n.d). Also, acoustic tools have become widespread in ocean monitoring due to the possibility to investigate the entire water column, and among others detect scattering layers (a layer with high density of marine organisms reflecting sound; Geoffroy et al., 2019; Kloser et al., 2009; Moyer, 2022). However, remote sensing alone can detect biology, but not on a species level, and therefore it is not suitable as a biodiversity monitoring method on its own (Ershova et al., 2021). Therefore combination of methos such as autonomous collection and molecular analysis can be beneficial and desirable (Danovaro et al., 2016). Molecular science is perhaps one of the most likely sources of innovation in marine biodiversity monitoring techniques, and it is increasingly used. It has also shown to result in cost effective and practical advantages when analysing organisms in the environment (Bourlat et al., 2013).

1.2.1 Monitoring methods

When we are increasing human actions in the costal and oceanic areas, and implementing more MPA's there will be a higher need for monitoring, and efficient ways to conduct the monitoring programs (Cicin-Sain & Belfiore, 2005; Pendleton et al., 2020; Weller et al., 2019). Traditionally, monitoring for example zooplankton has relied on plankton net tows and following classical microscopy, which is costly, time consuming and requires high expertise in taxonomy (Arashkevich et al., 2015; Borja & Elliott, 2013; Danovaro et al., 2016). In addition, several taxa are hard or impossible to identify to low taxonomic levels (species/genus level), due to high resemblance between similar species in the same genus as well as difficulty in connecting early and late life stages (Stefanni et al., 2018). For example, Tanaka (2007) found that our understanding of the diversity and life cycles for the species in the Gnathiidae Leach, 1814 family (isopod crustaceans) have evolved slowly due to problems connecting juveniles and adults. Other species have been underestimated by taxonomy, due to difficulties in the sampling and handling approaches, for instance gelatinous zooplankton (phrase describing planktonic species with transparent and fragile features; Haddock, 2004). Gelatinous zooplankton species are often fragile (break easily e.g., when sampled and hard to preserve (Harbison et al., 1978)), are highly variable in body size or live just above the seafloor which is area hard to sample with plankton nets. Hence, including these gelatinous organisms into biodiversity assessments and monitoring programs by plankton net is difficult (Haddock, 2004). Sometimes, even the most studied species are difficult to distinguish (Choquet et al., 2018). One of the most studies copepod genus *Calanus* Leach, 1816 has over 100 scientific publications (Web of Science, n.d*.)*, and it is still difficult to distinguish between different species which co-occur in the same regions

(Hirche et al., 1994). Taxonomic identification of *Calanus* species relies mainly on standardized size tables, redness of antennas and structure of the fifth pair of swimming legs. However, none of these can give a reliable identification (Choquet et al., 2018).

Molecular methods have shown promising results in species detection and estimation of species diversity (Jerney et al., 2023). Molecular approaches often refer to two different approaches: single species detection (two methods: DNA barcoding and quantitative polymerase chain reaction, qPCR) and multiple species detection (DNA metabarcoding; Cristescu, 2014). These approaches are dependent on DNA markers, short homologous DNA fragments, e.g., COI, 16S, 18S, to name a few. DNA barcoding refers to the identification of one single specimen, using a single standardized DNA fragment (Deiner et al., 2017; Hebert et al., 2003; Valentini et al., 2009). The method is divided into two different parts (I) Taking sequences from a specimen of a known species, to build a molecular refence library, and (II) Matching barcode of an unknown specimen to the reference library for identification (Kress & Erickson, 2012). qPCR refers to a method where amount of DNA from a single species is detected in a sample using a species-specific DNA marker (LeBlanc et al., 2020; Taniguchi et al., 2022). DNA metabarcoding refers to identification of multiple species from a mixed sample (bulk sample or environmental DNA) (Liu et al., 2020), and requires taxonomical complete reference libraries with DNA sequences for each species (Bucklin et al., 2016). All the methods rely on amplification of DNA markers in PCR. DNA barcoding and metabarcoding are further dependent on sequencing of the amplicons while qPCR is just detecting PCR amplification success. PCR was invented as a method for amplifying specific DNA sequences. The method is often referred to as simple and fast at the same time it has high sensitivity and specificity (Newton et al., 1997). The PCR processes are reliant on, among others, distinct temperature steps:

- Denaturation: The double-stranded DNA are desaturated by heating, to separate to single strands, typically at 94ºC
- Annealing: rapidly cooling of the reaction, to allow the primers to hybridize to the template.
- DNA synthesis: reaction is heated, usually 72ºC, so the DNA polymerase can conduct efficient DNA synthesis.

Each cycle the PCR template strands give a new duplex, and each of the next cycles will double the number of copies of the target region (McPherson & Møller, 2006). PCR is a qualitative method, whereas qPCR is used for quantification (Bustin, 2010).

DNA samples can be collected by either as community DNA metabarcoding sample (hereafter bulk DNA sample), which is a sample of the organisms themselves (e.g., net tows or organisms isolated from sediment samples), or environmental DNA metabarcoding sample (hereafter eDNA), which is samples from the environment (e.g., water, air or soil, to name a few) (Creer et al., 2016; Deiner et al., 2017). In bulk samples, the DNA is extracted from the tissue of the specimens itself, whereas eDNA uses the DNA that the specimens have shed into the environment (e.g., mucus, cells, and skin, to name a few), without the presence of the organism itself (Klymus et al., 2015; Taberlet et al., 2012). Despite very similar principles and sample processing technique in the laboratory, they are very different in quantity and quality of the used DNA. Where the bulk DNA samples have the whole genome from species, eDNA often just have small fractions (Deiner et al., 2017). The methods can also be used for quite different purposes: as the bulk samples often are used where samples are easy to obtain, eDNA can be used in more remote areas, targeting

organisms that are difficult to sample, e.g., endangered or rare species (van der Loos & Nijland, 2021). However, both methods have their opportunities (table 1) and limitations (table 2).

Table 1: opportunities with different monitoring methods; morphological species identification, Imaging, bulk DNA metabarcoding, eDNA metabarcoding and qPCR. (Andruszkiewicz Allan et al., 2021; Barnes & Turner, 2016; Beja-Pereira et al., 2009; Biggs et al., 2015; Sarah J Bourlat et al., 2013; Bucklin et al., 2016; Davy et al., 2015; Dejean et al., 2011; Ficetola et al., 2019; Taniguchi et al., 2022; Thomsen & Willerslev, 2015; van der Loos & Nijland, 2021)

	Opportunities					
Method	**Species identification**	**Life stages**	**Quantification**	**Time**	**Cost**	**Additional notes**
Morphological species identification	Generally good, see all the organisms in the samples	They can generally be identified	Can count the species in the sample			Simple and reliable method for trained personnel
	Know that the species was alive at time of sampling					
Imaging (Silcam, UVP6)	Generally good, limited to certain size classes	They can generally be identified	Can count the species in the sample	With AI methods can be relatively fast	Cheap	Requires good quality reference library and time to train the AI
Bulk DNA metabarcoding	Detection of many species	Not possible, except meroplanktonic larvae can be separated from benthic adult life stages	There is a weak correlation between DNA reads and relative abundance	Faster than traditional methods, increasing number of samples does not necessarily increase laboratory time	Often more cost effective than traditional methods, since cost don't increase with increasing number of samples	If there are questions about the reliability, and samples are not destroyed or if they are split before DNA analysis, they can be morphologically verified
				Entire samples can be sequenced as once, instead of single specimens		Easy to compare, if one use standardized protocol and implementation
eDNA metabarcoding	Detection of many species (Bucklin et al., 2016), Sample many species at the same time from a relatively small sample.	Not possible, except meroplanktonic larvae can be separated from benthic adult life stages	There might be weak correlation between DNA reads and relative abundance	Faster than traditional methods, increasing number of samples does not necessarily increase laboratory time	Often more cost effective than traditional methods, since cost don't increase with increasing number of samples	Non-invasive method, can study a population without disturbing or inflict damage on species or habitats
	Possible to detect rare, endangered, potentially invasive or low-density species			Entire samples can be sequenced as once, instead of single specimens		Perhaps the most likely source of innovating marine monitoring techniques
	Can sample in habitats with limited access					
Quantitative PCR	Only Single species/group detection	Not possible	If standard curve is good, the amount of DNA can be quantified	Yes	Cheap	Good for detecting invasive species in large spatial and temporal scales if the species-specific primers exist.

Table 2: Table 2: limitations with different monitoring methods; morphological species identification, imaging, bulk DNA metabarcoding, eDNA metabarcoding and qPCR (Alberdi et al., 2018; Aylagas et al., 2016; Beng & Corlett, 2020; Bucklin et al., 2016; Dawson et al., 1998; Ficetola et al., 2019; Hansen et al., 2018; Post et al., 1993; Sigsgaard et al., 2020; Strickler et al., 2015; Taniguchi et al., 2022; Zizka et al., 2018)

Limitations						
Method	**Species identification**	**Life stages**	**Quantification**	**Time**	**Cost**	**Additional notes**
Morphological species identification	Requires trained personnel	Identification keys are often to certain life stages		Very time consuming	High as it requires trained personnel and is slow	
	Identification keys are scattered in literature					
Imaging (Silcam, UVP6)	Requires good quality reference library	Limited to certain size		Time consuming to train the AI		
Bulk DNA metabarcoding	Dependent on the marker region and primer (the specificity, sensitivity, and efficiency) used	No data on population composition (e.g., sex ratios, life stage, health condition, size)	No abundance data, which is important to provide more information about populations/ community/ environment	Relatively high starting price, since consumables and sequencing is costly		Dependent on a complete reference library
	The subsample must be well-mixed to represent all species			DNA quality degrade quickly, especially in high temperatures		Difficult catching rare or endangered species
eDNA metabarcoding	Dependent on the marker region and primer (the specificity, sensitivity, and efficiency) used	No data on population composition (e.g., sex ratios, life stage, health condition, size)	No abundance data, which is important to provide more information about populations/ community/ environment	Relatively high starting price, since consumables and sequencing are costly		Degradation of DNA in the environment both abiotic and biotic factors (e.g., grazing, enzyme break down, temperature and UV-light)
	Dependent on a complete reference library		DNA shed differently from species to species	False positives (eDNA detected, but the targeted species is absent) and false negatives (eDNA not detected, but the targeted species is present)		
	Environment influence the shedding, degradation, persistence transport and location of DNA					
	Shedding rate of different animal forms, e.g., hard/soft body (fish versus gelatinous species)					
Quantitative PCR	Only Single species/group detection	Can't detect life stage				

When designing a molecular species identification, or even molecular monitoring, an important concern to take into account is the targeted species group, since this will affect the approach, marker, primers and reference library used. The different markers target different fragments of genes and different organisms groups (Hebert et al., 2003). Markers can either target the mitochondrial DNA (mtDNA; e.g., COI and 16S; Hebert et al., 2003; Pereira et al., 2006) or nuclear DNA (nDNA; e.g., 18S; Berntson et al., 2001; Hoot et al., 1999; Zrzavý et al., 1998). MtDNA is a maternally inherited short circular DNA molecule (Guo et al., 2022; Sato & Sato, 2013; Smeitink et al., 2001), whereas nDNA is a linear DNA molecule, inherited from all ancestors (Christensen et al., 2014). For example, Cytochrome oxidase I (COI) markers target the mitochondrial gene coding part of a large transmembrane protein complex of a respiratory electron transport chain of cells (Hebert et al., 2003; Watson & McStay, 2020), and 18S ribosomal RNA (rRNA) gene markers target the nuclear gene coding ribosomal RNA (Krieger & Fuerst, 2002). They are two of the most commonly used markers for metazoans (van der Loos & Nijland, 2021), whereas 16S ribosomal RNA gene markers are alternatives to the COI markers (Devloo-Delva et al., 2018). Both nDNA and mtDNA can be used for species identification (Stewart-Clark et al., 2009), but mtDNA have preferably been used, due to for example mutations which differentiate closely related species from each other (Guo et al., 2022).

A primer is a short single-stranded DNA fragment, used in PCR to amplify a chosen marker (Shchelochkov, 2023). Primers are both forward and reverse and they are bonded to specific locations, where the forward primer attaches the beginning and the reverse primer to the end of a DNA strand, after which polymerase will copy the part of target sequence between the two primers (Chuang et al., 2013). qPCR is dependent on species-specific primers (Taniguchi et al., 2022; Wood et al., 2019) while DNA barcoding can be done both using species-specific or universal primers (Li et al., 2022). DNA metabarcoding is dependent on universal primers, which can detect millions of DNA fragments from a wide range of species through multiple trophic levels (Djurhuus et al., 2020; Kim et al., 2021). Species detection using eDNA usually rely on relatively short fragments of DNA, which can be prone to biases. Primers used to amplify these fragments are therefore extremely important (Beng & Corlett, 2020), and the amplification relies on primer specificity, sensitivity and efficiency. Samples usually have a high diversity of taxa and DNA variations, which makes it difficult to achieve complementarity between primer and the sequence target during the PCR (Stadhouders et al., 2010), for example primer mismatch can lead to a higher amplification of non-targeted species compared to targeted species or amplification of short sequences compared to long sequences (Nichols et al., 2018). Amplification strategies in PCR can also influence the detection of species (Beng & Corlett, 2020), for example the number of PCR cycles can influence the results: a high number of cycles can lead to observation of more rare molecules, but this can also interfere with the relative abundance results (Nichols et al., 2018).

Creation of good reference libraries is essential for DNA barcoding and metabarcoding, they contain species name and DNA barcodes, for species identification (Bourlat et al., 2013). "*The taxonomic identification of taxa is no better than the reference database used*" (Thomsen & Sigsgaard, 2019, p. 1676). Public reference databases such as Barcode of Life Database (BOLD; Ratnasingham & Hebert, 2007) and GenBank (Sayers et al., 2020) contain DNA barcode data for animals, plants and fungi, and important tools for molecular assessments. GenBank is a sequence repository and BOLD both store sequences and work as a curation tool (Meiklejohn et al., 2019). The International Barcoding of Life (iBOL) was established as a collaboration to create reference library of DNA barcodes for multi-cellular life. With the main goal to extend the taxonomical and geographical coverage of BOLD and

speed the discovery of new species, track species dynamics and interaction (International Barcode of Life n.d). The Norwegian Barcode of Life (NorBOL) is a part of the iBOL project. NorBOL work as a national collaborative DNA-barcoding network for research institutions and have generated barcodes for over 20 000 species, which is available in BOLD. This contributes to over 30 % of the estimated species diversity in Norway (Norwegian Barcode of Life n.d). Even with these projects, there are still gaps remain in reference libraries, and many marine taxa is lacking (Weigand et al., 2019). Ctenophore *Bolinopsis infundibulum* (Müller, 1776*)* is common ctenophore in Norwegian and Arctic waters, whereas *Mnemiopsis leidyi* A. Agassiz, 1865 is highly invasive species and occurs in Norwegian coast up to Bodø. (Hosia & Falkenhaug, 2015). COI of *M. leidyi* is in the reference library but *B. infundibulum* is not. Molecular methods give the closest match and since these two species have strong relation misidentification is highly possible, which can give wrong interpretation of the presence species (Faasse & Bayha, 2006; Johansen 2019).

1.3 Zooplankton

Species diversity will enhance the ecosystem stability, and the productivity of a marine ecosystem is strongly linked to the biodiversity (Worm et al., 2006). Plankton communities are highly diverse, ranging from unicellular plants; phytoplankton, to generally small animals; zooplankton, but which can also be several meters in size (Brierley, 2017). Plankton includes all organisms that drift passively with water (Hays et al., 2005), or are unable to swim effectively against horizontal water movements (Hays et al., 2005; Weikert, 1987). Secondary producers, which channel energy from primary producers to higher trophic levels, depend on a diverse food source (Worm et al., 2006). Phytoplankton is the main primary producer in marine systems, and zooplankton plays a key role, as the main primary consumer, to funnel the energy up the food chain (Berge, 2020; Brierley, 2017; Richardson, 2009). Zooplankton are the most abundant animal group on the planet, outnumbering insects of magnitude. They also form a critical food source for fish, seabirds and whales, as well as benthic communities like anemones, crabs and fish through faecal pellets and carcases (Richardson, 2009). The extremely diverse zooplankton assemblage (Bucklin et al., 2010) contains a vast variety of species characteristics in sizes, life cycles, feeding ecologies, physiologies and evolutionary histories (Hagen & Auel, 2001; Richardson, 2009; Richardson, 2008). Despite their generally small size, they perform a variety of important ecosystem functions like being an important contributor to the biological carbon pump (Richardson, 2009; Richardson, 2008).

Zooplankton can be divided into two main groups, holoplankton and meroplankton. Holoplankton have their whole lifecycle as plankton, and meroplankton spend part of their lifecycle in a planktonic stage before graduating either to nekton or benthos (Richardson, 2009). Due to the size diversity, ranging from micrometres to several meters, zooplankton are also divided based on the size: (I) Mesozooplankton (0,2 – 20 mm), (II) macrozooplantkon (2 – 20 cm) and (III) megazooplankton (20 – 200 cm) (Hays et al., 2005), as well as some life stages of mesozooplankon, egg and larva, are even smaller (Bucklin et al., 2021). Zooplankton occupy several trophic levels, but their primary niche is primary consumers, and zooplankton is then considered as a link between the lower and higher trophic levels (Berge et al., 2020). However in reality, the role of zooplankton in the ecosystems is highly variable and they have different feeding strategies, like predation, grazing and filter feeding (Richardson, 2009; Richardson, 2008). For example, the deep-sea jellyfish *Periphylla periphylla* (Péron & Lesueur, 1810) is a tactile predator dependent on close contact between the prey and its body (Ugland et al., 2014). Filter feeders are found amongst many planktonic taxa and size groups, and they are dependent on

suspension of phytoplankton or bacteria as food source. For example, the copepod *Arcartia tonsa* Dana, 1849 is a filter feeder which makes small currents by breathing and swimming motions to catch food (Kiørboe, 2011). Many medusae can also be classified as filter feeders, for instance individuals of the hydrozoan genus *Obelia* Péron & Lesueur, 1810*,* makes small currents towards the mouth for catching food particles (Boero et al., 2007; Boero & Sarà, 1987). Hence, zooplankton can be considered one of the key organisms for all marine life (Berge, 2020; Richardson, 2008; Weikert, 1987), and functions as a good indicator for climate change for a few reasons (Richardson, 2009; Richardson, 2008). For example, (I) zooplankton physiological processes (respiration, ingestion and reproductive development) are highly sensitive to temperature change (Taylor et al., 2002), (II) they are short lived species, and therefore less influences by persistent individuals, population changes can therefore be linked to climate changes (Hays et al., 2005), (III) not commercially exploited so changes in genetics can be linked to environmental changes (Hays, 2003; Richardson, 2008).

The global assemblage of zooplankton is exceptionally diverse, with 15 phyla and 41 orders and classes, and includes also several undescribed taxa (Bucklin et al., 2021). Today there are described about 5700 species of metazoan holoplankton and it has been estimated that about 1600 species are still not discovered (Wiebe et al., 2010), when including meroplankton the estimated number of described species increase to about 30 000 metazoan species (Larink & Westheide, 2006). Species diversity assessment of zooplankton presents challenges, due to groups of sibling species, for example in the class Copepoda, which are difficult to separate with morphological characters (Bucklin et al., 2011; Choquet et al., 2018). Many species have a broad biogeographical distribution and span over multiple ocean basins (Bucklin et al., 2011), therefore some areas can contain 10% of the global known taxa, especially in regions classified as biological hotspots (McGowan & Walker, 1979).

Crustaceans are often the dominating group of zooplankton, with copepods being the most abundant group. Copepods are one of the most essential parts of the lower levels of the marine food web, forming a link between primary producers and higher trophic taxa (Berge et al., 2020). This means they play a key role in energy transport in the marine food web. Copepods are extremely diverse, and one of the most abundant multi-cellular organisms on earth (Richardson, 2008). The adult copepod is usually free living, with a body size ranging between 1 to 5 mm (Boxshall & Defaye, 2008). Copepods in the genus *Calanus* are often the most dominant in terms of biomass in the North Atlantic and Arctic waters (Falk-Petersen et al., 2007). *Calanus finmarchicus* (Gunnerus, 1770), *Calanus glacialis* Jaschnov, 1955 and *Calanus hyperboreus* Krøyer, 1838 coexist in these areas (Freer et al., 2022). *Calanus finmarchicus* is classified as an Atlantic species (Jaschnov, 1970), whereas *Calanus glacialis* and *Calanus hyperboreus* are both classified as Arctic (Hirche & Niehoff, 1996; Jaschnov, 1970). However, *C. hyperboreus* is mainly found in the oceanic part of the Arctic, while *C. glacialis* is predominantly on the Arctic continental shelf and slope (Fossum et al., 2012; Timofeev, 2001). *C. finmarchicus* and *C. glacialis* are often linked to Atlantic (*C. finmarchicus)* and Arctic water (*C. glacialis),* and therefore used to understand the spatial distribution of the two water masses (Unstad & Tande, 1991), and an indicator for "Atlantification" (the increased influence of Atlantic water in the Arctic region due to climate change, resulting in increasing water temperature and salinity) (Polyakov et al., 2017; Årthun et al., 2019). In addition, the species *Calanus helgolandicus* Tanaka, 1956 also occupy Atlantic water, *C. helgolandicus* usually occurs in warmer water than *C. finmarchicus,* but the two species have some overlapping geographical range (Bonnet et al., 2005; Fleminger & Hulsemann, 1977).

Identification of the different species of *Calanus* can be very challenging. For example, *C. glacialis* is bigger than *C. finmarchicus*, and prosome length is therefore commonly used (Broms et al., 2009; Choquet et al., 2018; Gabrielsen et al., 2012). However, Choquet et al. (2018) found that the latitude and body size of *C. glacialis* correlate. *C. glacialis* found in the Norwegian fjords overlaps with the body size of *C. finmarchicus,* but these *C. glacialis* were significantly smaller than in Arctic waters. Largescale misidentifications have led to wrong conclusions regarding the biogeography (Choquet et al., 2017; Choquet et al., 2018; Gabrielsen et al., 2012). Correct identification is crucial when studying life strategy, productivity, and distribution, especially for species that can indicate changes in the ecosystem (Choquet et al., 2018). The latitude correlation with body size may be the effect of temperature, and therefore climate change and ocean warming can affect the size of *C. glacialis* (Atkinson & Sibly, 1997; Choquet et al., 2018). The body size can also be connected to the length of *C. glacialis* life cycle, which is often two years, but one- and three-year life cycle have also been observed (Gabrielsen et al., 2012; Tande, 1991). Correct identification will either way be important to understand the life history *of C. finmarchicus* and *C. glacialis*, how they evolve and are affected by climate change. Molecular tools can therefore be highly important for reliable species identification (Choquet et al., 2018).

Many taxa of zooplankton can perform vertical migration. In the 1800's Cuvier (2018) observed movement of zooplankton, which later was described as diel vertical migration (DVM), and seasonal vertical migration (SVM) (Bandara et al., 2021; Brierley, 2014; Hays, 1995). DVM is periodical with a duration up to one day and is one of the world's largest migrations of biomass. Zooplankton generally migrate up in the water column during dusk, and down during dawn, and this movement allows organisms to feed in shallow water by night, and avoid visual predators by day (Brierley, 2014; Williamson et al., 2011). SVM is periodically up to one year, and organisms migrate downwards at the end of the productive season, usually early autumn, to overwinter, and up for the spring bloom (Berge et al., 2020). SVM can also be described as an ontogenetic migration, dependent on life stage, sex, and biological rhythm (Berge et al., 2020; Dorenbosch, 2006; Hirche, 1989). For example, the genus *Calanus* is known for SVM, where juveniles and adults spend winter in deep waters. During this time, they enter diapause, minimising their feeding, development, and respiration (Berge et al., 2020). Also, meroplanktonic larvae are commonly considered to occur in the water masses only during very short period during the most productive period (Descôteaux et al., 2021). Despite these common descriptions of DVM and SVM some species conduct these migrations with the opposite pattern (Ohman et al., 1983), and DVM can be performed even in 12 hours cycles or only during part time of the year and some meroplanktonic species can occur in low numbers outside of the main peak time, also DVM pattern may vary between different development stages within a species (Basedow et al., 2010; Forward & Cohen, 2010; Lampert, 1989; Ohman, 1990). The diverse patterns of both DVM and SVM effect which species are abundant in the different regions and in the different depths, and might have indications on what species are found at the time of the sampling (Bandara et al., 2021; Basedow et al., 2010).

1.4 Zooplankton diversity assessment on a biodiversity hotspot

The Norwegian coast is one of the most productive in the world, and it is therefore difficult to decide which one is the most valuable area to protect. The Norwegian Institute of Marine Research have made some recommendations for which areas in the Norwegian oceans should be classified as "particularly valuable and vulnerable", and therefore also possibly be potential MPAs. The area of Mausund Bank (63.8° – 64.2°N, 8.2° – 9.0° E), used in

this study, is an area of significantly important biological diversity and production, and therefore a part of this classification (Eriksen et al., 2021).

The overall aim of this thesis is to test applicability of DNA-based methods in biodiversity assessment and future monitoring zooplankton community in a biodiversity hotspot, and whether DNA can be used in a quantitative manner (qPCR and eDNA metabarcoding). To do this, eDNA samples were compared with traditional zooplankton net samples identified by morphological approach and evaluated if the eDNA based results can be quantified. To make comparable results *Calanus finmarchicus* was used as a key study species. The samples were taken during the spring bloom, and the data was also used to look if the relative abundance of different taxa makes sense for the season, and to describe the overall diversity of zooplankton in the area. The study also aimed to look at if the time point of the sampling (day versus night) would affect the biodiversity in the eDNA and morphology samples.

Based on previous knowledge, it was hypothesized that; (I) The species table for eDNA samples and morphological samples will correlate with each other, (II) qPCR can be used as a tool for quantifying *Calanus* samples, and (III) sampling at night versus day will show a pattern of diel vertical migration and will affect the species recorded in the area.

2 Materials and methods

This thesis was connected to the project Light as a *Cue for Life in Arctic and Northern Seas (LightLife)* which is funded by the Norwegian Research Council (2021-2024). The main aim of the project is to understand the role of light in the visual ecology of Arctic and Nordic zooplankton. This thesis has close connection to work package 1: "*Species dynamics and role of light climate on photo-biological responses in the Arctic*". The main goal of this WP is to gain knowledge about the zooplankton biodiversity during diel vertical migration in relation to the ambient light climate. To be able to understand how individual species react on changes in light climate, correct species identification is needed, and therefore knowledge on morphological and DNA-based species identification is required.

2.1 Sampling location

The physical environment is an important part of the marine environment and for the species living in it. Depth, bathymetry, temperature, salinity, and currents determine which organisms can survive in the ecosystem, and where they can live and interact with others. In general, the Norwegian coast is influenced by the Norwegian Coastal Current (NCC), the water masses originating primarily from fresh water runoffs from both the Baltic Sea and the Norwegian coast (Sætre, 2007). The coast is also influenced by Atlantic Water (AW) from the North Atlantic Ocean. This inflow is forced by atmospheric conditions, like the North Atlantic Oscillation (NAO), and transports warm, saline, and nutrient-rich water towards the coast (Ingvaldsen & Loeng, 2009; Sætre, 2007). AW is transported by two main branches to the Norwegian coast, one branch from the south and one from the north of the Faroe Islands (Hansen et al., 2008; Sætre, 2007). AW covers the area under and on the oceanside of the NCC, following the edge of the Norwegian continental slope (Orvik & Niiler, 2002). The two currents have a dynamic interaction and can form circular currents in some areas (Sætre, 2007). The transported volume of water is season dependent, with almost twice as high volume in the winter compared to the summer (Orvik et al., 2001). The circulation of AW is influenced by the bathymetry (Orvik & Niiler, 2002), which is also highly important for circulation and vertical mixing. Salinity is generally used to classify between the different waterbodies, where AW is classified with a salinity greater than 35, and Norwegian Costal Water with a salinity below (Sætre, 2007).

The study area of Mausund Bank (63.8° – 64.2°N, 8.2° – 9.0° E) is located in Froan Archipelago, Frøya county, mid-Norway. The area is shallow with strong tidal mixing and winds. The high geodiversity and complex bathometry contribute to the physical diversity, which is fundamental to support a rich ecosystem and a hotspot for primary production and marine life. The water flowing in and out of the Mausund Bank is dominated by two currents, the NCC dominating the surface layer and The North Atlantic Current flowing underneath. The NCC comes from the south and mixes with freshwater run-offs from the Norwegian coast, while the North Atlantic Current brings warm, saline and nutrient-rich water from the deep (Fragoso et al., 2019; Sætre, 2007). The deep Halten Bank is located outside of the Froan Archipelago making upwelling events when meeting the shallow Mausund Bank (Sætre, 2007). The high productivity in the area makes it one of the most important locations for commercial fishing industry in Norway (Ervik et al., 2018). Despite

this, the dynamics and distribution of plankton species in the area is a huge knowledge gap (Fragoso et al., 2019).

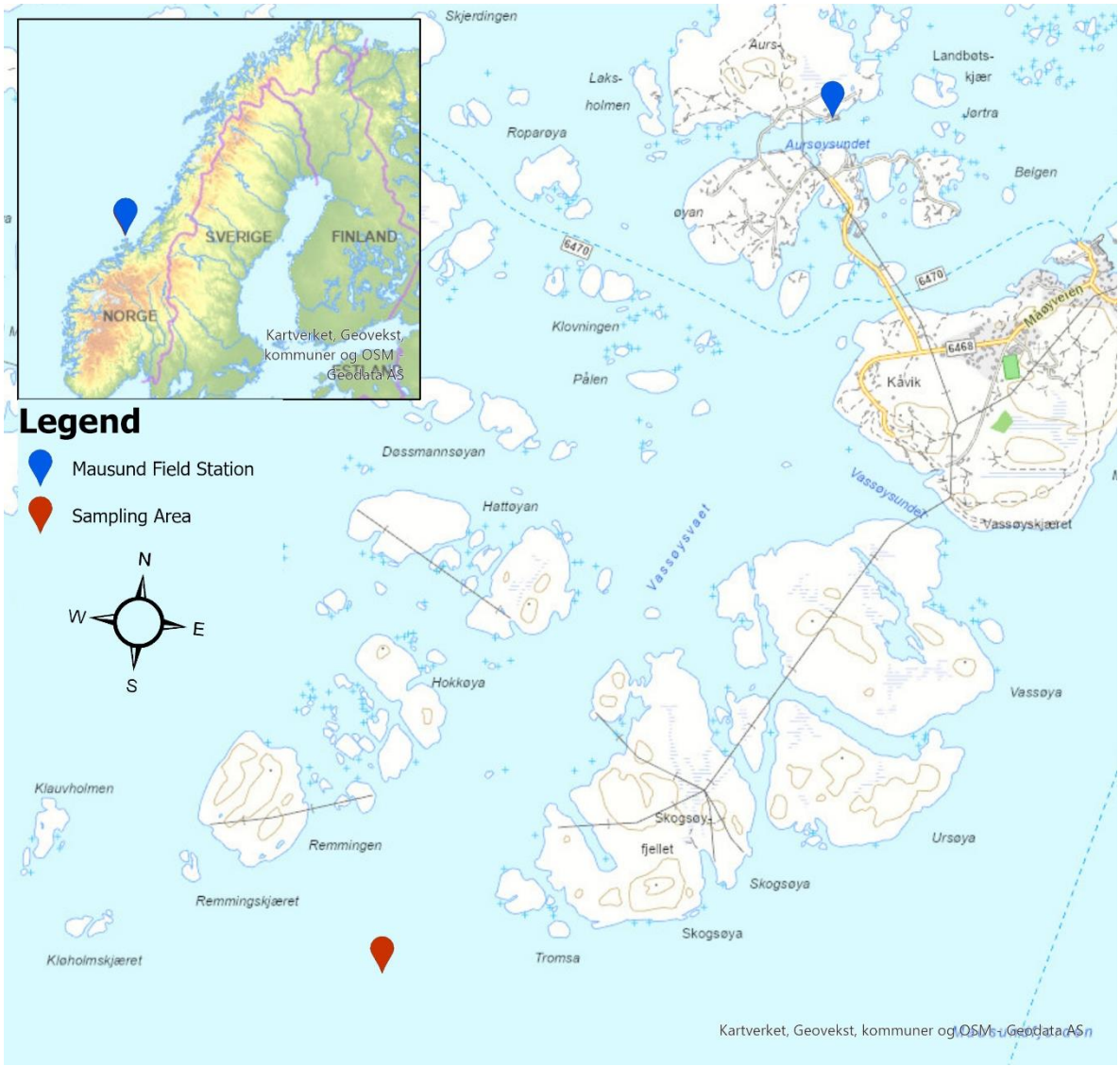

Figure 1 Map showing sampling location. The red tear pin represents the sampling area, and the blue tear pin where Mausund Field Station is located. Map made using ArcGIS PRO (Kartverket, Geodata AS)

2.2 Sampling

Samples for seawater for environmental DNA and zooplankton community composition were collected on the 31st of March 2022, at one station at the Mausund Bank (63.8° – 64.2°N, 8.2° – 9.0° E) (fig 1). The samples were collected at two timepoints, one targeting midday and one midnight. Duplicated water samples were collected with a custom made 5 L water sampler, at the surface, 10-, 25- and 50-meters depth. The water from each depth was stored in buckets and filtered through Glass Fiber Filter with binder (2.0 µm pore size, hydrophilic glass fiber binder resin, 47 mm diameter AP2504700, Millipore) with a vacuum pump in the laboratory (fig 2). To avoid cross contamination between samples, all equipment were disinfected with 10 % bleach between use. Two replicates were filtered from each depth and timepoint. The filters were preserved in 5 mL Eppendorf tubes prefilled with 4050 µL ATL-buffer (Qiagen) and stored in room temperature. Zooplankton

net sampling was conducted using a handheld zooplankton net with app. 200 µm mesh size with closed cod-end, at 0-5, 0-10, 0-25- and 0-50-meters depth. The samples from the net hauls were preserved in 96% ethanol (EtOH) in 200 mL bottles and stored in room temperature for later analysis of the zooplankton communities. A conductivity, temperature, and depth sensor (CTD, SAIV model SD204) was deployed at both timepoints, from the surface down to 50 meters.

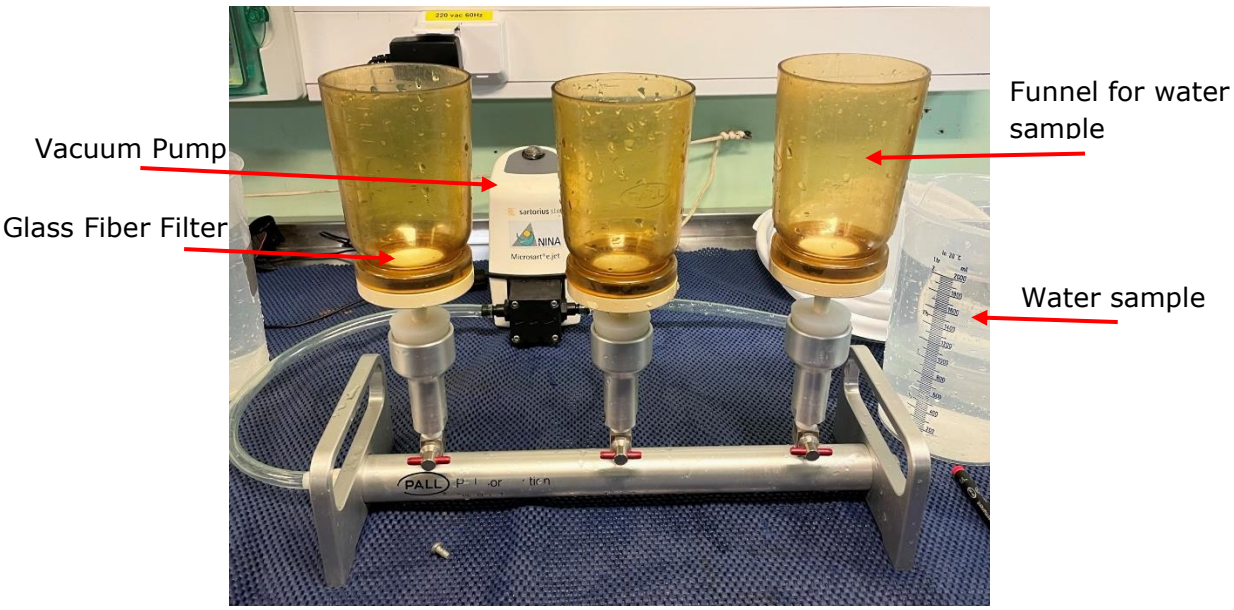

Figure 2: Sea water filtering set with 500 mL funnel with glass fibre filter and vacuum pump.

2.2.1 Zooplankton identification

In the laboratory, all specimens in the zooplankton samples were counted and identified to the lowest taxonomical level possible under a stereomicroscope (Leica), and using laboratory compendia (Daase, n.d.; Larink & Westheide, 2006). To count specimens from the genus *Calanus*, samples were split, using a 500 ml beaker filled with 100 ml diluted zooplankton. After mixing the sample, fractions of the samples were taken out with 5 ml pipette, depending on the size and density of the sample. From each fraction approximately 100 individuals were used for identification. To identify, 100 individuals' prosome length was measured and urosome segments and number of swimming legs were counted (fig 3). All *Calanus* were measured with x1.6 zoom at the microscope. The remaining *Calanus* of the split sample was counted. A fixed length table by Broms et al. (2009) for *Calanus finmarchicus* and *Calanus glacialis,* including all copepodite life stages, was used to identify the species (table 3). The total amount of both *C. finmarchicus* and *C. glacialis* was calculated.

Table 3: The size intervals of different stages of Calanus finmarchicus and Calanus glacialis. Standardized by the Institute of Marine Research, Bergen, Norway. Table source: Broms et al., 2009.

	Calanus finmarchicus	***Calanus glacialis***
CI	–	– 0.95
CII	– 1.1	1.2 – 1.4
CIII	– 1.5	1.6 – 2.1
CIV	– 2.1	2.2 – 2.9
CV	– 2.9	3.0 – 4.2
CVI (male and female)	– 3.1	3.2 – 4.3

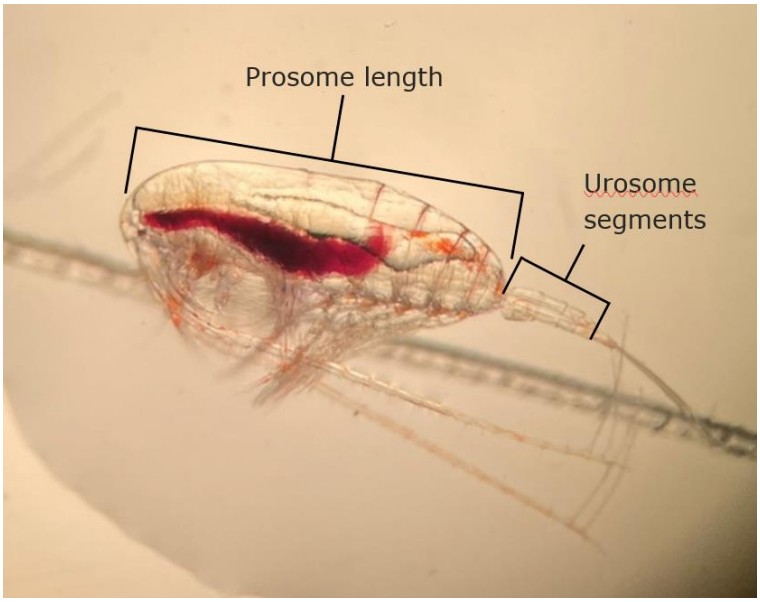

Figure 3: Calanus finmarchicus*. Prosome length and urosome segments. The prosome length is measured and the urosome segments counted to determine the* Calanus *life stage and species.*

2.2.2 Environmental DNA

From the Eppendorf tubes containing eDNA sample filters, DNA was extracted at NINAGEN, Centre for Biodiversity Genetics according to the NINAGEN protocol. 450 µL Proteinase-K (Qiagen) was added to all tubes before incubation in 56ºC overnight. The DNA isolation was then carried out using the NucleoSpin Plant II Midi kit protocol, with lysis- and cleaning buffers from Qiagen (Fossøy et al., 2020).

DNA metabarcoding of the eDNA samples was done at NINAGEN, following standard two-step Illumina 16S protocol (Anonymous, 2013). The amplifications were done using primers and PCR conditions from Leray et al. (2013) with slight modifications in the primer sequences (F: GGNACNGGNTGRACDSTNTAYCCNCC and R: TANACYTCNGGRTGNCYRAARAAYCA). The first amplification reactions included 12.5 µL 2x KAPA HiFI HotStart ReadyMix, 5 µL (0.4 µM final concentration) of each primer and 2.5 µL of DNA template. Each DNA sample was diluted to 10 ng/µL based on NanoDrop-measurements. In the second amplification step, samples were dual-indexed using IDT for Illumina DNA/RNA UD indexes. The PCR conditions were with a heated lid, 95 °C for 3 min, followed by a total of 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were cleaned with magnetic beads after each PCR step, using a ratio of 1:1 of beads and sample. In the end, indexed amplicons

were normalized and pooled into a sequencing library. The library was sequenced, using the 300 cycles NovaSeq 6000 SP Reagent Kit v1.5 (Illumina) at the Norwegian Sequencing Centre (NSC), Oslo, Norway.

The reads were processed with the R-package DADA2 1.18 (Callahan et al., 2016) in R 4.1.2 (R Core Team, 2021) at NINAGEN. Taxonomic affiliations of the generated ASVs (Amplicon Sequence Variants) were done in two steps at NINAGEN. First, taxonomic affiliations were identified, using blastn to search NCBI GenBank (Sayers et al., 2022), followed by the weighted lowest common ancestor algorithm in MEGAN 6.22.2 (Huson et al., 2016) with minimum bit score 400, top percentage 1.0 and minimum support 1. Secondly, all ASVs affiliated with Metazoa were searched against the BOLD database (S. Ratnasingham & P. D. Hebert, 2007). Only those ASVs were kept that were identified using BOLD.

2.2.3 Mock community experiments

Cultured *Calanus finmarchicus* from NTNU SeaLab Brattøra was used to quantify DNA content of individual specimens. The culture was established at SINTEF SeaLab in 2004. The culture originates from Trondheimsfjorden, from where life stages CIV and CV were collected with a zooplankton net. The cultures were moved in 2007 to new facilities at NTNU SeaLab Brattøra, where they are located today. The room is temperature-controlled with 10ºC, and the water is pumped from Trondheimsfjorden. *C. finmarchicus* are fed phytoplankton cultivated at SeaLab (SINTEF, 2007).

Individuals of adult female *C. finmarchicus* were carefully collected with wide-end pipet and kept in 1L water bottles for 24 hours. The water bottles contained approximately 1, 5, 10, 20 and 30 individuals (appendix 1). After 24h, all individuals from the bottles were collected in 2 mL Eppendorf tubes and preserved in EtOH and the water was filtered through 2.0 µm Glass Fibre Filters. Due to unforeseen events new specimens of *C. finmarchicus* was collected to be used as a mock community samples. The individuals used is therefore not the same as from the filtered water. The new batch of *C. finmarchicus* samples was not preserved in EtOH, but the samples were frozen overnight. DNeasy Blood and Tissue Kit (Quiagen) was used for DNA extraction both filters and individual samples. Excess water was removed from individual *C. finmarchicus* samples, and specimens were crushed before adding 180 µL ATL buffer and 20 µL proteinase-K, after which the samples were stored in a heating cabinet at 56ºC for 3 hours in constant shaking. After shaking was turned off, the samples were stored in 56ºC for 18 hours. The DNeasy Blood and Tissue protocol was followed for the next steps. For the filter samples containing ATL buffer, 130 µm proteinase-K was added, before shaking and heating, 56ºC, together with the individual samples. After 18 hours, the filters were placed back for heating and shaking for three hours. 200 µL from the filter samples was pipetted into an Eppendorf tube before continuing the DNeasy Blood and Tissue protocol. Three subsamples were taken from two of the samples. Filters and individual samples were stored in fridge until further analysis.

A Thermo scientific nanodrop 1000 spectrophotometer (fig 4) was used to measure the nucleic acid (DNA) concentration in all samples (*C. finmarchicus* individuals and filters). 2 µL water was used to make a blank and 1 µL sample was measured, from all samples. The results from these measurements were used to calculate the amount of DNA needed for qPCR.

qPCR assays were designed to look at the DNA amount in filtered water samples and individual samples of *Calanus finmarchicus.* The qPCR was preformed using the iTAQ

Universal SYBR green Supermix, together with forward and reverse primers, targeting the 16S gene (Tarrant et al., 2008). The conditions for the qPCR were after the program from Tarrant et al. (2008): 95ºC for 3 min, 95ºC for 40 cycles 15 seconds and 66º for 45s, and then cooling. Each reaction contained 20 µL master mix, constituting of 10 µL iTAQ Universal SYBER green Supermix, 1 µL forward primer, 1 µL reverse primer, 6 µL H2O and 2 µL DNA sample. Samples were diluted to the same DNA concentration (approximately 10 ng/µl) before running the qPCR assays (fig 4).

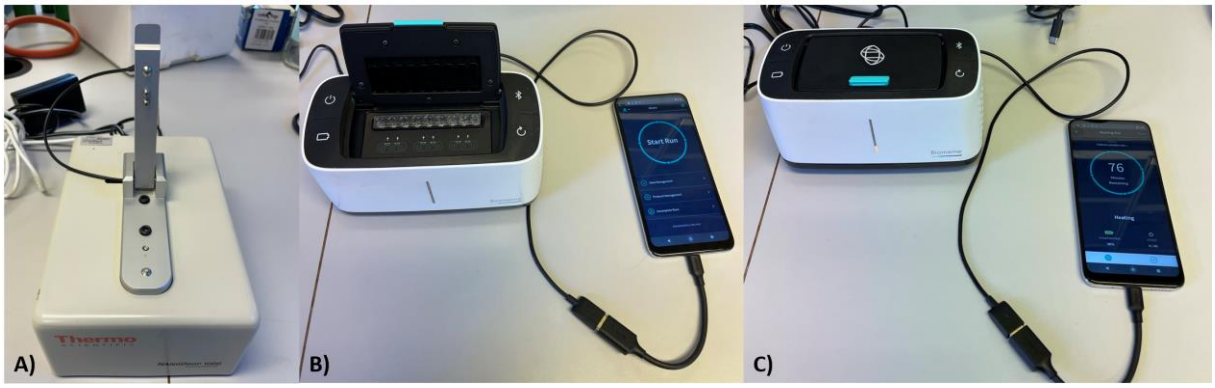

Figure 4: A) nanodrop 100 spectrophotometer used for measuring nucleic acid (DNA) concentration. B, C) Biomeme Franklin qPCR. B) setup in the qPCR with samples, C) qPCR running, each round of samples had a 76 min laps with conditions after Tarrant et al. (2008).

2.3 Data analyses

All material from counting was registered in Excel sheet for overview. All data analyses were conducted using R studio and phyloseq package (McMurdie & Holmes, 2013). For plankton net samples, number of individuals per m-3 were calculated for each taxa using the number of specimens and sampled water volume by assuming homogenous distribution. For correlations, Spearman's correlation was calculated. Results from qPCR assays of the individual *Calanus finmarchicus* samples were used to calculate a standard curve. The Cq value (cycle number/threshold cycle value, where amount of target is above detection limit) from the qPCR is used to calculate this standard curve. Each Cq value is plotted against logarithm of number of *Calanus* individuals in each sample in a graph and a regression line is made. The standard curve gives the equation $y = mx + b$, where m = slope and b = intercept. The R squared value is calculated from the regression line, and the value indicates how well it fits the data (1 is perfect). The PCR efficiency can be calculated using the equation $E = 10^{(\frac{1}{slope})}$. The standard curve was used to estimate number of *Calanus finmarchicus* specimens in mock filter samples and eDNA samples.

3 Results

3.1 CTD data

Vertical profiles with CTD (fig 5) showed a relatively stable temperature and salinity throughout the whole water column. Both the salinity and the temperature were stable throughout the water column day and night. At midday there was a small change towards increasing salinity at the surface (increase from 33.6 to 33.8), stabilizes a couple meters depth. The temperature measured was between 6.1ºC and 6.2ºC, with lowest temperature measured at midnight, and the highest at midday.

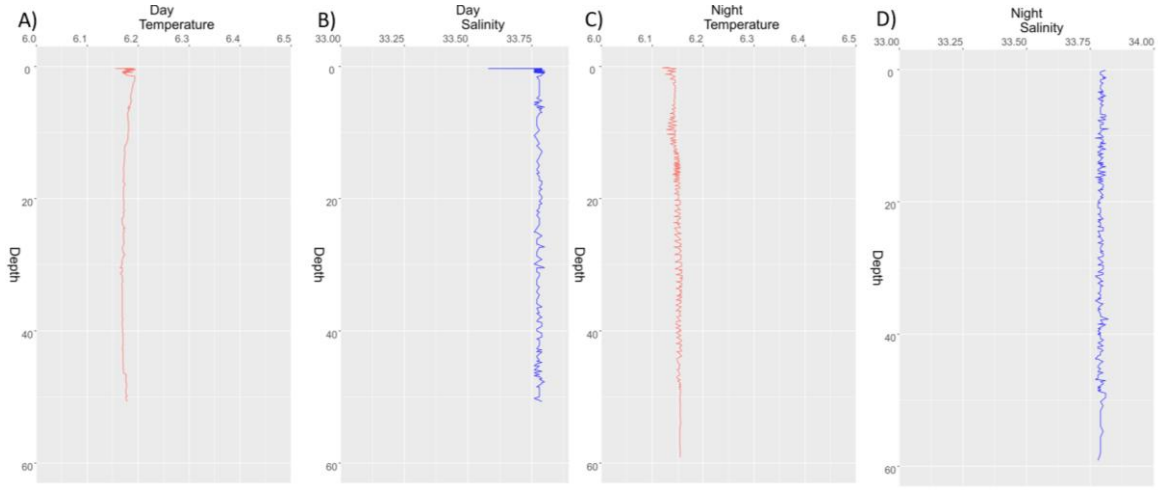

Figure 5: CTD profiles, the day cast ranging from 50 meter to surface, and the night samples from 60 meter to surface. A) profile of the midday salinity, B) profile of the midday temperature, C) profile of the midnight salinity, and D) profile of the midnight temperature.

3.2 Biodiversity with morphological identification

In total 29 different taxa were recorded in Mausund Bank (63.8° – 64.2°N, 8.2° – 9.0° E) during the sampling in March 2022. Three of these taxa were identified to species level, nine to genus, four to order, six to class level and three to phylum level (table 4), (appendix 2). In addition, eggs which are hard to identify to lower taxonomic level were recorded as "egg indet." and broken gelatinous zooplankton species as "jelly indet.". Arthropoda von Siebold, 1848 was clearly the dominating phylum in both day and night samples, and represented over 90% of recorded data (fig 6). In total 12 different taxa of Arthropoda were identified (table 4). The secound most abundant phylum was Chordata Haeckel, 1874, and the third was Echinodermata Klein, 1778. After Arthropoda, Echinodermata was the most abundant phylum in the surface layer, at daytime (fig 7), while Chordata was sligthly more abundant at the same depht at night. Annelida Lamarck, 1802 had the highest count during the day at 25 meter, at nigh the abundance of Annelida declined, and the counts of Cheatognatha Leuckart, 1854 increased at 5 meter, 10 meter and 25 meter. Mollusca Linnaeus, 1758 was present at 25 meters depth both day and night, but also present at five meters depth but only during night. The overall biodiversity seems to stabalize during the night, with m with multiple phyla having a relativily high abundance, whereas some phylum were less represented during the day and night.

Table 4: the 15 most common taxa detected counted in morphology samples. Sorted after highest abundance.

kingdom	**phylum**	**class**	**order**	**family**	**genus**	**species**
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	*Calanus finmarchicus*
Metazoa	Arthropoda	Thecostraca	NA	NA	NA	*Cirripedia* spp. nauplius
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	*Calanus glacialis*
Metazoa	Arthropoda	Malacostraca	Euphausiacea	NA	NA	*Euphausidacea* spp. nauplius
Metazoa	Egg indet.	NA	NA	NA	NA	*egg indet.*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	*Calanus* spp. nauplius
Metazoa	Echinodermata	NA	NA	NA	NA	*Echinodermata* spp.
Metazoa	Chordata	Appendicularia	Copelata	Fritillariidae	Fritillaria	*Fitellaria* spp.
Metazoa	Arthropoda	Malacostraca	Euphausiacea	NA	NA	*Euphausidacea* spp. calyptopis larvae
Metazoa	Arthropoda	Malacostraca	Decapoda	NA	NA	*Zoea larvae* spp.
Metazoa	Arthropoda	Thecostraca	NA	NA	NA	*Cirripedia* spp. cypris larvae
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Obeliidae	Obelia	*Obelia* spp.
Metazoa	Chordata	Appendicularia	Copelata	Oikopleuridae	Oikopleura	*Oikopleura* spp.
Metazoa	Arthropoda	Hexanauplia	NA	NA	NA	*Copepod* spp. naulius
Metazoa	Mollusca	Gastropoda	Pteropoda	NA	NA	*Pteropoda* spp.

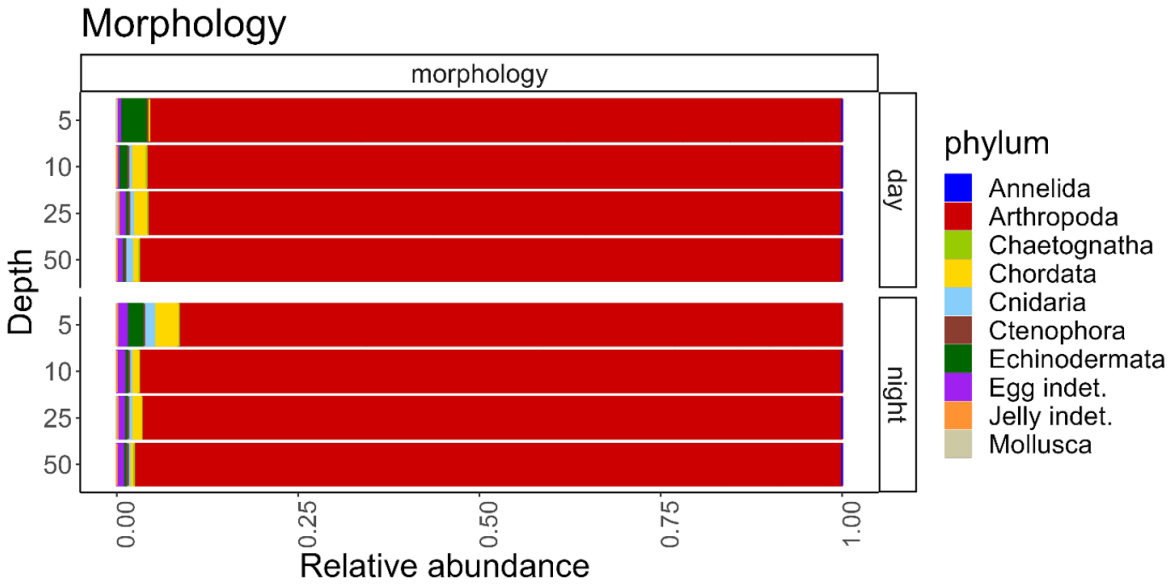

Figure 6: The relative abundance of different zooplankton phyla detected with morphological species identification, at day and at night, at 5-, 10-, 25 and 50-meters depth. The phyla are in alphabetical order, and the most abundant phylum occupying the biggest slot.

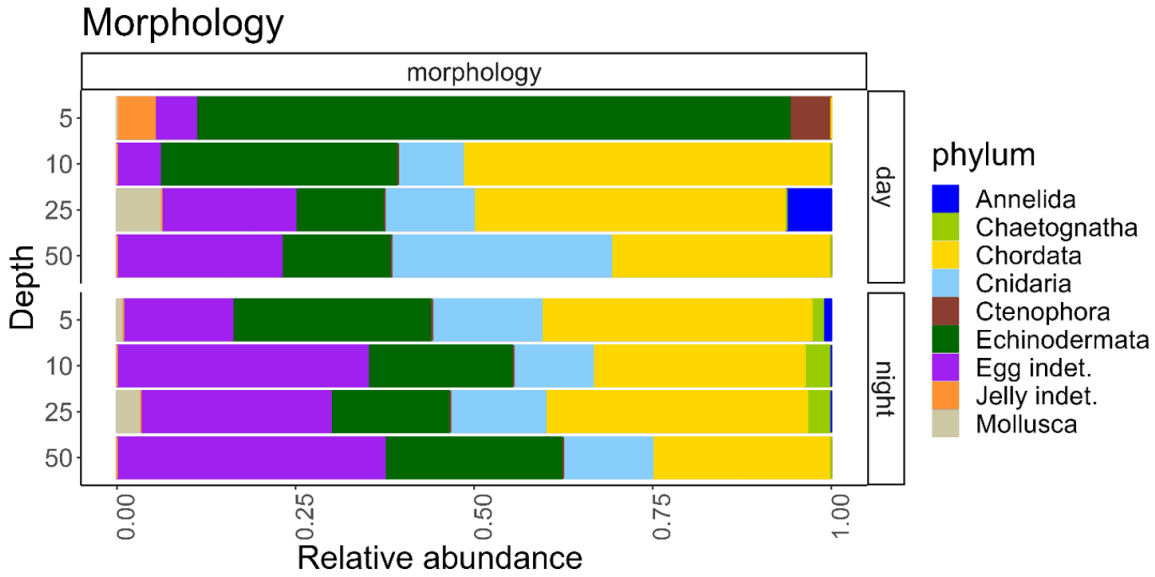

Figure 7: The relative abundance of phyla detected with morphological species identification except Arthropoda. For both day and night samples, at 5-, 10-, 25- and 50-meters depth. Plot arranged in alphabetical order, with the most abundant phyla occupying the biggest slot.

The phylum Arthropoda included four classes, of which Hexanauplia Oakley, Wolfe, Lindgren & Zaharof, 2013 was the most abundant (table 4). The class Hexanauplia contained five different taxa (fig 8) *Calanus* spp*. nauplius* Copepod spp*. nauplius* and Harpacticoida spp Sars G.O., 1903*.,* and different life stages of *Calanus glacialis* and *Calanus finmarchicus. C. finmarchicus* was clearly the most abundant taxon in the samples. The most abundant life stage of *C. finmarchicus* was copepodite stage II, III and IV at both day and night. *Calanus* spp. *nauplius* were present at five meters depth in both day and night, but also present at 10 meters during the day. The taxa with few speciemens in the samples included *C. glacialis,* Harpacticoida spp., and Copepod spp. *C. glacialis* was present

all depths and both timepoints, Harpacticoida spp. was present at all samples exept at 50 meter at night, and Copepod spp. was present in day 10 meter and night 10 meter and 25 meter samples.

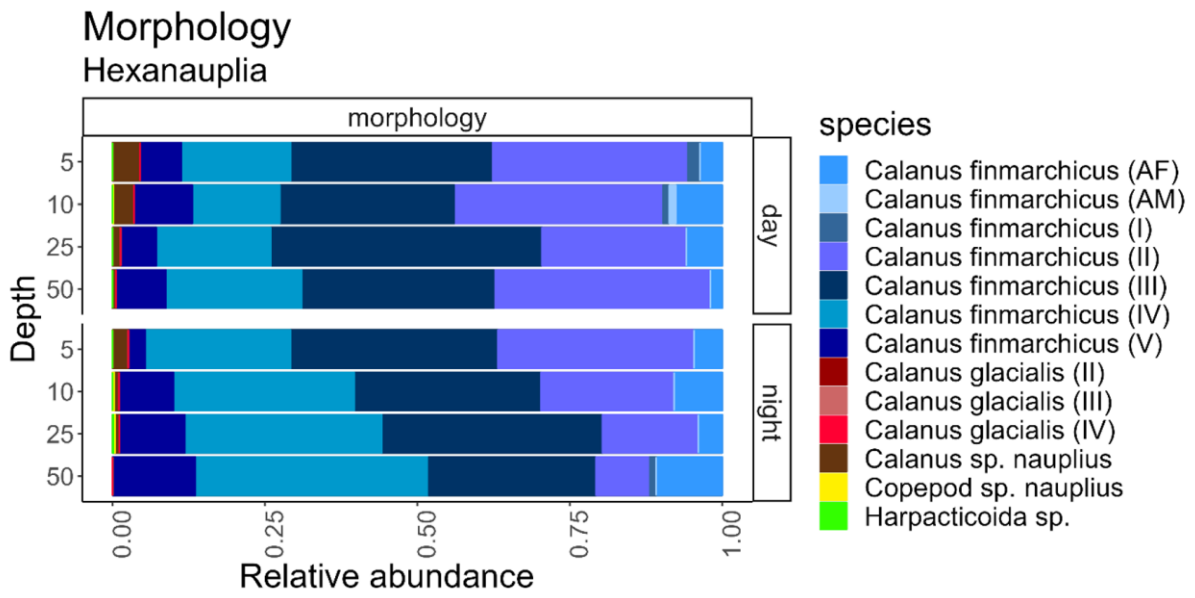

Figure 8: The relative abundance of different species in the class Hexanauplia detected with morphological species identification, in the day and night samples at different depths. The relative abundance of different life stages of C. finmarchicus and C. glacialis *are represented in different shades of blue and red respectively. Plot arranged in alphabetical order, with the most abundant species occupying the biggest slot.*

3.3 Biodiversity with eDNA approach

In total, 5 607 255 sequences were obtained using environmental DNA metabarcoding. Of which 771 737 sequences were classified as sequences originating from the targeted species (Metazoa Haeckel 1874), and 4 835 517 sequences were discarded from the further analyses as non-targeted (e.g., Fungi (L.) R.T. Moore, Plants) (fig 9).

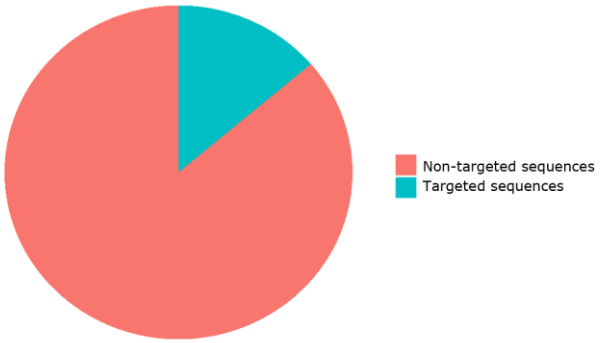

Figure 9: The portion of targeted sequences from all sequences detected in the eDNA samples. Red: non-targeted sequences, consisting of 4 835 517 sequences and blue: targeted sequences, consisting of 771 737 sequences.

In total 180 taxa were identified using eDNA. These taxa belonged to 11 different phyla. The four most abundant phyla were Arthropoda, Annelida, Bryozoa Ehrenberg, 1831 and Echinodermata (table 5), and Arthropoda was the dominating phylum (fig 10). At daytime,

Annelida was relatively most abundant at 50 m depth, whereas this shifted at the night, when the relative abundance of Annelida was higher at the surface (fig 11). The relative contribution of Bryozoa in the day samples was high at five meters depth, decreasing with depth. At night, the relative abundance of Bryozoa was lower, being the highest at 10 meters. At 50 meters at night Echinodermata had the highest relative abundance, compared to all other phyla, excluding Arthropoda. Mollusca was mainly detected during the night, at 10 meters and 25 meters depth. Both Cnidaria and Chordata had the highest relative abundance during the day, Chordata at surface and 10 meters depth and Cnidaria at 10- and 25-meters depth. Cnidaria Hatschek, 1888 was also present in the night sample, at 10 meters depth.

Table 5: the 15 most common taxa detected by eDNA. Sorted after highest abundance.

Kingdom	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Metazoa	Arthropoda	Hexanauplia	Cyclopoida	Oithonidae	*Oithona*	*Oithona similis*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus*
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	*Malacoceros*	*Malacoceros* sp. 1
Metazoa	Arthropoda	Thecostraca	Balanomorpha	Balanidae	*Semibalanus*	*Semibalanus balanoides*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	*Pseudocalanus*	*Pseudocalanus elongatus*
Metazoa	Echinodermata	Ophiuroidea	Amphilepidida	Ophiactidae	*Ophiopholis*	*Ophiopholis aculeata*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus* sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Metridinidae	*Metridia*	*Metridia lucens*
Metazoa	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	*Thysanoessa*	*Thysanoessa inermis*
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Membraniporidae	*Membranipora*	*Membranipora membranacea*
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Obeliidae	*Obelia*	*Obelia* sp. 1
Metazoa	Annelida	Polychaeta	NA	Capitellidae	*Capitella*	*Capitella* sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	*Microcalanus*	*Microcalanus pusillus*
Metazoa	Arthropoda	Hexanauplia	Cyclopoida	Oithonidae	*Oithona*	*Oithona atlantica*
Metazoa	Echinodermata	Asteroidea	Forcipulatida	Asteriidae	*Asterias*	*Asterias rubens*

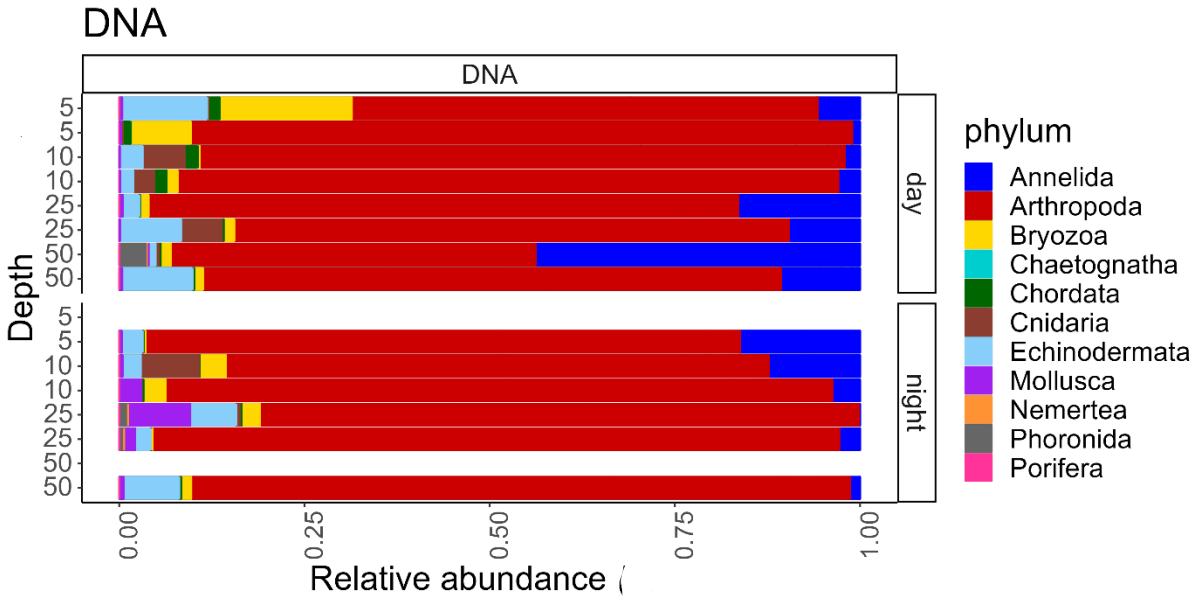

Figure 10: The relative abundance of different zooplankton phyla detected with eDNA, at day and at night, at 5-, 10-, 25 and 50-meters depth. The phyla are in alphabetical order, and the most abundant phylum occupying the biggest slot. All eDNA samples had 2 replicates, which is shown here as duplicate in each depth.

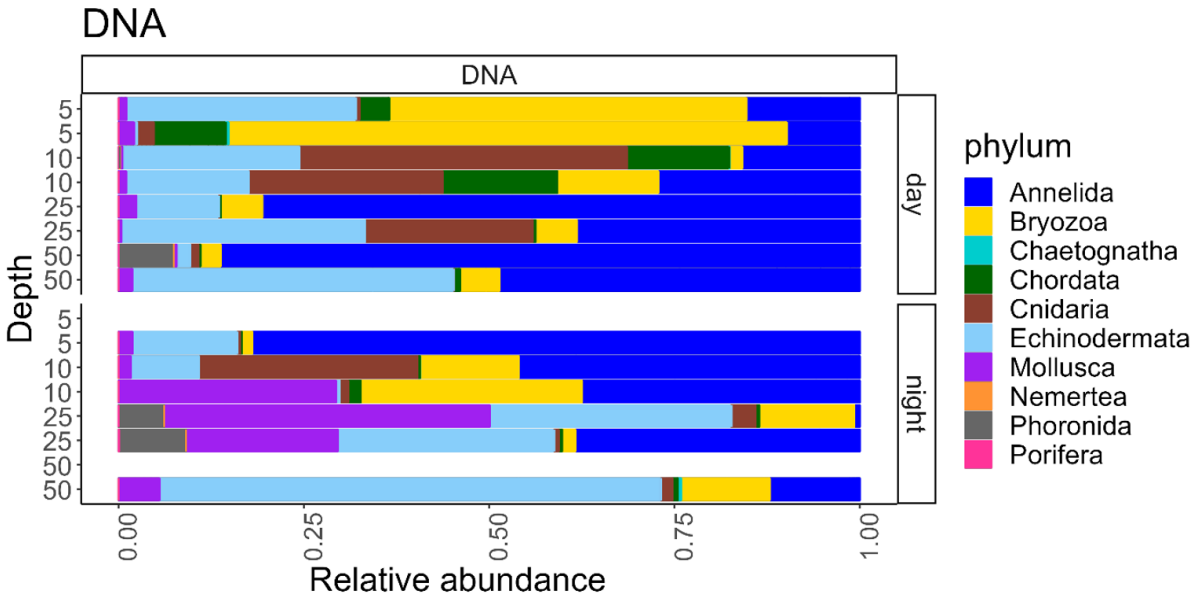

Figure 11: The relative abundance of different zooplankton phyla, excluding Arthropoda, detected with eDNA, at day and at night, at 5-, 10-, 25 and 50-meters depth. The phyla are in alphabetical order, and the most abundant phylum occupying the biggest slot. All eDNA samples had 2 replicates, which is shown here as duplicates in each depth.

The richest class of Arthropoda was Hexanauplia, which contained 27 taxa (Appendix 3). The two most dominating species of the class Hexanauplia were *Calanus finmarchicus* and *Oithona similis* Claus, 1866 (fig 12). The relative amount of *C. finmarchicus* was the highest at 50- and 10-meters samples during the day, while the relative contribution of *C. finmarchicus* was overall lower during the night. *O. similis* was the most abundant species, with the highest relative abundance at five- and 25-meters depth during the day, and at 50- and 25-meters depth during the night, but the relative contribution of *O. similis* was high at all the depths, both at day and night. At night, the relative abundance of

Pseudocalanus elongatus (Brady, 1865) increased at the same depths as the species was present at during the day (10- and 25-meters depth).

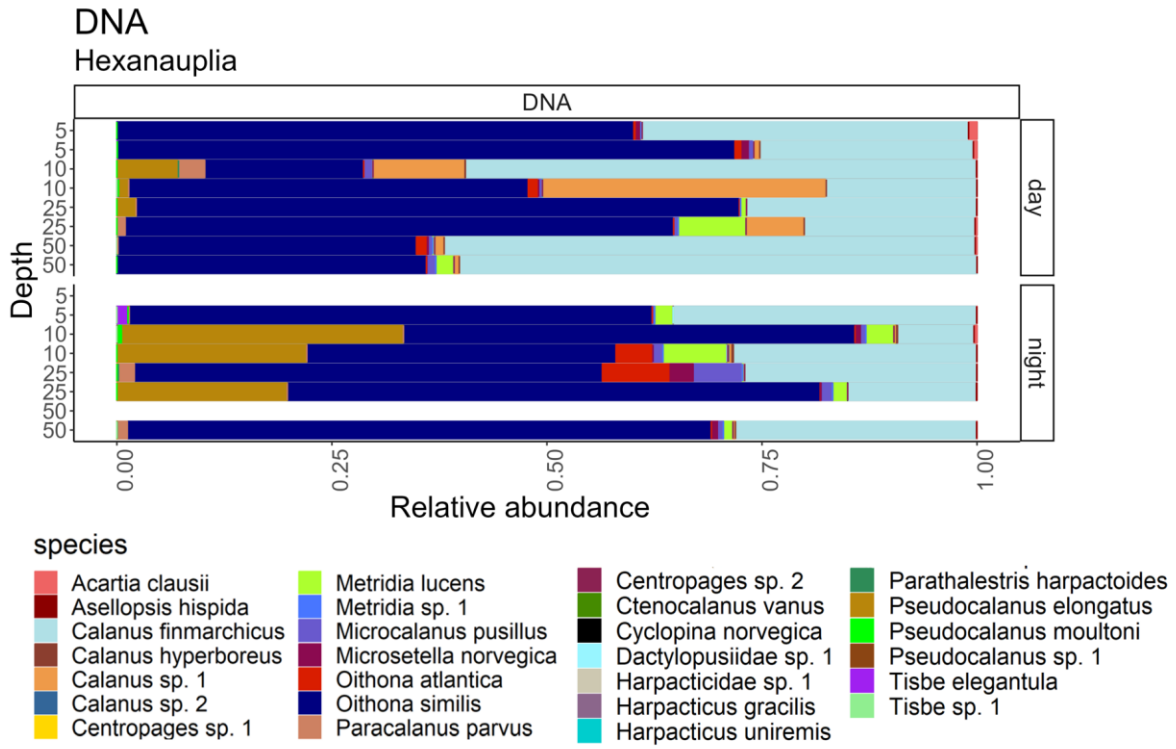

Figure 12: The relative abundance of different zooplankton species detected with eDNA, at day and at night, at 5-, 10-, 25 and 50-meters depth. The species are in alphabetical order, and the most abundant species occupying the biggest slot. All eDNA samples had 2 replicated, which is shown here as duplicated in each depth.

Within the genus *Calanus,* four taxa were identified; *Calanus finmarchicus, Calanus hyperboreus, Calanus sp. 1* (identified as *C. helgolandicus* or *C. euxinus* Hulsemann, 1991*)* and *Calanus sp. 2* (identified as *C. hyperboreus* or *C. glacialis*) (fig 13). *C. finmarchicus* had the relative highest number of reads at all depths and timepoints, except at 10 meteres depth, where *Calanus* sp. 1 represent about 60% of the total *Calanus* reads. This taxon was also detected at surface, 25- and 50-meters depth, but with a lower relative sequence number. *Calanus hyperboreus* was detected at all depths, the highest relative contribution was at 10 meters depth at night. The number of reads of *Calanus sp. 2* was low at both day and night, with two detections at night (10- and 50-meter) and all depths during the day.

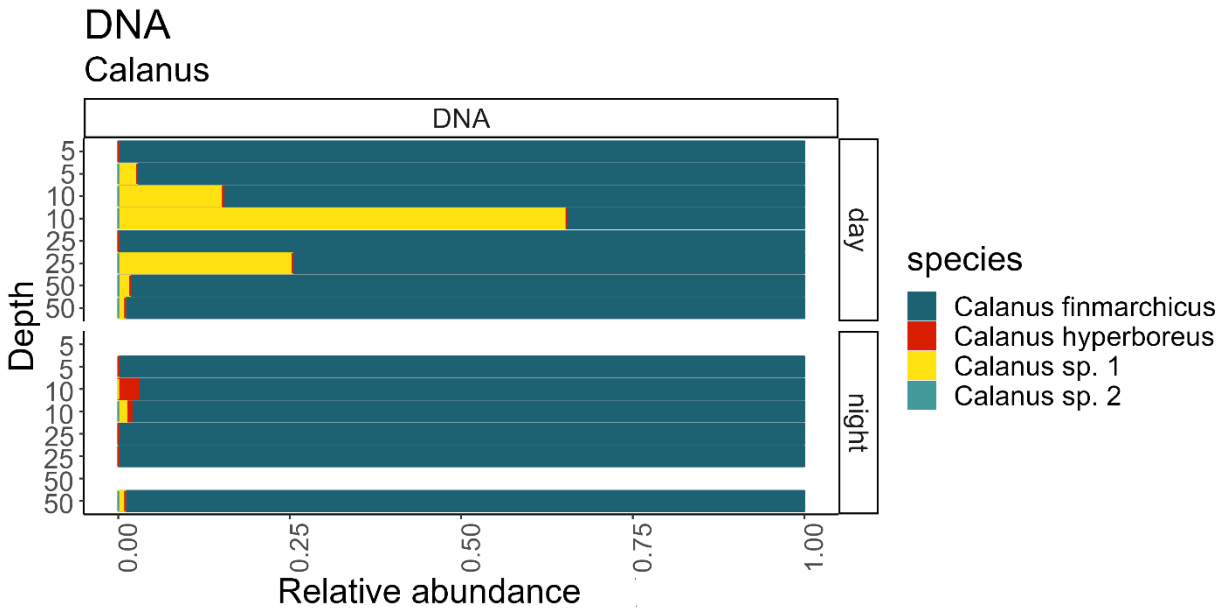

Figure 13: The relative abundance of different Calanus *species detected with eDNA, at day and at night, at 5-, 10-, 25 and 50-meters depth. The species are in alphabetical order, and the most abundant species occupying the biggest slot. All eDNA samples had 2 replicated, which is shown here as duplicated in each depth.*

3.4 Morphological species identification versus eDNA

In total, 29 taxa were recorded using morphology and 180 with DNA. From these three were identified to species level when using morphological species identification approach, and 128 based on molecular species identification analysis. For the morphology most taxa were identified to genus level and for DNA to species level. The morphology-identified taxa originated from nine phyla, while the DNA taxa belonged to 11 different phyla (appendix 2 & 3). The phylum Arthropoda was dominating both in morphology and in DNA results (fig 14), but the dominance was more pronounced in morphology. Annelida was the phylum with the second most reads in the DNA samples, whereas in the morphology the phylum was mostly present just at daytime at 25 meters depth (fig 15). Chordata was detected as one of the most abundant phyla for all depths both at day and night, except five meters during the day in the morphology data, but the phylum included low number of reads in the DNA data. If excluding Arthropoda, almost 70 % of the DNA reads was Echinodermata at 50 meters depth in night, where Annelida (10 %), Bryozoa (10 %), and Mollusca (5 %) represented almost all other reads, for this same depth. In contrast, the phylum Chordata and Cnidaria represent 50 % each at 50 meters depth in morphology. In the surface layer, Echinodermata was detected as the most abundant in morphology samples (about 75 %) if excluding Arthropoda. In the DNA samples at the same depth, Echinodermata was detected with about 25 % of the sequences.

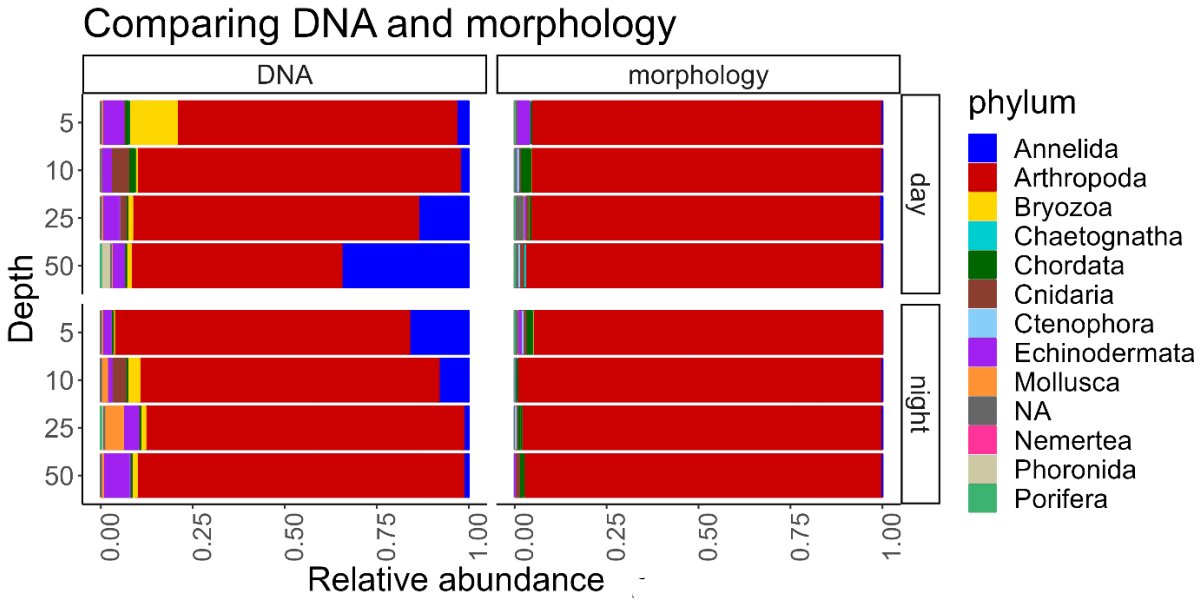

Figure 14: Comparison of the relative abundance of phyla detected with morphological species identification and eDNA at all depths during the day and night sampling, at 5-, 10-, 25 and 50-meters depth. The phyla are in alphabetical order, and the most abundant phylum occupying the biggest slot.

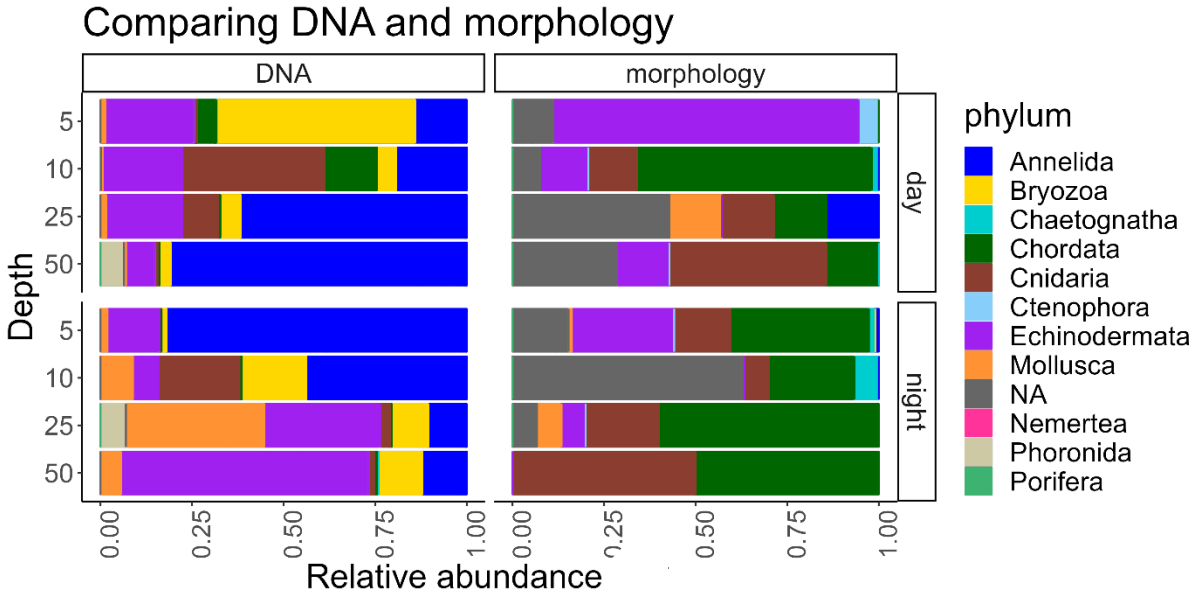

Figure 15: Comparison of the relative abundance of phyla, except Arthropoda, based on morphological species identification and eDNA at all depths during the day and night sampling, at 5-, 10-, 25 and 50-meters depth. The phyla are in alphabetical order, and the most abundant phylum occupying the biggest slot.

Of Arthropoda, four classes were detected by morphology, and five by DNA (fig 16). Hexanauplia taxa were detected and 27 taxa in DNA (appendix 3)). The samples of morphology were highly dominated by the genus *Calanus,* the eDNA samples also had a high number of *Calanus* reads, but the species *Oithona similis* had even more reads (table 4 & 5). *Psedocalanus elongatus* was detected at 10- and 25-meters depth, for both day and night in the eDNA samples, this species was not detected by morphology.

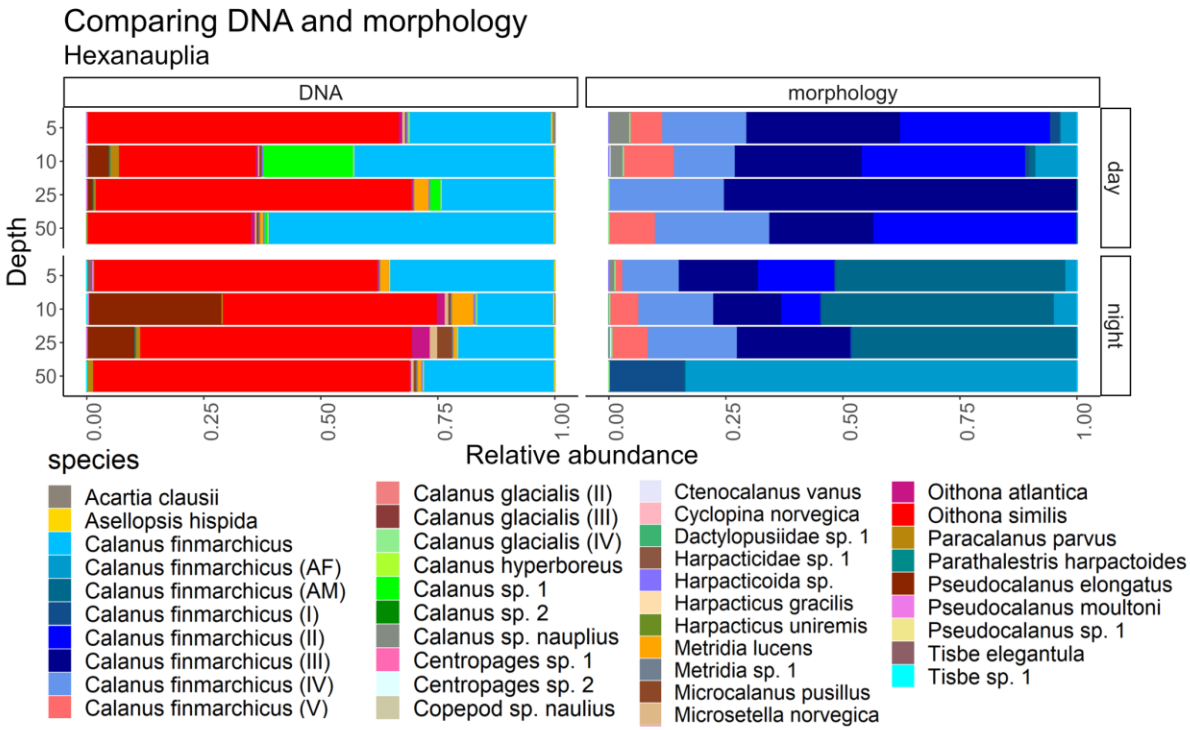

Figure 16: Comparison between species in the Hexanauplia class based on morphological species identification and eDNA at all depths during the day and night sampling, at 5-, 10-, 25 and 50-meters depth. The species are in alphabetical order, and the most abundant species occupying the biggest slot.

The genus *Calanus* had a high detection with both approaches. Three species of *Calanus* was detected in plankton nets and four in eDNA samples (fig 17). *Calanus finmarchicus* dominated all samples, with counting almost 100% at the certain depths. Six life stages of *C. finmarchicus* and three life stages of *C. glacialis* were detected using morphology, whereas DNA only detected *C. finmarchicus* without information of life stages. *C. glacialis* was not identified at all in eDNA samples whereas *C. hyperboreus* was recorded only in the eDNA samples, as well as *Calanus* sp. 1 and sp. 2.

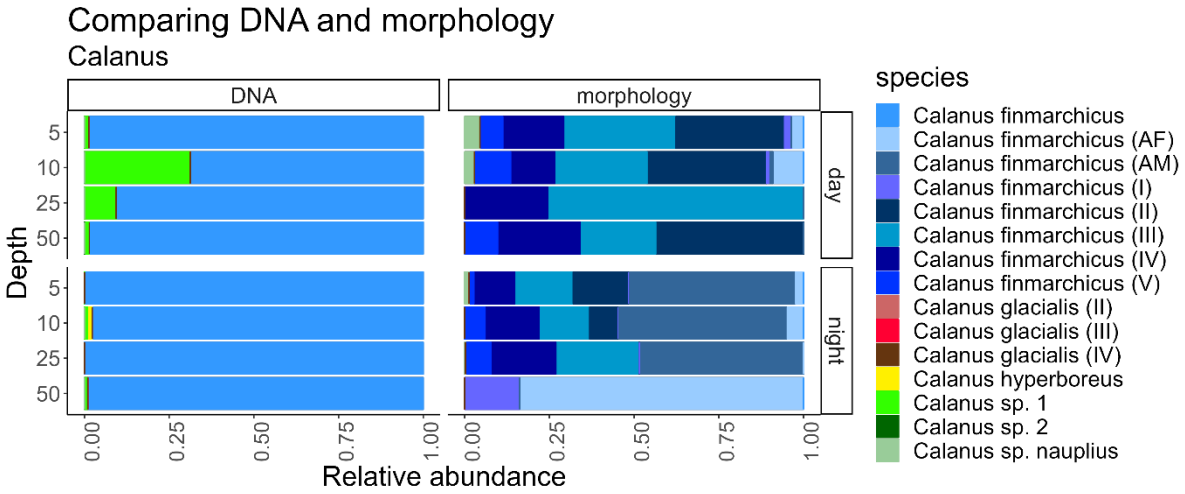

Figure 17: Comparison between Calanus *species based on morphological species identification and eDNA at all depths during the day and night sampling, at 5-, 10-, 25 and 50-meters depth. The species are in alphabetical order, and the most abundant species occupying the biggest slot.*

3.4 The quantification of *Calanus finmarchicus*

In total 24 samples of *C. finmarchicus* mock communities and nine eDNA samples were successfully analysed with qPCR. In general, the DNA concentration in the individual mock community samples was a lot higher than in the filters (fig 18). For the individual samples the concentration of DNA increased with the increase of individuals; one individual gave the mean values -0.42 ng/µl (standard deviation = 0.51, five individuals 1.88 ng/µl (SD = 1.90), 10 10.93 ng/µl (SD = 5.93), 20 23.50 ng/µl (SD = 5.25) and 30 34.89 ng/µl (SD = 11.75) of DNA. For 30 individuals, the range of values withing the replicates was high, with the lowest value being 22.11 ng/µl and the highest 50.80 ng/µl. For all filter samples the concentration of DNA was between -2.79 ng/µl and 9.88 ng/µl, with the mean value 0.24 ng/µl (SD = 1.91).

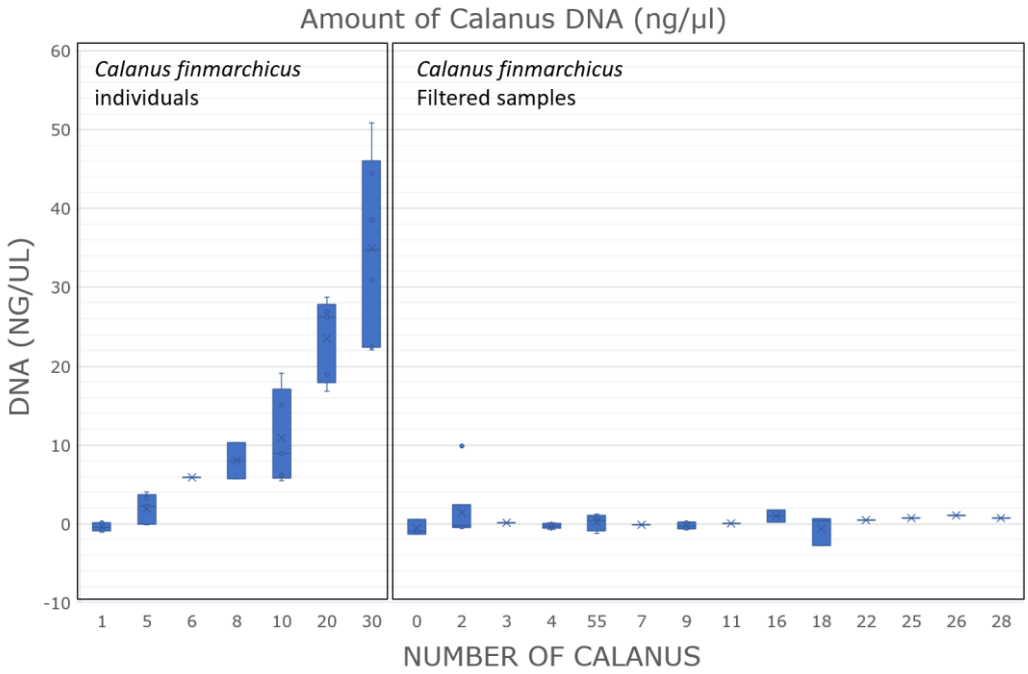

Figure 18: The concentration of DNA in all samples, both Calanus finmarchicus *individual samples and eDNA filter samples (experimental water) in the mock community experiment. The concentration of DNA was higher in the individual samples, than in the filter samples.*

The estimated number of *Calanus finmarchicus* in the Mausund eDNA samples was calculated using the formula $y = -3.1181x \cdot 19.914$ that was derived from the individual mock samples (fig 19). No significant correlation between the number calculated based on this formula (table 6) and the counted numbers from the Mausund samples using morphology were recorded (correlation value = - 0.67, significance level = 0.08). There is also no significant correlation between the qPCR numbers and the eDNA metabarcoding data (correlation value = - 0.23, significance level = 0.40), or correlation between morphology and eDNA metabarcoding (correlation value = -0.21, significance level = 0.62).

Table 6: Calculation of the abundance of Calanus finmarchicus *in all Mausund samples, and the average of individuals at each depth. Calculated eDNA sequence counts in* Calanus/m^3 *from the metabarcoding of the eDNA samples. The* Calanus/m^3 *(Average, depth) is the average sequence count (average of* Calanus/m^3*) for each depth at day and night (two rows to the left). Morphology* Calanus/m^3 *is the counted* Calanus *in the morphological samples. The counts were then calculated to* m^3*.*

Depth	**Time**	**eDNA *Calanus*/m³**	***Calanus*/m³ (Average, depth)**	**Morphology *Calanus*/m³**
50 m	Day	28.2	458.7	367.2
50 m	Day	889.1		
25 m	Day	797.3	462.8	304.8
25 m	Day	128.2		
10 m	Day	195.7	232.6	657.0
10 m	Day	269.4		
5 m	Day	135.9	144.7	339.7
5 m	Day	153.3		
50 m	Night	64.6	64.6	297.7
25 m	Night	5.0	6.0	811.9
25 m	Night	7.0		
10 m	Night	4.5	4.5	1452.8
10 m	Night	-		
5 m	Night	8.6	8.6	947.5

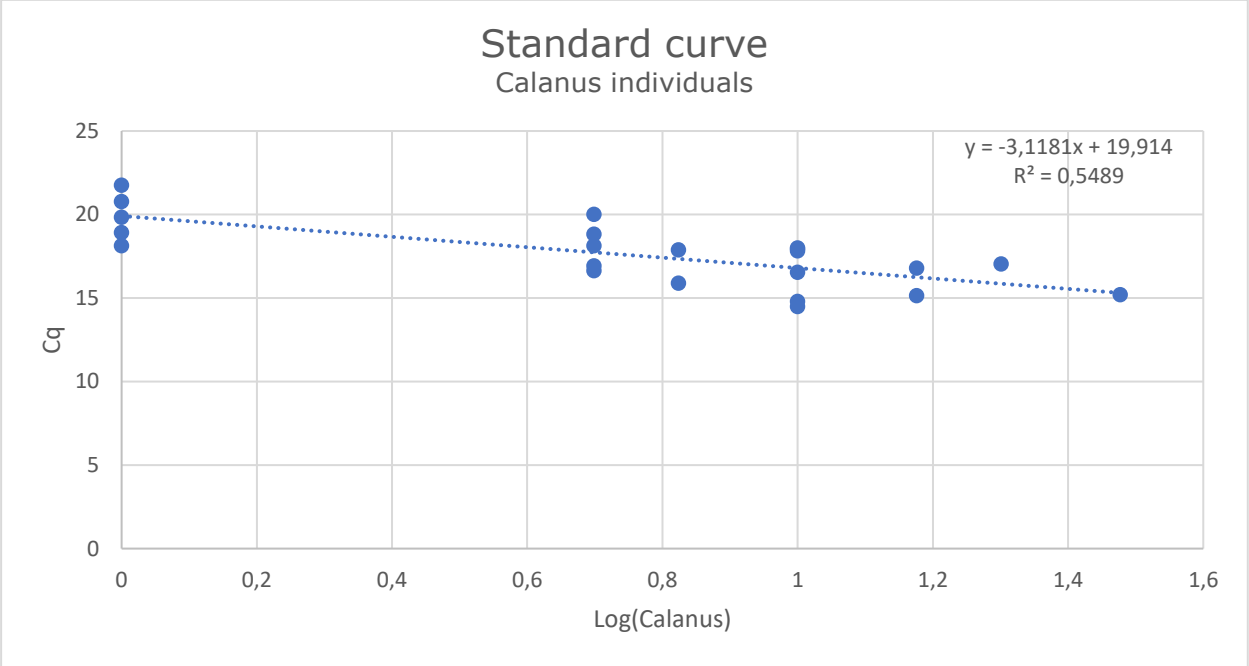

Figure 19: Standard curve calculated from qPCR based on the Calanus *individuals. The formula was used to calculate the estimated number of* Calanus finmarchicus *in samples collected at Mausund. R-squared measures how well the regression line fit. The Cq value: cycle number/threshold cycle value, where amount of target is above detection limit.*

4 Discussion

Improving the understanding of structure and function of the marine environment is and will be extremely important, not only to manage it but also to secure ecosystem health. However, we cannot understand or manage something that cannot be measured and therefore developing better observation and monitoring programs will be a crucial step forward (Estes Jr et al., 2021). Zooplankton are some of the most important organisms in the marine realm and connects the different trophic levels (Berge et al., 2020). Different species of zooplankton are also good indicators for the climate change (Richardson, 2008). Hence, monitoring of zooplankton diversity and abundance can give critical insights to the state of the marine environment. Whereas morphological species identification methods have been traditionally used for observation surveys (Danovaro et al., 2016), the DNA based approaches have grown and shown good suitability for improved species identification. That was also evident in this study, as in total 180 species were detected using environmental DNA and only 29 using morphological species identification. However, quantification of mock *Calanus finmarchicus* communities showed no significant correlation with the eDNA samples collected from the field. Hence, emphasizing the difficulty in quantification when using molecular methods, especially when compared to morphological identification where all specimens are visible and easy to count.

4.1 Biodiversity assessment with morphology and eDNA

Generally, it has been considered that DNA-based species identification methods have improved taxonomic resolution in different taxonomic classes. Hexanauplia, Bivalvia Linnaeus, 1758, Gastropoda Cuvier, 1795 and Polychaeta Grube, 1850 are examples of classes where molecular methods have shown a hidden diversity compared to knowledge only based on morphological species identification (Lindeque et al., 2013). This was also evident in this study, as only four taxa were identified to species level from the 29 morphologically identified taxa (appendix 2). On the other hand, for eDNA, 180 taxa were identified, of which 128 were identified to species level. In the eDNA data, the phylum Mollusca had the lowest taxonomic resolution: 11 out of the 52 not species-level identified taxa belonged to the phylum Mollusca. Also, the relative abundance of different taxa varied. For example, the phylum Arthropoda was detected as the dominating in both methods, but the relative abundance of other phyla varied a lot. Annelida was only detected with one taxon in the morphology samples, whereas in the DNA samples 35 taxa were identified and 27 of them to species level. Annelida was also the only phylum detected with DVM pattern. Identification possibilities of different zooplankton taxa varies widely. For example, species in the class Polychaeta (phylum Annelida), haves been classified as hard to identify with morphology (Lindeque et al., 2013). Lindeque et al. (2013) found that molecular methods can reveal a greater number of Polychaeta species than morphological approaches. This was also supported by the findings in this study, where 34 taxa of Annelida were Polychaeta, and only 8 could not be identified to species level, compared to morphology results where the only one Annelida taxa was found to be Polychaeta and could not be identified further.

The main limiting factor in morphological species identification is the lack of taxonomic knowledge, both in the case of overall species identification, as well as in the case of

identifying the different life stages (Stefanni et al., 2018). The sampling for this study was performed in late March, when the zooplankton community in the coast of Norway is represented by large numbers of small organisms from early life stages (eggs, nauplii and larvae stages) of different taxa (Coguiec et al., 2021). These early life stages are generally very hard to identify, both since two different species can look similar, and since the larval stage of organisms may be very different from their adult stage. For example, in this study all Hexanauplii nauplii were classified as either *Calanus* spp*.* nauplii or Copepod spp*.* nauplii (table 4), and more detailed level species identification was not possible. In addition, the difference in these two groups were made only based on body size of the nauplius; Copepod spp. was clearly smaller in size compared to *Calanus* spp. nauplii. It is common in taxonomic studies to find this all-encompassing groups of taxa or identification to a broad taxonomic level, like phylum or class (Descôteaux et al., 2021).

The benefit of morphological identification is the opportunity for life stage level identification. For instance, *Calanus* species can be classified to different copepodite and adult stages by counting urosome segments, number of legs and prosome segments (Broms et al., 2009). In this study, all copepodite life stages of *C. finmarchicus* and *C. glacialis* were detected only with this method. A known limit to eDNA is the inability to distinguish between different life stages, since it often only tells what is present by pooling juveniles and adults together (Beng & Corlett, 2020; Danovaro et al., 2016). However, different life stages give valuable information about the ecological status of the organism, and can give answers to the breeding activity, sex ratio and life history (Beng & Corlett, 2020), which can be important information for effective population management (Lande & Barrowclough, 1987). A few attempts of DNA based life stage detection studies exist. A study by Crane et al. (2021) on the effects of life stages in eDNA detection for the crab species *Carcinus maenas* (Linnaeus, 1758) showed no significant difference between DNA detection in different life stages, except for ovigerous crabs, suggesting that eDNA can be used to understand and estimate reproduction strategies. Descôteaux et al. (2021) studied the meroplankton diversity at the Barents Sea using DNA metabarcoding, focusing on identifying early life stages. Larvae were classified after body size before DNA assessment. They found that DNA metabarcoding can give a high taxonomic resolution and detect species which are usually difficult to classify. This study also shows how molecular approaches can help with identification with the bulk samples, where at the same time this cannot be conducted without some form of manual separation of the sample. Hence, distinguishing life stages using DNA sampling is limited.

The overall knowledge about zooplankton biodiversity also reflects what taxonomic level taxa are identified to, where morphological identification to genus is the most common level. However, even with the best taxonomical expertise available identification to species level is often difficult (Pereira et al., 2021). This study suggests that using DNA-based species identification techniques a finer taxonomic resolution than traditional morphological species identification is possible. The finer taxonomic resolution in this study suggest that DNA-based methods can also detect a higher taxonomic diversity than morphological identification. For example, Arthropoda contributed 95 % of abundance in morphology samples, while in the eDNA samples the relative abundance of the phylum varied from 50 % to 90 %. Pereira et al. (2021) found when studying phytoplankton, zooplankton, and macroinvertebrate communities, that "*eDNA yielded finer taxonomic resolution than morphology*" as DNA enabled identification of 70% of the taxa to species level, whereas 63% of the taxa were determined to species level with morphology. Similarly, Ershova et al. (2021) also found that molecular methods gave the highest yield of biodiversity when studying zooplankton communities in the Norwegian Sea, Barents Sea

and fjords at Svalbard. Ershova et al. (2021) molecular identification resulted in 80 – 100 unique taxa categories at each sampling station, where morphological study gave 20 – 30 unique taxa. However, a study comparing morphology and eDNA on phytoplanktonic group, diatoms, were able to identify more taxa with morphology, both to genus and species level (Kulaš et al., 2022). Hence, demonstrating that there are still limits in the use of eDNA-method. This also demonstrates that DNA-based methods are dependent on a good-quality reference database, and incompleteness can lead to misidentification of taxa or lack of detection. For example, the possibility of misidentification between the closely related ctenophores *Bolinopisis infundibulum* and *Mnemiopsis leidyi.* where only COI of *M. leidyi* is in the reference library (Faasse & Bayha, 2006; Hosia & Falkenhaug, 2015; Johansen, 2019). The non-targeted taxa in this study (fig 9) includes all sequences that were not classified to Metazoa based on the reference database used, meaning that the non-targeted taxa include for example bacteria but also can contain zooplankton species which are not currently in the reference databases.

4.1.1 Evaluating the time and cost of the biodiversity assessement

Morphological species identification is also time consuming, which can be seen in this study as well since the most abundant samples took more than a whole day to assess. This was also evident in this study when the samples contained high number of early life stages. As a result of the high requirements of time there can be many months between collecting data and finishing analysis, which makes it difficult to detect changes in time to perform good management, especially when using a large set of samples. This also limits the opportunity to examinate and monitor long term changes (Ershova et al., 2021). Toresen et al. (2019) conducted a study of shifts in the Northern Sea, with zooplankton samples, where only a fraction was analyzed due to *"limiting capacity at the laboratory".* With sequencing DNA it is possible to analyze a lot of samples at the same time, within a few days (Biggs et al., 2015; Rees et al., 2014). Jerney et al. (2023) estimated the time difference between DNA-based method and traditional methods for phytoplankton monitoring, and they suggest that DNA-based monitoring would take approximately 0,87 hour per sample, while traditional monitoring would take approximately between 8 and 9 hours. This is showing the benefit of DNA-based methods when it comes to time if a larger set of samples is to be analyzed.

The price of DNA-based methods and morphological species identification is also often discussed, and studies suggest that molecular methods are often more cost-effective than traditional methods (Biggs et al., 2015; Davy et al., 2015; Sigsgaard et al., 2015). Biggs et al. (2015) found, in a study of *Triturus cristatus (Laurenti, 1768)* (great crested newt), the price of eDNA (field and laboratory costs included) was about 1700 NOK per site, compared to 17 000 NOK per site when conducting traditional identification methods. A study by Sigsgaard et al. (2015) monitoring the *Misgurnus fossilis* (Linnaeus, 1758) (European weather loach) using eDNA found that laboratory and fieldwork with eDNA was almost 60 000 NOK cheaper than traditional methods. Magnussen & Navrud (2021) estimated that the Norwegian insect monitoring program costs approximately 20 million NOK each year, whereas if this monitoring was conducted with traditional methods, the price would be approximately 480 million each year. Whereas Davy et al. (2015) found that the cost of traditional methods being the cheapest, in a study detecting freshwater turtles: the price of eDNA was about 54 000 NOK, while traditional field surveys ranged from approximately 1 200 NOK to 45 000 NOK. These four studies suggest that DNA-based methods usually cost less than morphology. However, DNA-based methods, are as mentioned, dependent on reference libraries, where the price for to build and maintain

each species (this includes process, sorting, and storage) are approximately 10 000 NOK to 150 000 NOK, insects often being on the cheaper side of the spectrum, and marine deep-sea species being some of the most expensive (Magnussen & Navrud, 2021). The prices vary mostly due to the cost of collecting the specimens. These costs should be considered an investment and a cost-benefit, since the information gathered is important to build reliable reference libraries (Magnussen & Navrud, 2021), and provide better knowledge about species and ecosystems (Jerney et al., 2023). It's also noteworthy that the price of DNA-based assessing methods decrease when increasing the number of samples, while for traditional methods the price would most likely increase (Jerney et al., 2023). Monitoring programs have earlier undergone cuts due to high cost (Borja & Elliott, 2013), but perhaps the difference in price in favor for DNA-based monitoring would help continuing on-going and starting new monitoring programs.

4.1.2 Accurate species identification

A good example of the need for accurate species identification is the species in the genus *Calanus.* In this study two species of *Calanus* were detected in plankton net samples based on morphological approach: *C. finmarchicus* and *C. glacialis.* To discriminate between these two *Calanus* species, fixed length classes were used as described in the methods section (table 3). However, this approach has been questioned as overlapping prosome length were found for *C. finmarchicus* and *C. glacialis* in the Norwegian fjords (Choquet et al., 2018; Gabrielsen et al., 2012). This means that the detected number of *C. finmarchicus* and *C. glacialis* may not be the reality, and due to potential misidentification, the distributions of these species might be misunderstood and in some locations the numbers of *C. glacialis* or *C. finmarchicus* could be higher. For example, in Arctic waters, studies found that the size difference between *C. finmarchicus* and *C. glacialis* are possibly bigger, but this can also be an underestimation of smaller *C. glacialis* individuals (Choquet et al., 2018). Using molecular methods to compare with the measurement, could possibly be a better method to distinguish between *Calanus* species. In this study, the DNA results detected four species of *Calanus*: *C. finmarchicus, C. hyperboreus*, *Calanus sp. 1* and *Calanus sp. 2, and C. finmarchicus* and *Calanus sp. 1* were the most abundant (figure 13). Between 95 - 100% of the reads during the night were identified as *C. finmarchicus,* and overall, *C. finmarchicus* was the most dominant *Calanus* species*. Calanus sp. 1* shifted as the most dominant species at 10m in the day sample representing 65% of the detected reads, although this species was mostly detected at night. Interestingly, no *C. glacialis* was detected based on the DNA analysis in any of the eDNA samples. *C. glacialis* is rarely observed in the open Atlantic waters, but occur in many Norwegian fjords, as far south as 60ºN (Choquet et al., 2017), and was also observed in Trondheimsfjorden by Choquet et al. (2017). In the eDNA samples two species, *Calanus* sp. 1 and *Calanus* sp. 2 were not identified to species level. Based on further analysis, *Calanus* sp*.* 1 could be identified as *C. helgolandicus* or *C. euxinus* and *Calanus sp. 2* could be identified as *C. hyperboreus* or *C. glacialis*. Which could indicate that there would be *C. glacialis* in the eDNA samples after all. Surprisingly *C. hyperboreus* was detected in the samples, with relatively small number of sequences at the highest at about 5%. This species is considered an Arctic species, with its key area in the open ocean around Greenland. The distribution of the species is expected to start north of Jan Mayen (Fossum et al., 2012). Hence, eDNA metabarcoding gave somewhat contradictory results compared to morphological identification and it is very important to have pre-knowledge of the organisms and ecosystems in question.

4.1.3 Are we able to quantify with eDNA samples?

Many studies emphasize that morphology is the main method for quantification of zooplankton communities (Beng & Corlett, 2020; Ershova et al., 2021). In this study qPCR and Nanodrop was conducted on *Calanus finmarchicus,* both from individuals and filtered samples, to examine if eDNA samples could be used for quantification. Here, the result shows an increasing amount of DNA when increasing the number of individuals in the mock community bulk samples. However, there was also an increasing variability in the DNA values. In contrast to the individual samples, the filtered samples had no increasing trend of DNA concentration when increasing the number of individuals, and thus indicating that quantification in the eDNA samples would not be possible.

Similarly, both eDNA and morphology samples were compared to the qPCR result, to understand if it is possible to quantify DNA samples and understand if there was any correlation between the number of DNA copies detected and the counted morphology samples (appendix 4 and 5). The morphology samples showed no significant correlation with the numbers estimated in qPCR. Neither did DNA metabarcoding results show significant correlation with the numbers estimated in qPCR. Klymus et al. (2015) studied the possibility to quantify eDNA shedding rates of *Hypophthalmichthys nobilis* (Richardson, 1845) (bighead carp) and *Hypophthalmichthys molitrix* (Valenciennes, 1844) (Silver carp), the study found that quantification of eDNA samples can be highly variable even when using the same individual and when conducted in a controlled environment. However, other studies suggest that qPCR can be used as a tool for quantifying biomass (Evans et al., 2016; Takahara et al., 2012). Evans et al. (2016) suggest that species richness can be quantified using qPCR and eDNA samples. Takahara et al. (2012) estimated fish biomass using eDNA and found a correlation between DNA copies and fish biomass. Mertz (2022) conducted a study on quantification of *Lepeophtheirus salmonis* (Krøyer, 1837) (salmon lice) and *Caligus elongatus* Heegaard, 1943 (sea lice). The study found that *L. salmonis* samples had an increasing amount of DNA which correlated with the increasing amount of *L. salmonis* in the sample. Whereas in the case of *C. elongatus* situation was less clear, especially in samples with high density of copepods. Mertz (2022) discussed if this could be caused by problems with the homogenization during the lysis step or alternatively related to the species genetics. Hence, showing that although promising method, qPCR still has limited applicability in ecosystem monitoring.

4.2 Future perspectives

Molecular methods show a huge potential for species identification and increasing the taxonomic resolution, but there is still limitations and research is needed for the method to be improved. The main limitation is quantification and the ability to separate the different life stages and investigate should focus more on these limitations. As a way forward from this study, it would be interesting to study if there is a difference in correlation when increasing numbers of specimens in the DNA quantification. For example, to see if adding samples that contain 50, 100, 500 and 1000 individuals would make a difference. Also, different species and different life stages of these organisms should also be investigated to understand if there are differences between hard and soft bodied species (e.g., gelatinous species and copepods) or with different life history strategies (meroplanktonic and holoplanktonic).

The use of molecular methods for environmental monitoring have been discussed in multiple studies (Basedow et al., 2010; Bourlat et al., 2013; Danovaro et al., 2016; Jerney et al., 2023), and emphasized as the most likely way on innovation in the field of

monitoring (Bourlat et al., 2013). There are continuously new developments in this field. For example, use of eDNA in combination with AUV's for *in situ* sampling is already possible and could be the future for monitoring as well. Today, there are already technologies which autonomously can sample water for molecular identification (Scholin et al., 2017; Yamahara et al., 2019). In addition, where DNA-based method cannot detect species diversity, it would be possible to combine this with imaging for life stages detection (Greer et al., 2021).

5 Conclusion

The aim of this study was to test the applicability of DNA-based methods to assess the zooplankton community and look at the opportunity to use this in future monitoring programs by comparing eDNA and morphological species identification. Based on previous knowledge it was hypothesized that the species list for eDNA samples and morphological species identification would correlate with each other. The list of species in morphological analysis correlated with eDNA samples in the matter that many taxa also were identified with eDNA, however with some exceptions (e.g., *Calanus glacialis*). However, the molecular method gave a much higher number of taxa and higher taxonomic resolution than the morphologically identification. The aim of this study was also to investigate if DNA can be used in a quantitative matter, with qPCR and *Calanus finmarchicus* was used as a key study species. Based on previous studies and knowledge it was hypothesized that qPCR can be used as a tool for quantifying *Calanus* samples. The results gave no significant correlation between the number of *Calanus* and the number of DNA copies, this study thus found that it is not possible to quantify the abundance of Calanus based on DNA-based method. Based on previous knowledge it was also hypothesized that sampling at night versus day would show a DVM pattern, and affect which species were recorded. In this study the only DVM pattern found was for the phylum Annelida, where the relative abundance in the DNA samples shifted from highest in the deep samples (50 meters) in the day, to higher abundance in the surface at night. DVM patterns were not detected in other species or did not seem to affect the species recorded, however this could change if sampled would have been taken from deeper waters as well.

This study shows that DNA-based methods are suitable to detect presence of different species and can identify most taxa with high taxonomic resolution. However, as for now, quantification of DNA-based methods seems to be insufficient, and more research needs to be conducted to understand if there is a connection between the number of DNA copies and the number of individuals present.

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Appendix

Appendix 1

Table of all mock community samples used for nanodrop and qPCR.

Appendix 1: Samples for qPCR, the amount of specimens in each sample

***C. finmarchicus* Water sample**	**Number of individuals**	***C. finmarchicus.* individual sample**	**Number of individuals**
1A	1	1Ae	1
1B	1	1Be	1
1C	1	1Ce	1
1D	1	1De	1
2A	5	1Ee	1
2B	5	2Ae	5
2C	3	2Be	5
2D	4	2Ce	5
2E	4	2De	5
3A	7	2Ee	5
3B	9	3Ae	10
3C	9	3Be	10
3D	9	3Ce	10
3E	9	3De	10
4A	18	3Ee	10
4B	16	4Ae	20
4C	16	4Be	20
4D	18	4Ce	20
5A	22	4De	20
5B	25	4Ee	20
5C	26	5Ae	30
5D	28	5Be	30
6A	11	5Ce	30
6B	5	5De	30
6C	5	5Ee	30
		6Ae	6
		6Be	8

Appendix 2.

All species detected with morphology. From kingdom to species level.

kingdom	phylum	class	order	family	*genus*	*species*
Metazoa	Annelida	Polychaeta	NA	NA	*NA*	*Polychaeta sp. larvae*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus sp. nauplius*
Metazoa	Arthropoda	Thecostraca	NA	NA	*NA*	*Cirripedia sp. nauplius*
Metazoa	Arthropoda	Thecostraca	NA	NA	*NA*	*Cirripedia sp. cypris larvae*
Metazoa	Arthropoda	Malacostraca	Euphausiacea	NA	*NA*	*Euphausidacea nauplius*
Metazoa	Arthropoda	Malacostraca	Euphausiacea	NA	*NA*	*Euphausidacea calyptopis larvae*
Metazoa	Arthropoda	Malacostraca	Decapoda	NA	*NA*	*Zoea larvae sp.*
Metazoa	Arthropoda	Hexanauplia	NA	NA	*NA*	*Copepod sp. naulius*
Metazoa	Arthropoda	Branchiopoda	Onychopoda	Podonidae	*Evadne*	*Evadne sp.*
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	NA	*NA*	*Harpacticoida sp.*
Metazoa	Arthropoda	Malacostraca	Amphipoda	Hyperiidae	*Hyperia*	*Hyperia sp.*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (I)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (II)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (III)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (IV)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (V)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (AF)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus finmarchicus (AM)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus glacialis (II)*

Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus glacialis (III)*
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	*Calanus*	*Calanus glacialis (IV)*
Metazoa	Chaetognatha	NA	NA	NA	*NA*	*Chaetognatha sp.*
Metazoa	Chordata	Appendicularia	Copelata	Oikopleuridae	*Oikopleura*	*Oikopleura sp.*
Metazoa	Chordata	Appendicularia	Copelata	Fritillariidae	*Fritillaria*	*Fritillaria sp.*
Metazoa	Chordata	Actinopteri	NA	NA	*NA*	*Fish larvae*
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Obeliidae	*Obelia*	*Obelia sp.*
Metazoa	Cnidaria	Hydrozoa	NA	NA	*NA*	*Hydrozoa sp.*
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Corymorphidae	*Corymorpha*	*Corymorpha nutans*
Metazoa	Cnidaria	Siphonophorae	NA	NA	*NA*	*Siphonula larvae sp.*
Metazoa	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	*Beroe sp.*
Metazoa	Ctenophora	Tentaculata	Lobata	Bolinopsidae	*Bolinopsis*	*Bolinopsis sp.*
Metazoa	Ctenophora	NA	NA	NA	*NA*	*Ctenophora sp.*
Metazoa	Echinodermata	NA	NA	NA	*NA*	*Echinodermata sp.*
Metazoa	Mollusca	Gastropoda	Pteropoda	NA	*NA*	*Pteropoda sp.*
Metazoa	Jelly indet.	NA	NA	NA	*NA*	*Jelly indet.*
Metazoa	Egg indet.	NA	NA	NA	*NA*	*egg ind. (big)*
Metazoa	Egg indet.	NA	NA	NA	*NA*	*egg ind. (small)*

Appendix 3.

All species detected with eDNA. From kingdom to species level.

kingdom	phylum	class	order	family	genus	species
Metazoa	Annelida	Clitellata	Enchytraeida	Enchytraeidae	Mesenchytraeus	Mesenchytraeus flavus
Metazoa	Annelida	Polychaeta	Capitellida	Arenicolidae	Arenicolides	Arenicolides ecaudata
Metazoa	Annelida	Polychaeta	Eunicida	Amphinomidae	Paramphinome	Paramphinome jeffreysii
Metazoa	Annelida	Polychaeta	Eunicida	Dorvilleidae	Ophryotrocha	Ophryotrocha maculata
Metazoa	Annelida	Polychaeta	Eunicida	Onuphidae	Nothria	Nothria conchylega
Metazoa	Annelida	Polychaeta	NA	Capitellidae	Capitella	Capitella sp. 1
Metazoa	Annelida	Polychaeta	NA	Chaetopteridae	Chaetopterus	Chaetopterus sp. 1
Metazoa	Annelida	Polychaeta	NA	Protodrilidae	Protodrilus	Protodrilus ciliatus
Metazoa	Annelida	Polychaeta	Phyllodocida	Hesionidae	Nereimyra	Nereimyra punctata
Metazoa	Annelida	Polychaeta	Phyllodocida	Hesionidae	Psamathe	Hesionidae sp. 1
Metazoa	Annelida	Polychaeta	Phyllodocida	Nereididae	Nereis	Nereis zonata
Metazoa	Annelida	Polychaeta	Phyllodocida	Nereididae	Platynereis	Platynereis dumerilii
Metazoa	Annelida	Polychaeta	Phyllodocida	Pholoidae	Pholoe	Pholoe sp. 1
Metazoa	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eumida	Eumida sanguinea
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	Acanthicolepis	Acanthicolepis asperrima
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	Gattyana	Gattyana cirrhosa
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	Harmothoe	Harmothoe sp. 1
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	Harmothoe	Harmothoe sp. 2
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	Malmgreniella	Malmgreniella mcintoshi
Metazoa	Annelida	Polychaeta	Phyllodocida	Polynoidae	NA	Polynoidae sp. 1
Metazoa	Annelida	Polychaeta	Polychaeta_incertae_sedis	Paraonidae	Aricidea	Aricidea quadrilobata
Metazoa	Annelida	Polychaeta	Sabellida	Fabriciidae	Fabricia	Fabricia stellaris
Metazoa	Annelida	Polychaeta	Sabellida	Oweniidae	Galathowenia	Galathowenia oculata
Metazoa	Annelida	Polychaeta	Sabellida	Oweniidae	Owenia	Owenia fusiformis
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Aonides	Aonides oxycephala
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Aonides	Aonides pauchibranchiata
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Malacoceros	Malacoceros fuliginosus

Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Malacoceros	Malacoceros sp. 1
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Prionospio	Prionospio cirrifera
Metazoa	Annelida	Polychaeta	Spionida	Spionidae	Spiophanes	Spiophanes kroyeri
Metazoa	Annelida	Polychaeta	Terebellida	Ampharetidae	Ampharete	Ampharete octocirrata
Metazoa	Annelida	Polychaeta	Terebellida	Cirratulidae	Dodecaceria	Dodecaceria concharum
Metazoa	Annelida	Polychaeta	Terebellida	Terebellidae	Eupolymnia	Eupolymnia nesidensis
Metazoa	Annelida	Polychaeta	Terebellida	Terebellidae	Hauchiella	Hauchiella tribullata
Metazoa	Annelida	Polychaeta	Terebellida	Terebellidae	Nicolea	Nicolea venustula
Metazoa	Arthropoda	Arachnida	Sarcoptiformes	Ameronothridae	Ameronothrus	Ameronothrus lineatus
Metazoa	Arthropoda	Arachnida	Sarcoptiformes	Phenopelopidae	Eupelops	Eupelops sp.
Metazoa	Arthropoda	Collembola	Entomobryomorpha	Isotomidae	Anurophorus	Anurophorus laricis
Metazoa	Arthropoda	Collembola	Entomobryomorpha	Isotomidae	Pseudisotoma	Pseudisotoma sensibilis
Metazoa	Arthropoda	Collembola	Poduromorpha	Neanuridae	Anurida	Anurida maritima
Metazoa	Arthropoda	Hexanauplia	Calanoida	Acartiidae	Acartia	Acartia clausii
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	Calanus finmarchicus
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	Calanus hyperboreus
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	Calanus sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Calanus	Calanus sp. 2
Metazoa	Arthropoda	Hexanauplia	Calanoida	Calanidae	Ctenocalanus	Ctenocalanus vanus
Metazoa	Arthropoda	Hexanauplia	Calanoida	Centropagidae	Centropages	Centropages sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Centropagidae	Centropages	Centropages sp. 2
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	Microcalanus	Microcalanus pusillus
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	Pseudocalanus	Pseudocalanus elongatus
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	Pseudocalanus	Pseudocalanus moultoni
Metazoa	Arthropoda	Hexanauplia	Calanoida	Clausocalanidae	Pseudocalanus	Pseudocalanus sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Metridinidae	Metridia	Metridia lucens
Metazoa	Arthropoda	Hexanauplia	Calanoida	Metridinidae	Metridia	Metridia sp. 1
Metazoa	Arthropoda	Hexanauplia	Calanoida	Paracalanidae	Paracalanus	Paracalanus parvus
Metazoa	Arthropoda	Hexanauplia	Cyclopoida	Cyclopinidae	Cyclopina	Cyclopina norvegica
Metazoa	Arthropoda	Hexanauplia	Cyclopoida	Oithonidae	Oithona	Oithona atlantica

Metazoa	Arthropoda	Hexanauplia	Cyclopoida	Oithonidae	Oithona	Oithona similis
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Dactylopusiidae	NA	Dactylopusiidae sp. 1
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Ectinosomatidae	Microsetella	Microsetella norvegica
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Harpacticidae	Harpacticus	Harpacticus gracilis
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Harpacticidae	Harpacticus	Harpacticus uniremis
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Harpacticidae	NA	Harpacticidae sp. 1
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Laophontidae	Asellopsis	Asellopsis hispida
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Thalestridae	Parathalestris	Parathalestris harpactoides
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Tisbidae	Tisbe	Tisbe elegantula
Metazoa	Arthropoda	Hexanauplia	Harpacticoida	Tisbidae	Tisbe	Tisbe sp. 1
Metazoa	Arthropoda	Malacostraca	Amphipoda	Dexaminidae	Dexamine	Dexamine thea
Metazoa	Arthropoda	Malacostraca	Amphipoda	Ischyroceridae	Jassa	Jassa falcata
Metazoa	Arthropoda	Malacostraca	Amphipoda	Talitridae	Orchestia	Orchestia gammarellus
Metazoa	Arthropoda	Malacostraca	Decapoda	Galatheidae	Galathea	Galathea nexa
Metazoa	Arthropoda	Malacostraca	Decapoda	Hippolytidae		Hippolytidae sp. 1
Metazoa	Arthropoda	Malacostraca	Decapoda	Majidae	Hyas	Hyas sp. 1
Metazoa	Arthropoda	Malacostraca	Decapoda	Pandalidae	Pandalus	Pandalus sp. 1
Metazoa	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	Meganyctiphanes	Meganyctiphanes norvegica
Metazoa	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	Thysanoessa	Thysanoessa inermis
Metazoa	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	Thysanoessa	Thysanoessa raschii
Metazoa	Arthropoda	Malacostraca	Isopoda	Idoteidae	Idotea	Idotea neglecta
Metazoa	Arthropoda	Malacostraca	Isopoda	Porcellionidae	Porcellio	Porcellio scaber
Metazoa	Arthropoda	Thecostraca	Balanomorpha	Balanidae	Balanus	Balanus balanus
Metazoa	Arthropoda	Thecostraca	Balanomorpha	Balanidae	Balanus	Balanus sp. 1
Metazoa	Arthropoda	Thecostraca	Balanomorpha	Balanidae	NA	Balanidae sp. 1
Metazoa	Arthropoda	Thecostraca	Balanomorpha	Balanidae	Semibalanus	Semibalanus balanoides
Metazoa	Arthropoda	Thecostraca	Verrucomorpha	Verrucidae	Verruca	Verruca stroemia
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Candidae	NA	Candidae sp. 1
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Candidae	Scrupocellaria	Scrupocellaria scruposa
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Celleporidae	Omalosecosa	Omalosecosa ramulosa

Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	Electra	Electra pilosa
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Membraniporidae	Membranipora	Membranipora membranacea
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Smittinidae	Parasmittina	Parasmittina trispinosa
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Smittinidae	Smittoidea	Smittoidea prolifica
Metazoa	Bryozoa	Gymnolaemata	Cheilostomatida	Umbonulidae	Umbonula	Umbonula littoralis
Metazoa	Bryozoa	Gymnolaemata	Ctenostomatida	Alcyonidiidae	Alcyonidium	Alcyonidium mamillatum
Metazoa	Bryozoa	Gymnolaemata	Ctenostomatida	Vesiculariidae	Amathia	Amathia imbricata
Metazoa	Bryozoa	Stenolaemata	Cyclostomatida	Crisiidae	Crisia	Crisia eburnea
Metazoa	Bryozoa	Stenolaemata	Cyclostomatida	Crisiidae	Crisiella	Crisiella producta
Metazoa	Chaetognatha	Sagittoidea	Aphragmophora	Sagittidae	Sagitta	Sagitta elegans
Metazoa	Chaetognatha	Sagittoidea	Phragmophora	Eukrohniidae	Eukrohnia	Eukrohnia sp. 1
Metazoa	Chordata	Actinopteri	Clupeiformes	Clupeidae	Clupea	Clupea harengus
Metazoa	Chordata	Actinopteri	Gadiformes	Gadidae	Boreogadus	Boreogadus saida
Metazoa	Chordata	Actinopteri	Gadiformes	Gadidae	Gadus	Gadus morhua
Metazoa	Chordata	Actinopteri	Gadiformes	Gadidae	Melanogrammus	Melanogrammus aeglefinus
Metazoa	Chordata	Actinopteri	Gadiformes	Gadidae	Pollachius	Pollachius virens
Metazoa	Chordata	Actinopteri	Gadiformes	Gadidae	Trisopterus	Trisopterus minutus
Metazoa	Chordata	Actinopteri	Gadiformes	Lotidae	Molva	Molva molva
Metazoa	Chordata	Actinopteri	Gadiformes	Phycidae	Phycis	Phycis blennoides
Metazoa	Chordata	Actinopteri	Gobiiformes	Gobiidae	Lebetus	Lebetus scorpioides
Metazoa	Chordata	Actinopteri	Perciformes	Liparidae	Liparis	Liparis montagui
Metazoa	Chordata	Actinopteri	Perciformes	Sebastidae	Sebastes	Sebastes sp. 1
Metazoa	Chordata	Actinopteri	Pleuronectiformes	Pleuronectidae	Hippoglossus	Hippoglossus hippoglossus
Metazoa	Chordata	Actinopteri	Pleuronectiformes	Pleuronectidae	Microstomus	Microstomus kitt
Metazoa	Chordata	Actinopteri	Salmoniformes	Salmonidae	Salmo	Salmo salar
Metazoa	Chordata	Ascidiacea	Phlebobranchia	Ascidiidae	Ascidia	Ascidia conchilega
Metazoa	Chordata	Ascidiacea	Phlebobranchia	Ascidiidae	Ascidia	Ascidia sp. 1
Metazoa	Chordata	Ascidiacea	Stolidobranchia	Pyuridae	Boltenia	Boltenia echinata
Metazoa	Cnidaria	Anthozoa	Actiniaria	Gonactiniidae	NA	Gonactiniidae sp. 1
Metazoa	Cnidaria	Anthozoa	Actiniaria	Hormathiidae	Adamsia	Adamsia palliata

Metazoa	Cnidaria	Anthozoa	Actiniaria	Metridiidae	NA	Metridiidae sp. 1
Metazoa	Cnidaria	Anthozoa	Ceriantharia	Arachnactidae	Isarachnanthus	Isarachnanthus nocturnus
Metazoa	Cnidaria	Anthozoa	Malacalcyonacea	Alcyoniidae	Alcyonium	Alcyonium sp. 1
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Bougainvilliidae	Bougainvillia	Bougainvillia sp. 1
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Corynidae	Sarsia	Sarsia lovenii
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Eudendriidae	Eudendrium	Eudendrium rameum
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Eudendriidae	Eudendrium	Eudendrium sp. 1
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Tubulariidae	Ectopleura	Ectopleura larynx
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Tubulariidae	Hybocodon	Hybocodon prolifer
Metazoa	Cnidaria	Hydrozoa	Anthoathecata	Tubulariidae	Tubularia	Tubularia indivisa
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Campanulariidae	NA	Campanulariidae sp. 1
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Clytiidae	Clytia	Clytia hemisphaerica
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Haleciidae	Halecium	Halecium sp. 1
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Laodiceidae	NA	Laodiceidae sp. 1
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Laodiceidae	NA	Laodiceidae sp. 2
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Laodiceidae	Staurostoma	Staurostoma mertensii
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Mitrocomidae	Mitrocomella	Mitrocomella polydiademata
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Obeliidae	Obelia	Obelia sp. 1
Metazoa	Cnidaria	Hydrozoa	Leptothecata	Sertulariidae	Dynamena	Dynamena pumila
Metazoa	Cnidaria	Hydrozoa	Siphonophorae	Agalmatidae	Agalma	Agalma elegans
Metazoa	Cnidaria	Hydrozoa	Siphonophorae	Agalmatidae	Nanomia	Nanomia cara
Metazoa	Cnidaria	Scyphozoa	Semaeostomeae	Cyaneidae	Cyanea	Cyanea capillata
Metazoa	Echinodermata	Asteroidea	Forcipulatida	Asteriidae	Asterias	Asterias rubens
Metazoa	Echinodermata	Echinoidea	Camarodonta	Echinidae	NA	Echinidae sp. 1
Metazoa	Echinodermata	Echinoidea	Camarodonta	Strongylocentrotidae	Strongylocentrotus	Strongylocentrotus sp. 1
Metazoa	Echinodermata	Holothuroidea	Apodida	Synaptidae	Leptosynapta	Leptosynapta inhaerens
Metazoa	Echinodermata	Ophiuroidea	Amphilepidida	Ophiactidae	Ophiopholis	Ophiopholis aculeata
Metazoa	Echinodermata	Ophiuroidea	Amphilepidida	Ophiocomidae	Ophiocomina	Ophiocomina sp. 1
Metazoa	Echinodermata	Ophiuroidea	Amphilepidida	Ophiuridae	Ophiocten	Ophiocten affinis
Metazoa	Mollusca	Bivalvia	Adapedonta	Hiatellidae	Hiatella	Hiatella arctica

Metazoa	Mollusca	Bivalvia	Adapedonta	Hiatellidae	Hiatella	Hiatella sp. K
Metazoa	Mollusca	Bivalvia	Adapedonta	Pharidae	Ensis	Ensis sp. 1
Metazoa	Mollusca	Bivalvia	Mytilida	Mytilidae	Modiolus	Modiolula sp. 1
Metazoa	Mollusca	Bivalvia	Mytilida	Mytilidae	Modiolus	Modiolus modiolus
Metazoa	Mollusca	Bivalvia	Mytilida	Mytilidae	Mytilus	Mytilus sp. 1
Metazoa	Mollusca	Bivalvia	Pectinida	Pectinidae	Aequipecten	Aequipecten opercularis
Metazoa	Mollusca	Bivalvia	Pectinida	Pectinidae	Palliolum	Palliolum tigerinum
Metazoa	Mollusca	Bivalvia	Venerida	Arcticidae	Arctica	Arctica islandica
Metazoa	Mollusca	Bivalvia	Venerida	Mactridae	Spisula	Spisula subtruncata
Metazoa	Mollusca	Cephalopoda	Octopoda	Octopodidae	Eledone	Eledone cirrhosa
Metazoa	Mollusca	Gastropoda	Aplysiida	Aplysiidae	Aplysia	Aplysia sp. 1
Metazoa	Mollusca	Gastropoda	Cephalaspidea	Philinidae	NA	Philinidae sp. 1
Metazoa	Mollusca	Gastropoda	Littorinimorpha	Littorinidae	NA	Littorinidae sp. 1
Metazoa	Mollusca	Gastropoda	NA	Acteonidae	Acteon	Acteon tornatilis
Metazoa	Mollusca	Gastropoda	Neogastropoda	Nassariidae	NA	Nassariidae sp. 1
Metazoa	Mollusca	Gastropoda	Nudibranchia	Coryphellidae	Coryphella	Flabellinidae sp. 1
Metazoa	Mollusca	Gastropoda	Nudibranchia	Dendronotidae	Dendronotus	Dendronotus europaeus
Metazoa	Mollusca	Gastropoda	Nudibranchia	Goniodorididae	Goniodoris	Goniodoris nodosa
Metazoa	Mollusca	Gastropoda	Nudibranchia	Heroidae	Hero	Hero formosa
Metazoa	Mollusca	Gastropoda	Nudibranchia	Onchidoridide	Knoutsodonta	Knoutsodonta pusilla
Metazoa	Mollusca	Gastropoda	Nudibranchia	Onchidoridide	Onchidoris	Onchidoris bilamellata
Metazoa	Mollusca	Gastropoda	Nudibranchia	Tergipedidae	Tergipes	Tergipes tergipes
Metazoa	Mollusca	Gastropoda	Nudibranchia	Trinchesiidae	Trinchesia	Trinchesia foliata
Metazoa	Mollusca	Gastropoda	Trochida	Margaritidae	Margarites	Margarites groenlandicus
Metazoa	Mollusca	Gastropoda	Trochida	Trochidae	NA	Trochidae sp. 1
Metazoa	Mollusca	Polyplacophora	Chitonida	Tonicellidae	NA	Tonicellidae sp. 1
Metazoa	Nemertea	Enopla	Bdellonemertea	Malacobdellidae	Malacobdella	Malacobdella grossa
Metazoa	Nemertea	Enopla	Monostilifera	Tetrastemmatidae	Tetrastemma	Tetrastemma candidum
Metazoa	Nemertea	Pilidiophora	Heteronemertea	Lineidae	Pseudomicrura	Pseudomicrura afzelii
Metazoa	Phoronida	NA	NA	Phoronidae	Phoronis	Phoronis muelleri

Metazoa	Porifera	Demospongiae	Axinellida	Axinellidae	Axinella	Axinella rugosa
Metazoa	Porifera	Demospongiae	Poecilosclerida	Myxillidae	NA	Myxillidae sp. 1
Metazoa	Porifera	Demospongiae	Tetractinellida	Tetillidae	Craniella	Craniella sp. 1

Appendix 4

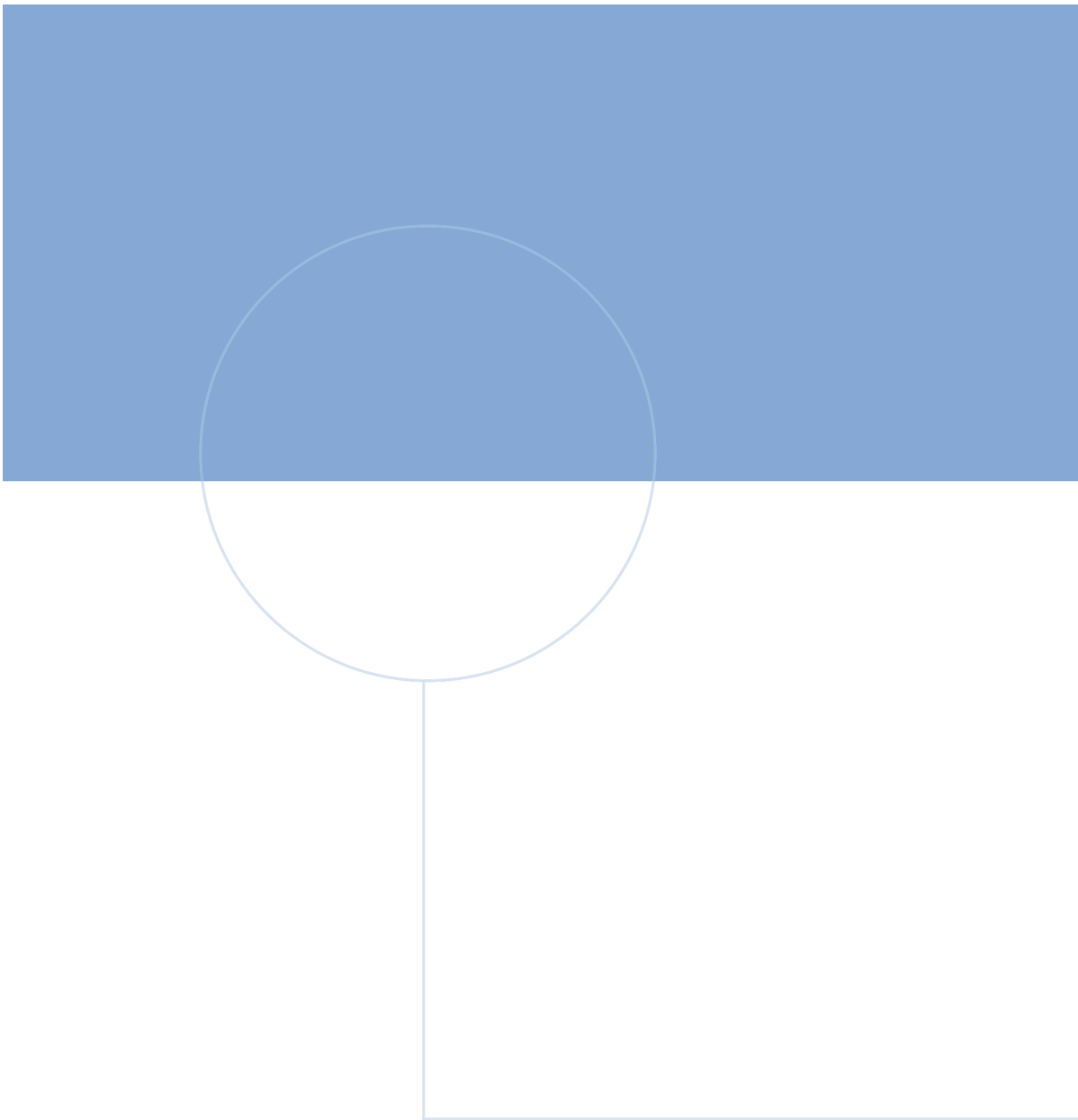
All *Calanus finmarchicus* counted with morphology, in the water column (*Calanus*/m^3).

Sample/species	Depth	CI	CII	CIII	CIV	CV	AF	AM	SUM
SA1	**50m**	0	131,1	116,2	82,4	30,0	7,5	0	367,2
SA2	**25m**	0	73,2	137,2	57,9	18,3	18,3	0	304,9
SA3	**10m**	6,5	234,2	195,1	97,6	65,0	52,0	6,5	656,9
SA4	**5m**	6,7	114,4	117,7	63,9	23,5	13,5	0	339,7
SA5	**50m**	2,9	26,0	80,9	112,7	40,5	31,8	0	294,8
SA6	**25m**	0	128,6	297,4	265,3	88,4	32,2	0	811,9
SA7	**10m**	0	319,6	450,4	435,9	130,8	116,2	0	1452,9
SA8	**5m**	0	312,9	330,7	232,4	26,8	44,7	0	947,5
SA9	**NA**	0	258,2	368,9	184,5	110,7	86,1	0	1008,3
SA10	**NA**	0	4,2	9,1	9,8	9,1	4,5	0	36,6
SA11	**NA**	0,2	2,3	4,2	1,6	0,5	0,5	0	9,3

Appendix 5

Calanus finmarchicus sequence reads, relative abundance, DNA.

Sample	**Sample ID**	**Depth**	***Calanus finmarchicus***
SA1A	**140**	50m	13467
SA1B	**141**	50m	21891
SA2A	**142**	25m	4413
SA2B	**143**	25m	10035
SA3A	**144**	10m	6661
SA3B	**145**	10m	34806
SA4A	**146**	5m	6184
SA4B	**147**	5m	7128
SA5	**148**	50m	12014
SA6Z	**149**	25m	6557
SA6B	**150**	25m	10933
SA7A	**151**	10m	6676
SA7B	**152**	10m	3261
SA8	**153**	5m	23918
SA9	**154**	NA	4129
AutoNaut10	**155**	NA	4304
Neg	**neg**		0
Neg	**neg**		0



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