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Zooplankton seasonality at high latitudes:

From community to behaviour

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Summary

The Arctic is a highly seasonal environment, with day length varying from the absence of day light in winter to the absence of darkness in summer. Seasonal variations in day length are associated to seasonal variation in temperature, currents, wind and sea ice cover. Due to these environmental constraints the periods offering favourable conditions for primary productions are relatively short at high latitudes and are followed by a long period of unfavourable conditions with highly limited primary production. To cope with long periods of limited food availability, the secondary producers and also higher trophic levels have evolved physiological and behavioural adaptations, such as entering diapause during the non-productive period or seasonal migration to escape unfavourable conditions. This thesis focuses on the seasonality of the mesozooplankton community, a direct link between primary producer and higher trophic levels. To fully understand the response of the mesoplankton community to seasonality, there is a need to develop new methods to investigate the entire community rather than only the most common species as commonly done. This thesis describes the seasonality of the mesozooplankton community in Ramfjord, a shallow high latitude fjord, in terms of diversity and behaviour by using a combination of new and traditional methods. This approach aimed to gain more insight into how the seasonality in environmental drivers, such as photoperiod and food availability, are shaping seasonal changes in mesozooplankton community structure and *Calanus* spp. behaviour.

Monthly sampling over an entire annual cycle revealed that the seasonal variability in the mesozooplankton community structure was manifested by changes in abundance, biomass and diversity of meroplankton and copepods. This seasonality was driven by seasonal changes in temperature and phytoplankton phenology. Using both visual identification and metabarcoding provided the most detailed species list of the mesozooplankton community for a Norwegian fjord to date. The use of metabarcoding as a quantitative tool established that unlike other Arctic ecosystems, where large copepods dominate in terms of biomass, in Ramfjord the small copepods were dominating in terms of biomass and abundance year-round while meroplankton were the most diverse group. These two groups are often overlooked in Arctic zooplankton studies and these finding highlight the importance of the small size fraction in shaping Arctic mesozooplankton communities. This study demonstrated the potential of using metabarcoding for quantification, however inconsistencies with the visual identification method have to be addressed before metabarcoding alone can provide reliable quantitative estimate of biomass of

the entire mesozooplankton community. Monthly monitor of *Calanus finmarchicus* swimming activity using locomotor activity monitors (LAM) under ambient day/night cycle during the overwintering period in an environment with shallow water depth (Ramfjord) revealed that *Calanus finmarchicus* remain active and enters a winter resting state rather than entering a diapause state as commonly observed in environments of deep-water where they can seek water layers of continuous darkness. Seasonal changes in *Calanus* spp. swimming behaviour were compared to diel vertical migration (DVM) behaviour of the zooplankton community monitored using active acoustics. The seasonal changes in DVM behaviour corresponded well with the seasonal changes in swimming activity of *Calanus* spp., indicating that swimming activity can be used as a proxy for DVM behaviour.

This thesis demonstrates that the combination of newly developed methods (locomotor activity monitor and metabarcoding as a quantitative tool) with more traditional methods, such as visual identification and active acoustic, can provide new understanding of seasonal changes in the mesozooplankton community structure and behaviour.

List of papers

Paper I:

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Paper III:

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Abbreviation

AZFP - Acoustic Zooplankton and Fish Profiler

BWSR - Biomass Weighted Sequence Reads

CCA - Canonical Correspondence Analysis

CTD - Conductivity Temperature and Depth profiler

CI to CV - Copepodite stage I to Copepodite stage V

DNA - Deoxyribonucleic

DVM - Diel Vertical Migration

DW - Dry Weight

InDels - Insertion-Deletion markers

IRB - InfraRed Beam

LAM - Locomotor Activity Monitor

LAPA - Lipid sac Area to Prosome Area ratio

MVBS - Mean Volume Backscattering strength

S_v - backscatter volume

SVM - Seasonal Vertical Migration

TL – Total Lipid

1 Introduction

1.1 Seasonality on earth

Seasonality refers to regular and periodic changes of the environment and can be defined in different ways. Astronomically, it is defined by the change in the Earth's position in relation to the sun (winter and summer solstice, autumnal and vernal equinox). Meteorologically its definition is based on weather patterns, with winter as the coldest months, summer as the warmest months and autumn and spring as the transitions between those seasons (Trenberth, 1983). In an ecological context, the definition most commonly used is by Lieth (1974) who defined seasonality as the occurrence of biotic and abiotic events within a defined period of time (Tonkin et al., 2017; Varpe, 2017). Seasonality is a result of the Earth's tilted rotation axis, which creates seasonal variation in day length (i.e., photoperiod), temperature, precipitations, wind and currents. The combination of these abiotic factors may generate windows of favourable conditions alternating with windows of unfavourable conditions for a given organism. Responses to seasonal changes in optimal conditions include latitudinal gradients in biodiversity, seasonal changes in community structure and, seasonal migrations. Many life history adaptations (e.g., timing of mating and reproduction, resource allocations) can only be understood in the context of seasonality (Corkeron and Connor, 1999; Tonkin et al., 2017 and references within). All of these processes are driven not only by the seasonality itself (i.e., the occurrence of an event), but also by the predictability of seasonality, i.e., the regularity of the appearance of the event of interest (Tonkin et al., 2017).

In the marine realm, the bloom of primary producers is one of the main biotic events structuring seasonality in the ecosystems (Varpe, 2017). Annual routines of marine organisms - herbivores in particular - are tightly coupled to the phenology in algae blooms. Light, temperature and nutrients are the main limiting factors for photosynthetic primary producers (Geider et al., 1997). Light is regulated by altitude of the sun and the cloud cover and, at high latitudes, sea ice. These factors vary seasonally. Seasonal changes in day length are highly dependent on latitude. For example, at the equator, the day length is 12 h year-round, and at the North Pole the entire year is one long day and one long night, each lasting six months (Berge et al., 2020). In the northern hemisphere above the Arctic Circle (66N°) (my geographical area of interest) the day length varies from polar night, i.e., the absence of daylight as the sun stays below the horizon for a full diel cycle, to mid-night sun, i.e., the absence of night as the sun stays above

the horizon. Consequently, at high latitudes, primary production is highly reduced during the polar night due to the absence of direct sunlight, but also because of the drastic reduction of light penetrating the water due to the presence of sea ice and the accumulation of snow on top of it during the winter period. This period is also referred to as the non-productive period. The productive period (i.e., the period when the conditions are favourable for primary producer to grow) becomes shorter with increasing latitude as the polar night gets longer. At high latitudes, the productive period also becomes less predictable due to the presence of sea ice, which regulates the light availability in the water column. Ice cover attenuates the light transmitted in the water column, making the ice break-up an essential event in the initiation of the productive period. However, the timing of this event has a high interannual variability as it is dependent on a combination of numerous abiotic factors including temperature, currents, precipitation and wind. Temperature varies seasonally along with wind and currents, and controls stratification and mixing of water masses. These two phenomena regulate nutrient availability and therefore productivity.

This extreme seasonality in availability of primary producer at high latitudes led to specific adaptation of secondary and higher trophic levels' life history traits, behaviour and physiology (Danks, 2004; Varpe, 2017). Such adaptations are found in a large range of organisms, from terrestrial and marine invertebrate to large mammals. They include:

- Spending the unproductive period in a state of diapause either as adult or near adult stage like in copepods of genus *Calanus* and in the fly *Drosophila auraria* (Kimura, 1984; Hirche, 1996), or as early developmental stages like in marine cladocerans and the Greenland seed bug *Nysius groenlandicus*(Onbé, 1991; Böcher and Nachman, 2001)
- Accumulation of large internal energy reserves to sustain organisms through the unproductive season like in the *Calanus* genus, but also in seals and polar bears (Falk-Petersen et al., 1986; Atkinson and Ramsay, 1995)
- Having an extended life cycle (Varpe et al., 2009; Barta, 2016) such as *Calanus hyperboreus* which has a life cycle up to 5 years against 1 year for more boreal sibling species such as *Calanus finmarchicus* (Berge et al., 2012a) or *Chironomus* species which have a 7 year-life cycle, the longest life cycle for an Arctic insect (Butler, 1982).

- Large scale horizontal and vertical migrations, such as Arctic terns (*Sterna paradisaea*) that migrate up to 20 000 km horizontally from pole to pole, and A*pherusa glacialis* an ice associated amphipod which migrate to the pelagic realm to avoid been advected out of the Arctic basin during free ice period (Berge et al., 2012b; Kunisch et al., 2020).
- A life cycle perfectly timed to the environmental seasonality, like in *Calanus glacialis* which times its reproduction to be able to utilised both the ice algae and the phytoplankton bloom, or the brent goose (*Branta bernicla hrota*) which timed its northward migration to coincide with the spring melt and early bloom conditions in its breeding areas (Søreide et al., 2010; Clausen and Clausen, 2013).

However, a change in the environment, such as an earlier sea ice break up impacting the timing of the algae bloom or an earlier start of the spring impacting the timing of the photosynthetic organisms, disturbs the strategies adopted by the organisms. Therefore, flexibility in life history adaptations is essential for these organisms to survive and successfully reproduce, despite encountering unfavourable timing at critical times. Climate change, one of the most documented environmental changes, provokes rapid long term environmental change, leading to questions about the ability of organisms to adapt to it.

In response to global warming, a northward migration of organisms (in the northern hemisphere) is observed and expected to become more prevalent as organisms follow their optimal temperature range (Beaugrand, 2003; Beaugrand and Reid, 2003; Fossheim et al., 2015). Arctic Atlantification, which is the increase of Atlantic water advected in the Arctic, is also predicted to play a role in this northward migration by increasing the advection of Atlantic species in the Arctic (Walsh et al., 2011). In terms of seasonality this means that organisms may eventually be exposed to different timing of seasonal cycle than the one they are adapted to. For Atlantic/boreal species in particular, this implies that they will be exposed to the extreme seasonality of the Arctic. In order to predict the consequences of such environmental changes it is important to understand the role that seasonality plays in shaping ecosystems and life history strategies.

1.2 Zooplankton

1.2.1 Zooplankton's role in the ecosystem

This thesis focuses on seasonality and zooplankton. The term zooplankton describes all heterotrophic organisms which are unable to swim actively against currents and consequently drift at the discretion of ocean currents (Daase et al., 2021). Therefore, zooplankton are particularly sensitive to hydrological changes as advection can drastically affect their distribution and fitness if advected into an unfavourable environment. Furthermore, most zooplankton are not harvested, consequently any change in the zooplankton community are related to environmental forcing rather than human intervention. This makes them a good indicator of the marine ecosystem state (Hughes, 2000; Taylor et al., 2002; Hays et al., 2005).

Zooplankton play essential roles in marine ecosystems. They constitute a direct link between the primary producer and the higher trophic levels, and thus are crucial in the energy transfer of marine food webs (Steele, 1974; Falk-Petersen et al., 2007; Daase et al., 2021)}. By conducting vertical migrations, zooplankton are exporting and releasing carbon, ingested in the surface water, to greater depth and thus play an important role in biogeochemical cycles, particularly in the biological carbon pump (Longhurst, 1991; Schnack-Schiel and Isla, 2005; Turner, 2015). Zooplankton are also important contributors to biodiversity as it is a highly diverse group not only in terms of numbers of species but also in terms of size and life history strategies.

Two main groups of zooplankton are commonly discriminated: holoplankton and meroplankton. Holoplankton are heterotrophs that spend their entire life cycle as plankton. Meroplankton, are heterotrophs that spend only part of their life cycle (usually the larval period) as plankton. Of the two groups, meroplankton is the least studied, and limited information is available on their phenology, population dynamics and diversity (Michelsen et al., 2017; Descôteaux, 2022). In boral and Arctic seas, the holoplankton community is largely dominated by copepods (Kosobokova et al., 2011), and are particularly well studied. Size is also a common criterion of discrimination of zooplankton organisms. This thesis focuses on the mesozooplankton, i.e., the zooplankton with a size included between 0.2 to 20 mm. The mesozooplankton category can be divided into two subdivisions. The large mesozooplankton which include all organisms with a size (as adult) > 1.5 mm, such as *Calanus* copepods, euphausiid, and the small mesozooplankton, which group mesozooplankton with a size (as

adult) < 1.5 mm, including most of the meroplankton previously introduced as well as small copepods such as *Oithona similis* or *Microsetella norvegica.* The smaller size fraction is particularly difficult to sample in an unbiased way because these organisms are often too small to be properly sampled with plankton nets commonly used to sample the mesozooplankton community, but too big to be properly sampled with the methods developed to sample microzooplankton (i.e., organisms between 20-200 µm, such as Radiolaria or heterotrophic dinoflagellate). As a consequence, small mesozooplankton abundance, biomass and community composition are often understudied and underestimated (Pasternak et al., 2008; Svensen et al., 2018).

1.2.2 Zooplankton vertical migration

One common behavioural adaptation of zooplankton to seasonality is a seasonal change in their vertical position. Seasonal vertical migration (SVM) describes a seasonal change in the depth distribution of a species (Figure 1). This most often consists of a descent to deeper water at the start of the non-productive period. Inhabiting dark and cold deep water enables the organisms to reduce the visual predation risk and to slow down their metabolism (Bandara et al., 2021). While they feed in the upper water during the productive period. Lipid-rich species also take advantage of the pressured environment at depth that acts on the lipid structure, enabling a neutral buoyancy. This reduces the energetic demand over the non-productive period since the organisms do not have to actively maintain their vertical position (Pond, 2012; Pond et al., 2014). However, that true only at specific depth, opening interrogations of SVM in shallow environment where organisms do not encounter neutral buoyancy (Pond, 2012).

Zooplankton can also change their vertical position over the daily cycle by performing diel vertical migration (DVM), which is the world's largest migration in terms of biomass (Hays, 2003). The most common form of DVM (classical DVM) is the presence of zooplankton in surface water during night time to feed and an active migration to depth during day time to avoid visual predation (Bandara et al., 2021). Predation risk and food availability are recognised as the ultimate cues (Lampert, 1993; De Robertis, 2002) while light is most commonly considered as the proximate cue (Cohen and Forward, 2009). However, the mechanisms behind the regulation of this behaviour are not fully understood and may vary between species and geographical regions, especially the cues that are initiating the ascent and descent. Amplitude and synchronicity of this behaviour vary primarily with the seasonal changes in day length and

food availability, but changes of DVM behaviour in response to changes in predation pressure, which can vary with seasons, have also been observed (Ohman, 1990). In the Arctic, a rather abrupt seasonal change into the characteristic of DVM have been observed with, classical DVM over the entire water column in spring and autumn, while in winter and summer the magnitude of the DVM behaviour is reduced (Berge et al., 2009; Daase et al., 2016; Ludvigsen et al., 2018). In winter, i.e., during the polar night, underwater light levels are low. Organisms that remain in the surface layers during the polar night exhibit a daily depth variation of only a few meters (Ludvigsen et al., 2018). This pattern can be impacted by the lunar cycle, with a migration to deeper waters during lunar nighttime (i.e., period centered around minimum lunar altitude) around the full moon (Last et al., 2016; Ludvigsen et al., 2018). In summer, the mid-night sun does not provide any specific favourable time of day to visit surface waters, leading to unsynchronized DVM behaviour in the population (Cottier et al., 2006). The magnitude of this DVM behaviour can be reduced (20 to 80 m of vertical migration as compared to several hundred meters under day/night cycle) and the upper surface may be totally avoided as the risk of visual detection by predators is high while it is often depleted of phytoplankton as it has started to sink (Daase et al., 2016).

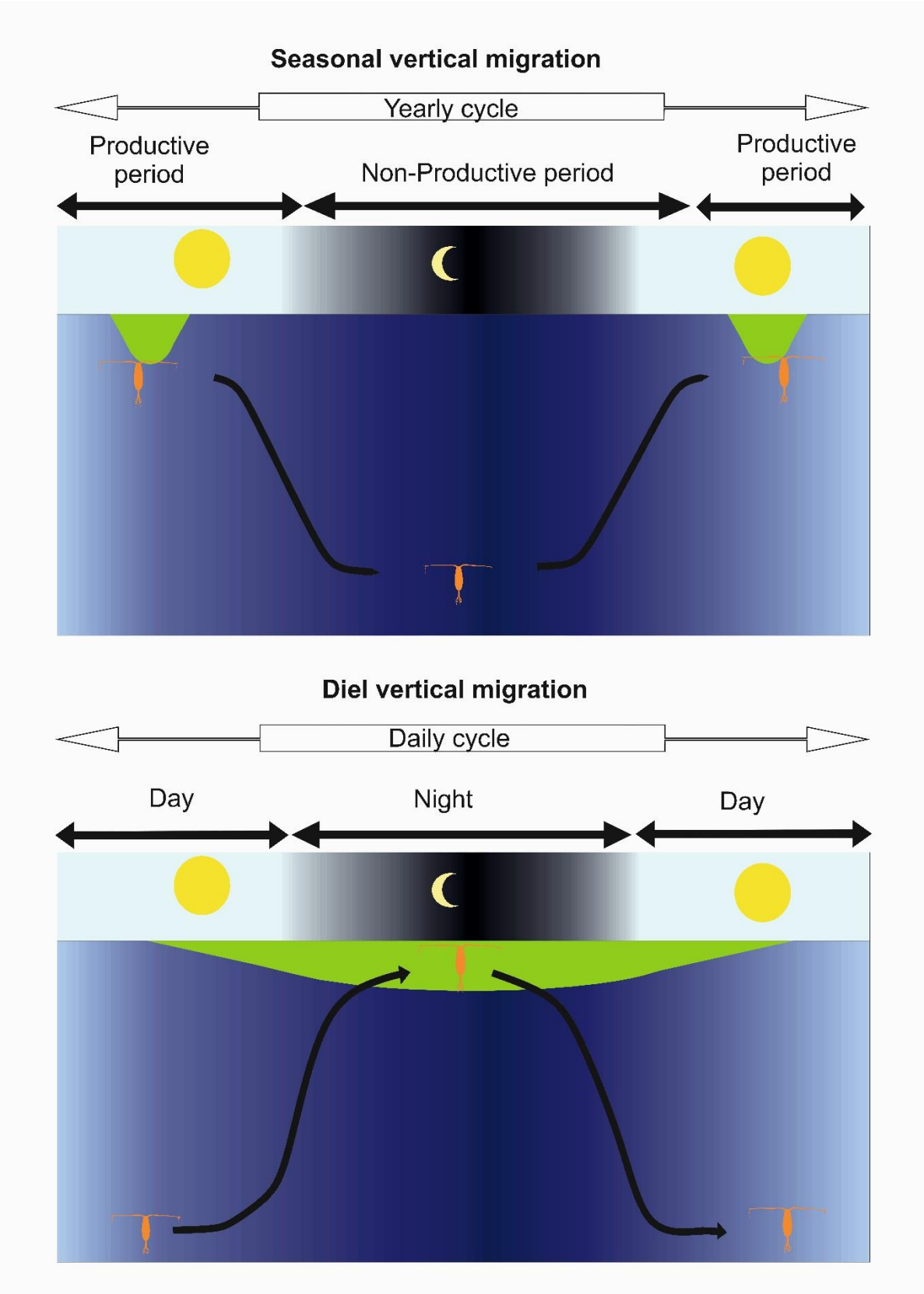

Figure 1: Schematic representation of vertical migration in zooplankton. The top panel illustrates seasonal vertical migration (SVM) with a descent to deep dark waters during the non-productive period. The bottom panel illustrates diel vertical migration (DVM) with a daily migration from the deep waters during daytime to the surface water during nighttime.

1.3 *Calanus* genus

At high latitudes, the mesozooplankton community is dominated, in terms of biomass, by large calanoid copepod, in particular those of the genus *Calanus*. Several *Calanus* species are encountered above the Arctic Circle (Choquet et al., 2017). Two of these species are negligible above the Arctic Circle: *C. marshallae* is found in low numbers in the Beaufort Sea (Wassmann et al., 2015), and *C. helgolandicus* is an Atlantic species only advected into sub-Arctic regions (i.e., area above the Arctic Circle not influenced by Arctic water masses). The *Calanus* complex above the Arctic Circle is largely dominated by three species: *C. finmarchicus, C. glacialis* and *C. hyperboreus* (Daase et al., 2021) (Table 1), hereafter referred to as the "*Calanus* species" in this thesis. These species have similar life strategies. They are primarily herbivorous and accumulate a large amount of lipid prior to the non-productive period (Falk-Petersen et al., 2009). Period during which *Calanus* spp. overwinter. *Calanus* species perform SVM (see 1.2.2 for more detail) and the overwintering period at depth is often associated with a state of a diapause (Hirche, 1996). This state is characterized by a disruption of the development, a slowing-down of the metabolism, and reduced swimming, feeding and enzymatic activity (Hirche, 1983). This strategy enables *Calanus* species to reduce their energetic cost of metabolic maintenance during overwintering and to preserve energetic reserves for moulting and reproduction after the overwintering period (Bandara et al., 2021). However, it is unresolved in how far *C. finmarchicus* enters a diapause. Some studies have reported non-diapausing population during the overwintering period with individuals continuing to feed and actively swim (Marshall and Orr, 1955; Hirche, 1983; 1996). A shift in dietary preferences from herbivorous to detritivorous and/or carnivorous has also been reported in these non-diapausing populations (Butler et al., 1970; Hirche, 1996).

Table 1: Summary of the origin area of distribution of the Calanus species found above the Arctic Circle

Species	Origin	Abundance above the Arctic Circle	references
Calanus marshallae	Pacific Ocean	Beaufort Sea	Wassman et al., 2015
Calanus helgolandicus	Atlantic	North Atlantic/Norwegian fjord	Choquet et al., 2018
Calanus finmarchicus	North Atlantic	North Atlantic + Amundsen Basin	Choquet et al., 2018
Calanus glacialis	Arctic	Arctic seas shelf + Norwegian fjord	Ershova et al., 2021
Calanus hyperboreus	Arctic	Arctic Ocean + Norwegian fjord	Choquet et al., 2018

The three *Calanus* species have different spatial distributions in the Arctic Ocean and are adapted to different seasonal environment resulting in three different life cycles (Figure 2)

(Falk-Petersen et al., 2009; Choquet et al., 2017) and differences in size. Past predation pressure is also a factor to consider in the evolution of the three distinct life cycle (Berge et al., 2012a). *C. finmarchicus* is a north Atlantic species that is advected into the Arctic Ocean. Its life cycle is adapted to a relatively long and predictable productive period, and it can extend its life cycle to a maximum of one-year. *C. finmarchicus* mainly overwinters at copepodite stage V (CV). During overwintering it moults to adult stage and then ascends in the water column at the end of the overwintering phase. Reproduction in spring is fuelled by the phytoplankton bloom. This strategy is called income breeding. The new generation's growth is also fuelled by the phytoplankton bloom. Under favourable conditions of relatively high temperature and a high phytoplankton availability, *C. finmarchicus* can produce up to 3 generations per productive period (before having to overwinter) (Michaud and Taggart, 2007). *Calanus finmarchicus* is the smallest of the three species due to its life strategy (short generation rather than growth leading to smaller body size). The relatively long food supply period and the relative warm temperature of its native Atlantic Ocean stimulates this short generation strategy. The life cycles of *C. glacialis* and *C. hyperboreus* are adapted to the less predictable and shorter productive periods typical of the Arctic. *Calanus glacialis* is mainly found in Arctic shelf seas and was until recently considered as an Arctic shelf species. However, recent evidence of biogeographical northwards shifts in *C. glacialis* (Ershova et al., 2021a) suggests that the balance between sea ice cover and open water is the factor determining *C. glacialis* distribution rather than depth itself. *Calanus hyperboreus* on the other hand, is considered an Arctic species mainly found in the deep Arctic basins (Falk-Petersen et al., 2009). For both species, the life cycle is longer than in *C. finmarchicus*. *Calanus glacialis* overwinters for the first time as copepodite stage IV (CIV), then ascends in the water column to develop to CV using the phytoplankton bloom before overwintering a second time as CV. After this second overwintering, *C. glacialis* moult to adults, ascend in the water column and reproduce prior to the phytoplankton bloom. Reproduction can be fuelled by energy reserves accumulated prior to the overwintering period; a strategy referred to as capital breeding. The growth, development and lipid accumulation of the new generation is fuelled by the phytoplankton bloom. Under favourable conditions, *C. glacialis* can switch to a one-year life cycle similar to *C. finmarchicus* (Falk-Petersen et al., 2009; Daase et al., 2013). *Calanus glacialis* can take advantage of both the ice algae, which fuels moulting, maturation and reproduction, and the phytoplankton bloom which fuels the new generation growth (Søreide et al., 2010). In the deep Arctic Ocean, the productive period is particularly short and unpredictable due to the dense sea ice cover. *Calanus*

hyperboreus has a multi-year life cycle, 3 to 5 years long (Hirche, 1997). Diapause are performed in stage CIII, CIV and CV. *Calanus hyperboreus* has a capital breeding strategy with a reproduction fuelled by the reserve accumulated during the previous productive period, enabling the new generation to take advantage of the extremely short productive period to fuel the growth (Hirche, 1997; Falk-Petersen et al., 2009; Daase et al., 2013). In *C. hyperboreus*, energy is invested in growth rather than fast turn over because of the unpredictability and shortness of the productive period, resulting in *C. hyperboreus* as the largest of the three *Calanus* species. While an overlap in distributional range and size between these species (particularly *C. glacialis* and *C. finmarchicus*) was known (Choquet et al., 2018), the extent to which *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* can live in sympatry and overlap in size has become more evident as molecular technique to distinguish the *Calanus* species have become more common, reinforcing that these sibling species are difficult to distinguish based on morphology alone (Choquet et al., 2018). However, *C. hyperboreus* possess a unique morphological feature, a spine on the last prosome segment from stage CIV, facilitating the identification of older life stages of this species.

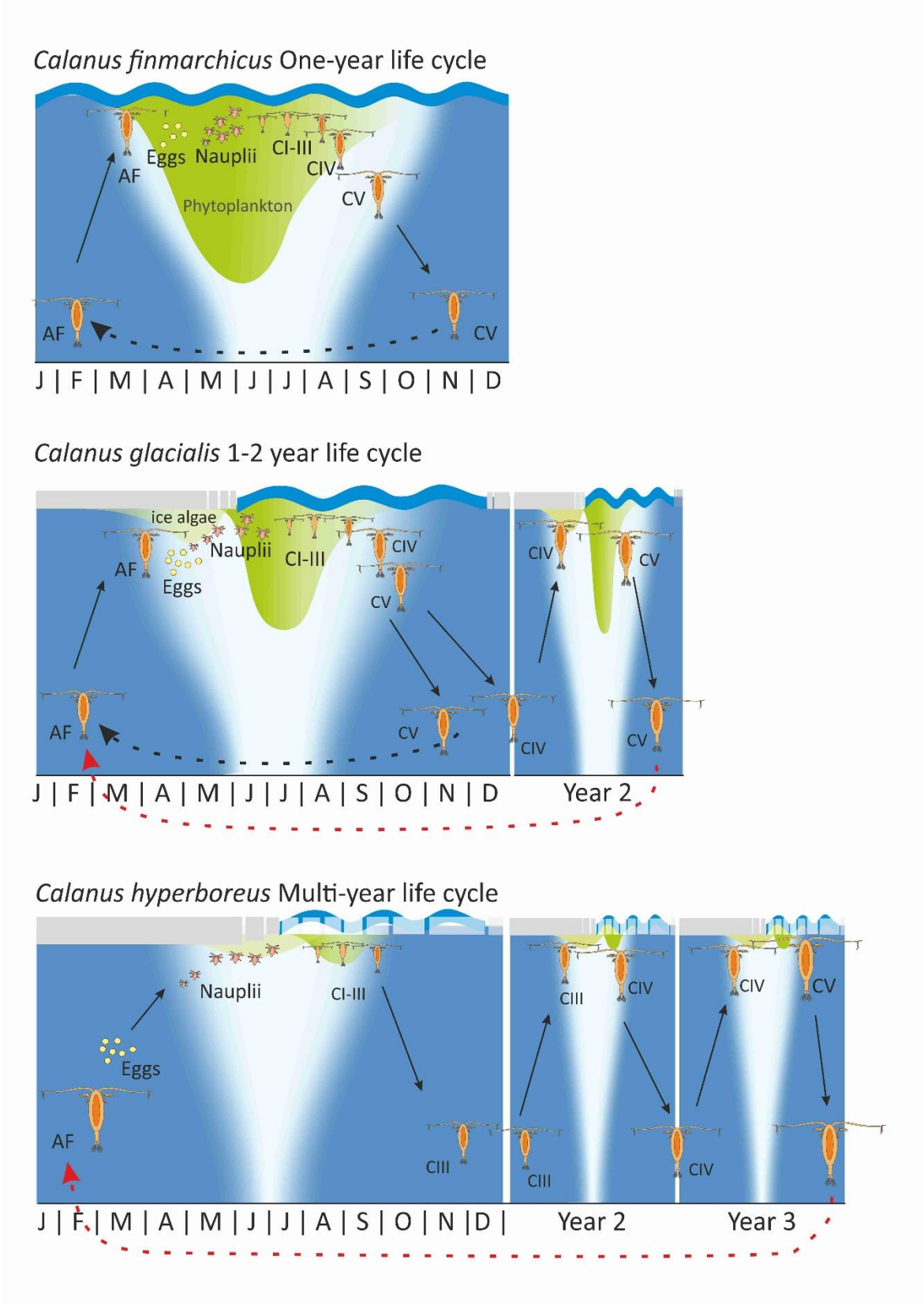

Figure 2: Schematic representation of the life cycle of the three main Calanus species found above the Arctic Circle. The top panel illustrates the one-year life cycle of Calanus finmarchicus. The middle panel illustrates a one to two- year life cycle found in Calanus glacialis. The bottom panel illustrates the multi-year life cycle of Calanus hyperboreus. Illustration Malin Daase (Daase et al., 2021).

1.4 Zooplankton study challenges

Zooplankton seasonality is challenging to study, especially so at high latitudes. First of all, it is a logistical challenge to sample year-round with high temporal resolution in often-extreme environments (Berge et al., 2015). The identification of zooplankton species is also time-consuming and requires taxonomic expertise, which is becoming a rarer skillset among biologists. The presence of a multitude of cryptic species, and difficulties in morphologically identifying even the most common copepod species, such as *Calanus* spp., often result in seasonal studies focusing on only one or few common species, while a large part of the community is neglected such as the meroplankton or small copepods (Walkusz et al., 2009; Gabrielsen et al., 2012; Daase et al., 2013; Weydmann et al., 2013). This limits our ability to document zooplankton biodiversity or species-specific life history strategies. Furthermore, due to their small size and migration patterns, zooplankton behavioural studies are also challenging. In situ observations are limited, and ex-situ studies face the technical challenge of reproducing a complex environment in the laboratory, which is often oversimplified and usually missing a realistic depth component essential for zooplankton behaviour. Methods do exist to solve some of these challenges of traditional net sampling and visual identification. For example, acoustic tools can be used to monitor zooplankton vertical migration. They can operate autonomously and thereby enable a high temporal resolution in remote areas. However, these methods have a low taxonomic resolution, as they record the entire zooplankton community. Molecular methods enable a high taxonomic resolution and are becoming more time and cost efficient, but lack the quantification component and do not provide details on life stages and size spectra.

Ramfjord is located close to Tromsø and its university, which offers the advantage of a relatively easy-to-access ecosystem that is exposed to a high-latitude seasonality. It provides the possibility to investigate the response of the mesozooplankton community to seasonality with a high temporal resolution. This opportunity of high temporal resolution motivated the investigation of zooplankton seasonality with a high degree of details using a diversity of method including traditional and newly developed methods. In this thesis, I investigated seasonal changes in community diversity and structure in the population dynamics and the vertical migration behaviours of *Calanus* spp., as well as in the diel vertical migration of the mesozooplankton community.

1.5 Thesis objectives

The overall objective of my thesis was to describe the seasonality in the mesozooplankton community in a high latitude fjord (Ramfjord) in terms of diversity and behaviour by using a combination of methods that were selected to gain a more complete picture, addressing knowledge gaps such as meroplankton dynamics, seasonal changes in mesozooplankton community and *Calanus* spp. vertical migration behaviour in a shallow high latitude fjord.

The following specific objectives (SO) and research questions (RS) were addressed and summarized in Figure 3:

SO1 To document the biodiversity and seasonal changes in the mesozooplankton community structure in Ramfjord using metabarcoding and taxonomy (Paper I)

- RQ1.1. How do seasonal changes in hydrography and the availability of microalgae affect the species composition?
- RQ1.2. What are the population dynamics of sibling species of common copepod species that are difficult to identify based on morphology alone (i.e., *Calanus* spp. and *Pseudocalanus* spp.)?
- RQ1.3. What advantages does the combination of metabarcoding, and visual taxonomy provide to ecological studies of zooplankton?

SO2 To describe the seasonal changes in the swimming activity of *Calanus finmarchicus* in shallow environment (Paper II)

- RQ2.1. Do *C. finmarchicus* diapause in shallow fjords?
- RQ2.2. Does the behaviour differ between boreal and Arctic locations?

SO3 To identify seasonal changes in diel vertical migration patterns of the mesozooplankton community using an autonomous acoustic zooplankton and fish profiler (AZFP).

- RQ3.1. How is DVM changing seasonally in Ramfjord?

SO4 To define the relation between the swimming behaviour in the laboratory and the DVM behaviour in natural conditions. (Paper III)

- RQ4.1. Are seasonal changes in *Calanus* spp. swimming behaviour reflected in DVM patterns?

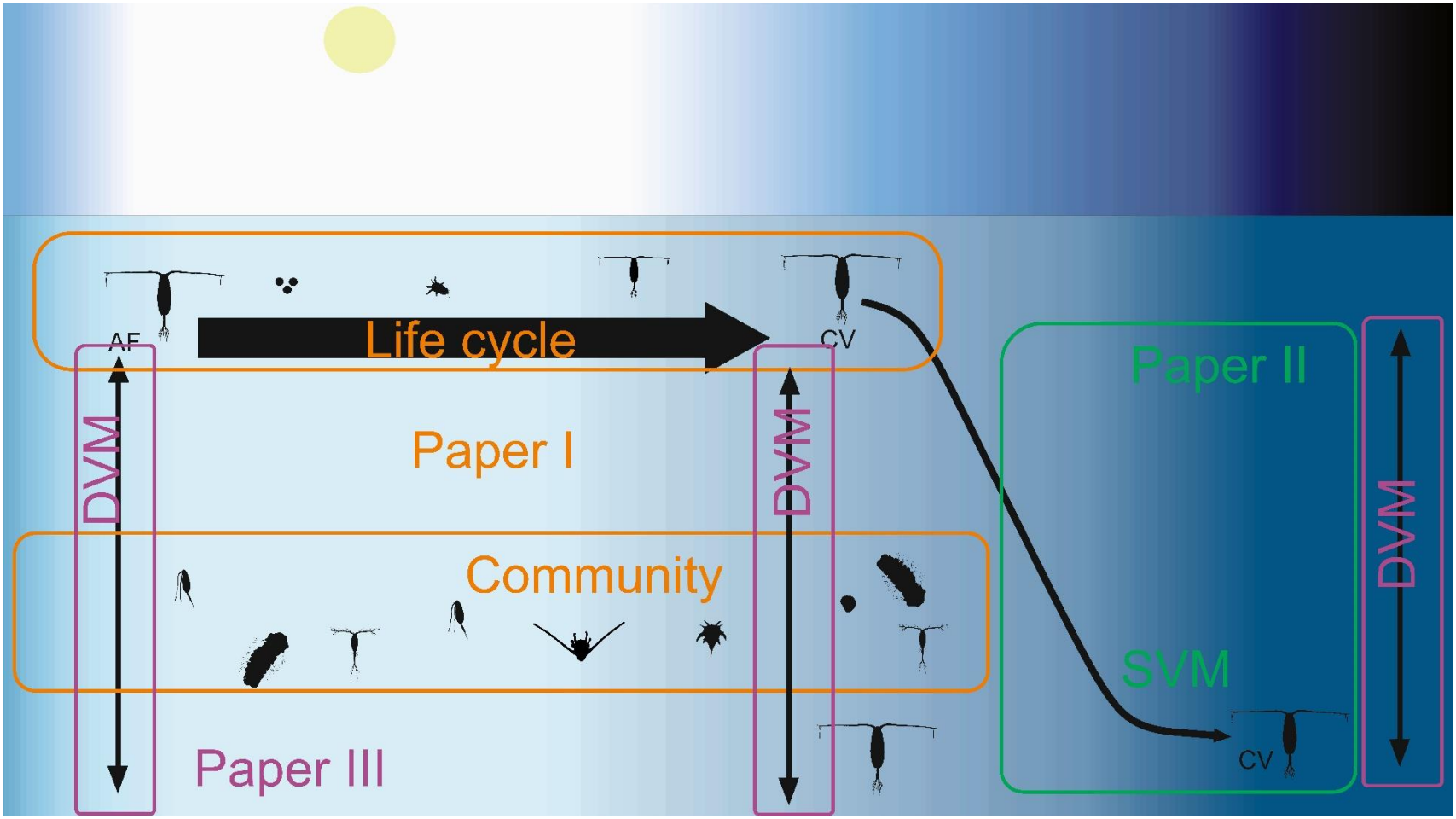

Figure 3: Conceptual presentation of the overall objective of each paper of my thesis. The orange boxes highlight the objectives of Paper I (defined Calanus *spp. population dynamics and the community composition), the green box highlight the objective of paper II (described the SVM behaviour of* Calanus*) and the purple boxes highlight the objective of Paper III (described the diel vertical migration of the mesozooplankton community).*

2 Methods

2.1 Study area

The work of this thesis was primarily conducted in Ramfjord, a northern Norwegian fjord located above the Arctic Circle close to the city of Tromsø (Figure 4). Ramfjord is a shallow fjord with two basins separated by a sill. The inner basin is the shallowest with a maximum depth of 55 m and is ice covered in winter, while the outer basin (the location of my sampling station) has a maximum depth of 130 m and is free of ice year-round. Ramfjord is exposed to an Arctic light regime, characterized by a period of polar night, from the 23rd of November to the 21st of January, and a period of midnight sun from the 19th of May to the 25th of July. While Ramfjord is considered one of the coldest fjords in Northern Norway with an average annual temperature of about 5°C (Oug and Høisœter, 2000), the fjord is not influenced by Arctic water masses and is therefore regarded as a sub-Arctic fjord.

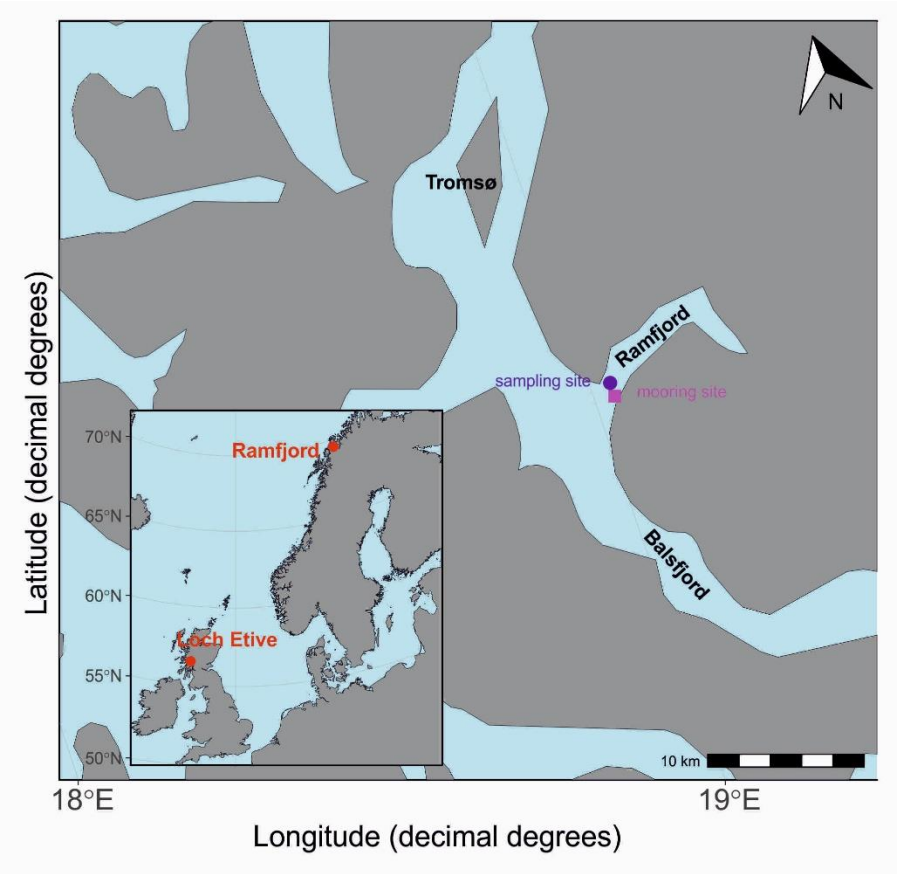

Figure 4:Map of the sampling stations used in the thesis, with a north European map in the bottom left corner to localized Ramfjord compared to Loch Etive, and a more detailed map of Ramfjord with the sampling station and the mooring site.

2.2 Sampling

The sampling station (69°31'49.9N, 19°02'11.9E) was established in the outer basin close to the fjord opening, at a depth of 125 m. Sampling was conducted monthly between November 2018 and December 2019 (Paper I, Table 1). During each sampling event, temperature, salinity, and fluorescence profiles were collected with a conductivity, temperature, and depth profiler (CTD, SBE 19 plus, seabird Electronics SBE, Bellevue, USA). Water samples were collected at 5, 10 and 15 m with a 5 L Niskin bottle (KC Denmark, Silkeborg, Denmark), to measure chlorophyll *a* concentration and identify the taxonomical composition of the phytoplankton community. Zooplankton were sampled with a WP2 net (Hydrobios, Kiel, Germany) with a maximum mesh size of 180 µm (Paper I, Table 1). Three types of samples were taken at each sampling event:

- Vertical net hauls of the entire water column, which were immediately preserved with 96 % ethanol for metabarcoding analysis (Paper I).
- Discrete vertical net hauls from 115 to 50 m (the deep layer) and 50 to 0 m (the shallow layer). These samples were fixed in a 4 % formaldehyde-sea water solution and used for taxonomic identification and species abundance determination of the mesozooplankton community (Paper I), as well as to measure the mesozooplankton biomass (Dry weight (DW)) (Paper I).
- A tow of the deep layer which was kept alive at ambient water temperature and kept in the dark while being transported to the lab to be used for the swimming activity experiment on *Calanus* spp. (Paper II and III).

2.3 Underwater observatory

In parallel, a moored underwater observatory (Figure 5) was deployed in the proximity of the sampling station (69°32.005'N, 19°02.904'E) (Figure 4), between the 18th of March 2019 and the 11th of June 2020. This observatory was equipped with a CTD (seabird Electronics SBE 16) at 18 m which continuously measured temperature, salinity and fluorescence at hourly intervals. In addition, temperature loggers (Seabird Electronics, SBE, Bellevue, USA) were placed every 10 to 15 m along the chain, measuring temperature every 12 min and computing an hourly average. To monitor vertical migration in the zooplankton community (Paper III), the

observatory had an upward-looking Acoustic Zooplankton and Fish profiler, (AZFP) (ASL Environmental Science, Victoria, Canada) equipped with a 125, 200 and 455 kHz transducer at a depth of 106m, emitting a ping every 20 s, (Paper III).

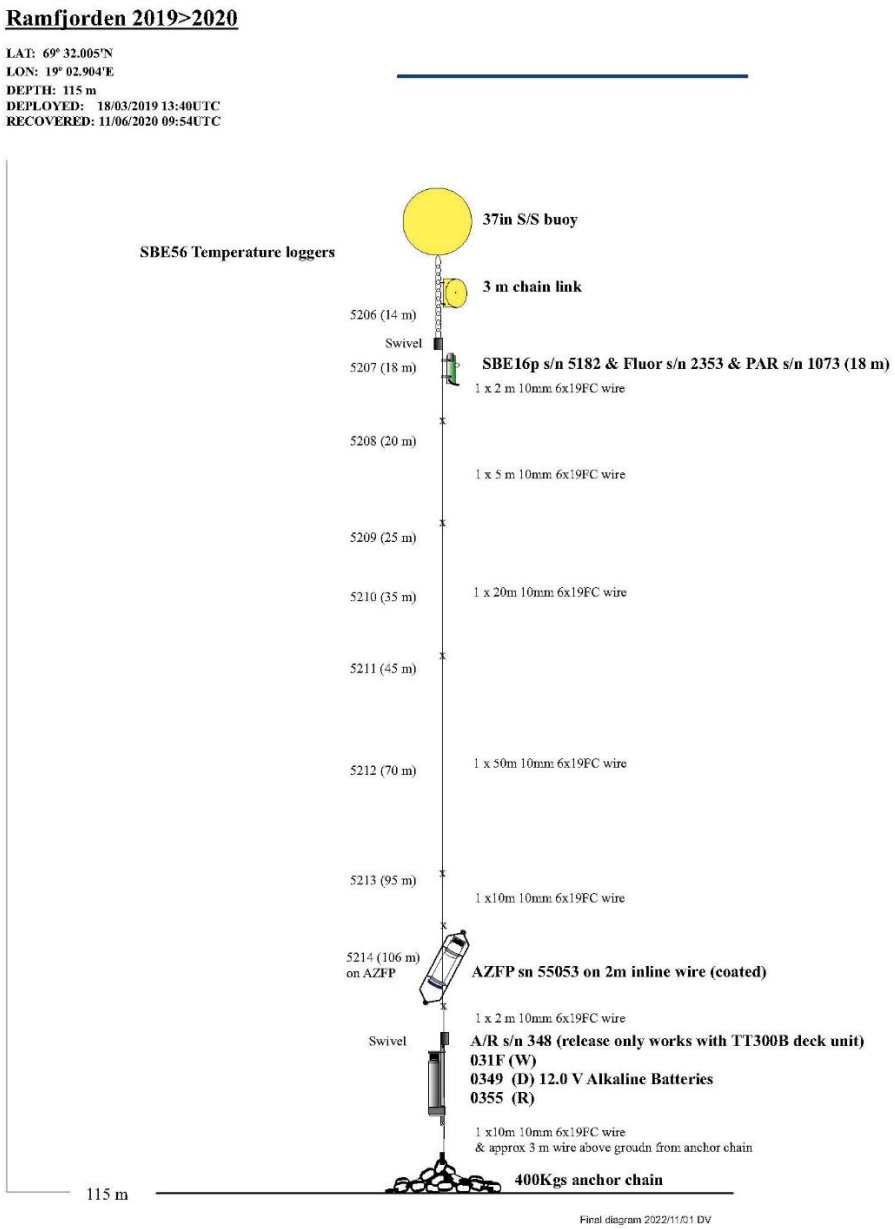

Figure 5: Diagram of the underwater observatory deployed in Ramfjord. Illustration Daniel Vogedes.

2.4 Swimming activity experimentation

Swimming activity experiments were run after each sampling event, for 3 to 8 days (Paper II, Table 1). Individuals of *Calanus* spp. (mostly of copepodite stage V (CV) and adults) were carefully picked and placed in transparent acrylic tubes with filtered sea water in order to monitor their swimming activity with a modified Locomotor Activity Monitor (LAM 10, TriKinetics, Waltham, USA) (Box 1). A maximum of four monitors were used with at least 16 of the 32 chambers filled with individuals from the depth layer of interest (Paper II Table 1). The monitors were kept in a temperature-controlled room under constant temperature and a light/ dark cycle similar to the natural environmental conditions at the time of sampling (Table 1 Paper II). At the end of each experiment, individuals were photographed using a Leica stereomicroscope fitted with a camera, and the pictures were used to confirm the developmental stage and to measure the prosome length, as well as the lipid sac and prosome area of each individual. From these measurements, the Lipid sac Area to Prosome Area ratio (LAPA) was calculated for each individual as an index of lipid fulness. Individuals were individually frozen (-80 °C) to run molecular analysis in order to discriminate *Calanus finmarchicus* from *Calanus glacialis*, both present in Ramfjord and overlapping in size. For Paper II, I used only the swimming activity data of individual CVs of *C. finmarchicus*. The G-150 marker of the Insertion-Deletion method (InDels) (Smolina et al., 2014; Choquet, 2017) does not identify *C. helgolandicus*, which can sometimes be confused for *C. finmarchicus*. While a slight contamination by *C. helgolandicus* is therefore possible. It can be considered as negligible due to the low abundance of *C. helgolandicus* in Ramfjord (Paper I). In paper III, I used the results from the same experiments but included individuals of all stages (mainly CV and Adult stage) and all *Calanus* species (*C. finmarchicus*, *C. glacialis*, and molecularly unidentified) in the analysis.

For paper II, in order to get a latitudinal comparison of the swimming behaviour of *C. finmarchicus*, the swimming activity data set was complemented with swimming activity data of *C. finmarchicus* from Loch Etive, Scotland (Figure 4). Similar to Ramfjord, Loch Etive is a shallow fjord with a maximum depth of 145 m encountered at the sampling station (56°45'N, 5°18W). The experiments were performed at the Scottish Association for Marine Science in Oban between August 2017 and March 2018 under local environmental conditions following a similar protocol as the one applied to the Ramfjord population. However, in Loch Etive the

Calanus community consists exclusively of *C. finmarchicus* (Choquet et al., 2017), making molecular analysis unnecessary.

Paper II focused on the period when *C. finmarchicus* is assumed to be at overwintering depth based on *Calanus* spp. population dynamics (Paper I). However, in order to account for the physiological change in *C. finmarchicus* during this period, the overwintering period has been divided into three phases: the early overwintering when part of the population is assumed to be at overwintering depth; the mid-overwintering when the entire population is assumed to be at overwintering depth; and the late overwintering when only part of the population is assumed to have ascended in the water column (i.e., exit overwinter). Those phases are equivalent to the initiation, induction and termination phases described by Hirche (1996).

Box 1: How to monitor swimming activity

Swimming activity of copepods is monitored with Locomotor Activity Monitors (LAM) originally designed to monitor flying activity in flies. The monitors are used horizontally instead of vertically. Each monitor has 32 chambers equipped with an infrared beam (IRB) in its middle (Figure B 1). A transparent acrylic tube filled with filtered sea water and occupied by a single copepod is placed in each chamber.

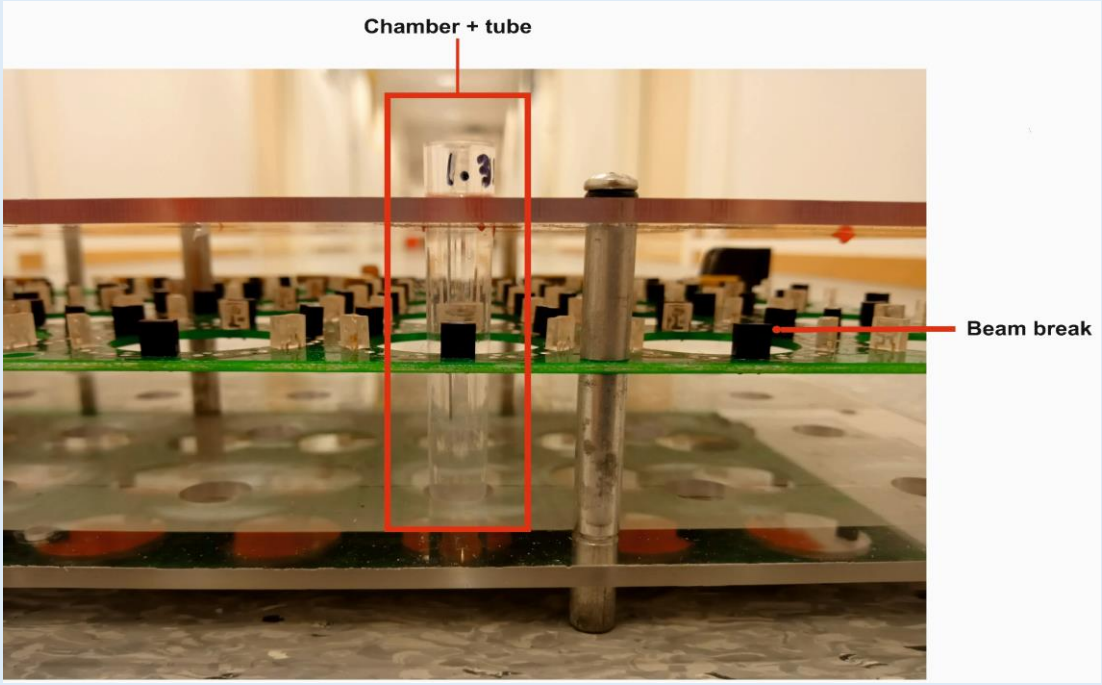

Figure B 1: Photo of the locomotor activity monitor (LAM) used to monitor Calanus spp. swimming activity. In the picture a chamber with an acrylic tube is highlighted as well as the position of the IRB in an empty chamber

Each time the copepod in the tube crosses the IRB, the beam is broken. The computer counts the number of times the beam is broken per 30 s period. During data processing, the number of beam breaks is summed up over 30 min. This parameter is called the activity intensity (breaks/ 30 min). From the activity intensity, the activity level can be obtained by averaging the activity intensity over the entire experiment. Those variables are obtained for each individual.

In order to visualize the change of swimming activity within a day, the activity intensity is averaged for each 30 min interval over the first three days of experiment. The 48 activity intensity values obtained this way represent the daily swimming activity intensity (Figure B 1Figure B 2). To identify the dominant pattern in daily swimming activity intensity in the population, it is averaged for all the studied individuals. Periodicity can then be investigated within those data.

Box 1: How to monitor swimming activity (continued)

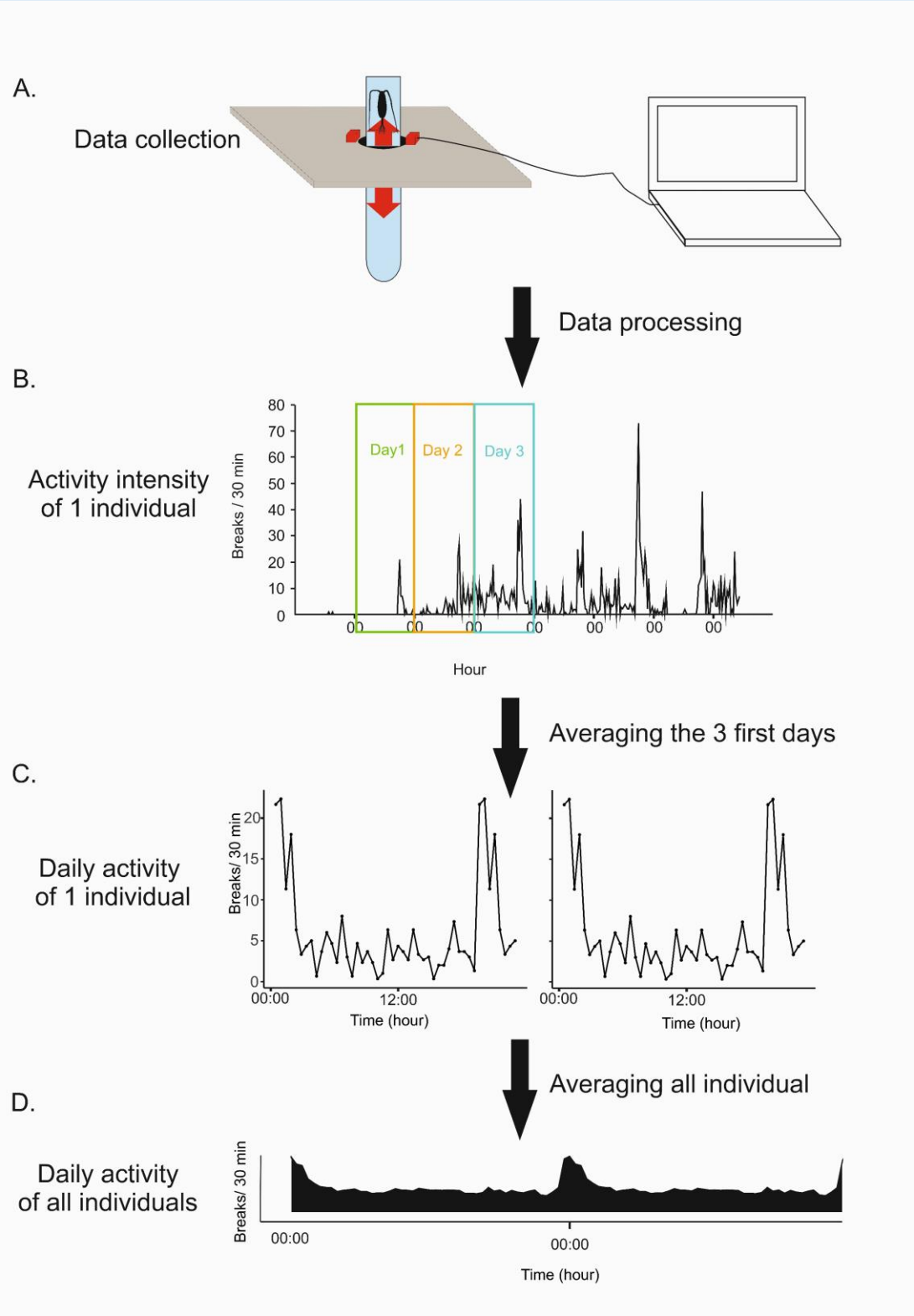

Figure B 2: Schematic illustration of the LAM method from the data collection to the obtention of a double actogram of the

2.5 Data analysis and statistic

For paper I, I combined the mesozooplankton biomass measurements with the proportion of sequence reads of each species acquired from the metabarcoding analysis to obtain biomass-weighted sequence reads (BWSR) as a proxy of each species biomass (Ershova et al., 2021b). The abundance of each sibling species of *Calanus* and *Pseudocalanus* complex was estimated using the genus abundance, and the targeted species proportion in the genus complex calculated based on the number of sequences read of each species. The abundances data (measured and estimated) were used to find seasonal pattern in community composition by applying a hierarchical cluster analysis and a Canonical Correspondence Analysis (CCA).

For papers II and III, the periodicity within the experimental and acoustical data sets was determined. To do so, *C. finmarchicus* CV swimming activity intensity (Paper II), the total *Calanus* spp. swimming activity, and the backscatter volume strength (S_v) at 102m (Paper III) were analysed using Lomb-Scargle periodogram analysis (VanderPlas, 2018) to identify circadian rhythms within the data as well as the period and the strength (PNmax) of those rhythms. To analyse the rhythms in more detail, a Rayleigh test was performed on the daily activity data set of each experiment to define if the data were uniformly distributed over 24 h. None of the data was uniformly distributed (p-value < 0.05), i.e., there was a peak in activity, so the mean resultant (ρ), i.e., the intensity of the activity, and the mean angle (μ), i. e., the time around which the peak of activity is centered, were determined.

For paper II, non-parametric univariate analyses were performed to compare our variables (prosome length, LAPA, Total Lipid, rhythm strength and activity level) between locations and overwintering phases.

For paper III, the S_v retrieved from the AZFP measurements were processed in Echoview (version 12.1.50). Several filters were applied to the S_v data to remove the noise generated by the method itself, such as background noise, transient noise, impulse noise, and attenuating signals, following recommendations by (Ryan et al., 2015). The data were visualized as an echogram and visually inspected. Signals generated by external factors, such as the introduction of air bubbles in the water column due to storms or a boat propeller, were removed manually. The data were then integrated over 20 min and 4 m cells. The data from 102 m depth (the deeper layer) were plotted as a double actogram (Box 2) to identify periods of synchronized diel vertical migration.

Box 2: Active acoustic data processing

Active acoustics is a method used to monitor the position of marine organisms in the water column. A transducer emits a sound, i. e., ping, at a chosen frequency and at a regular time interval. The frequency is chosen in relation to the targeted organisms. Higher frequencies enable the detection of smaller organisms, lower frequencies detect larger organisms. The ping travels through the water column and, when it encounters an obstacle (such as the sea bed, a fish or a swarm of zooplankton), it is scattered back to the transducer, which has switched to a "listening" mode. The backscattering signal is dependent on the nature of the obstacle, i. e., its shape, its size, its density, and the composition of its tissues (e.g., mostly composed of water [i.e., gelatinous taxa], filled with air [i.e., swim bladders] or having a hard exoskeleton). Scattering signal is also dependant on how long it takes to return to instrument hence provides a depth resolved dataset.

The backscattered signal is then processed (see Simmonds and MacLennan (2008) for more details) and the volume backscatter strength (S_v in dB re 1 m-1), a negative value indicating how much of the initial signal was backscattered, is extracted. These values can be averaged for a finite volume and are called mean volume backscattering strength (MVBS), often simplified to S_v, which is the case in this thesis. High S_v values indicate a high density of obstacles, i.e., a high level of backscattered acoustic signal.

This S_v value can be plotted as a function of depth and time to obtain a pixelized picture of the backscatter in the water column over time, called an echogram. When studying the vertical migration of zooplankton over time, both small-scale temporal resolution (to visualize change of vertical position within a day) and large-scale temporal resolution (to visualize change over season) are needed. It is not possible to visualize both scales simultaneously in echograms. To solve this problem, the data can be plotted as an actogram, a method commonly used in chronobiological research. This method was first used for acoustic data by Last et al.(2016) and is illustrated in Figure B 3. An actogram is constructed by plotting the S_v data for a single depth layer. The data are sorted into 24 h periods, and stacked vertically. Each row of an actogram is a day, and each column is a point in time. Often actograms are displayed doubled, which is the same day is plotted twice on the same row, in order to have a better visualization of the behaviour during the transition, i.e., around midnight.

Box 2: active acoustic data processing (continue)

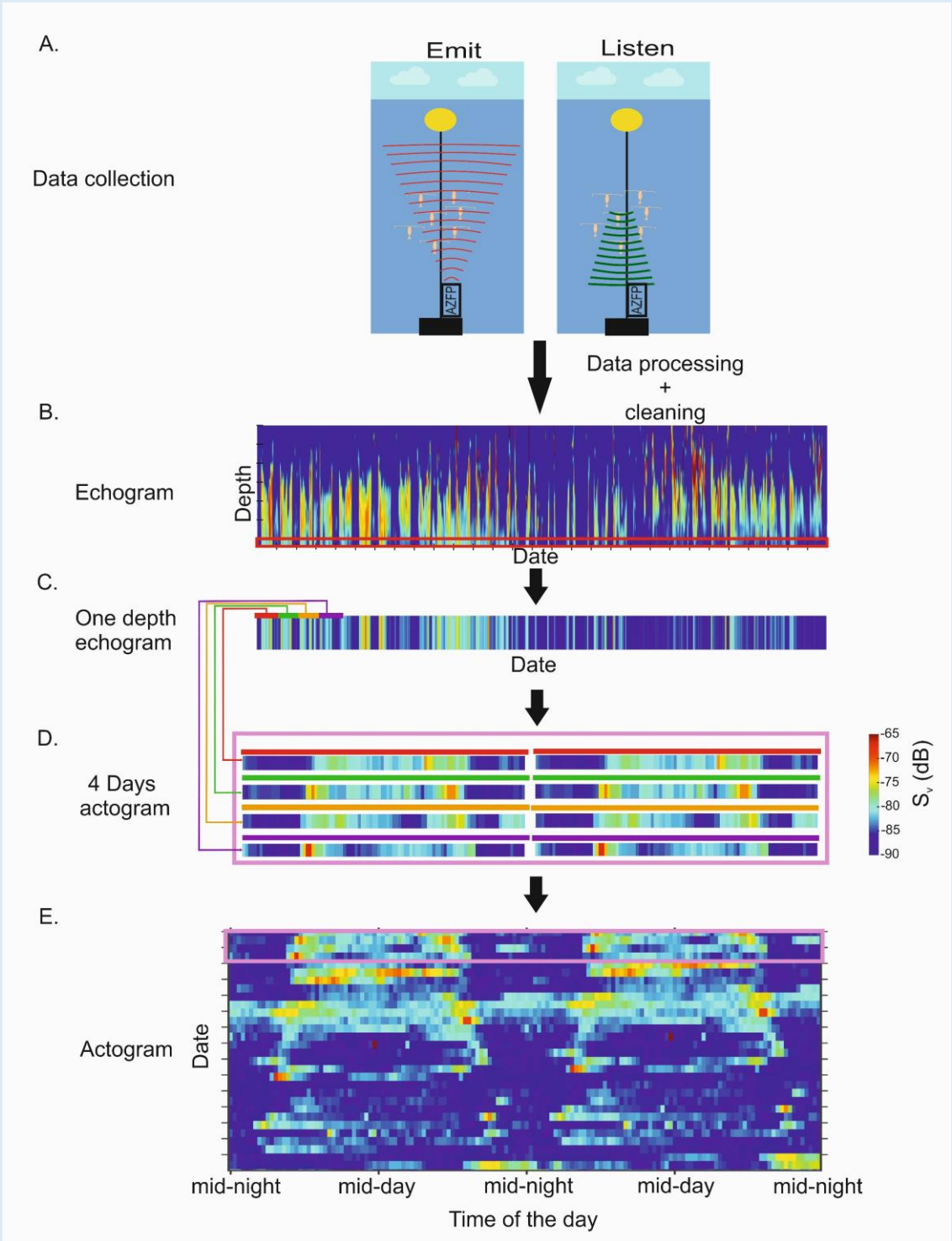

Figure B 3: Conceptual schema of the steps to get an actogram based on acoustic backscatter data. Panel A outline how the acoustic data is acquired. Panel B show an echogram (i.e., time vs depth) of one month of data where a DVM pattern can be observed. From this echogram the data from one depth (C.) are extracted and separated into days and then stacked on top of each other and doubled (Panel D) Once assembled, it forms an actogram (Panel E). The figures of this conceptual schema are based on the data collected for the thesis, but for a better visualisation of the different step only one month (April 2019) is shown here.

3 Results

3.1 Mesozooplankton community structure (Paper I)

In Ramfjord, the mesozooplankton community was very diverse, with 154 taxa identified over the study period. Meroplankton was the most diverse group with 90 taxa of which 46 were polychaeta. Copepod was the second group in terms of diversity with 27 taxa of copepod identified over the study period. Small copepods (prosome length of adult stages < 1.5 mm) largely dominated the mesozooplankton community in terms of abundance and biomass. *Oithona similis* and *Microcalanus pusillus* were dominating in terms of abundance year-round. *Pseudocalanus* spp. was dominating in terms of biomass year-round. Four species of *Pseudocalanus* were identified: (1) *P. acuspes*, the dominant species, which was present year-round and represented the highest proportion of biomass; (2) *P. elongatus* was also present year-round but with a low biomass; (3) *P. minutus;* and (4) *P. moultoni* two species only present in Autumn and winter with a relatively high biomass (Paper I, Figure 4). Large copepods (prosome length at adult stages > 1.5 mm) were present year-round and mainly represented by the *Calanus* genus. *Oithona similis* and *Pseudocalanus* spp. were mainly found in the surface water, while *M. pusillus* and *Calanus* spp. were found mostly in the deep layer at the time of sampling (around mid-day) (Figure 6).

Based on the mesozooplankton community and the environmental conditions four seasons were defined:

- The spring bloom season (SB in Paper I) in April, characterized by a cold (4°C) homogenously mixed water column, a pronounced phytoplankton bloom, high abundance of meroplankton (particularly Cirripedia nauplii, decapod larvae and Polychaeta larvae), which were present year-round but also more abundant during the spring bloom season as well as juvenile stage of euphausiid.
- The summer season (SS in Paper I) between May and September, characterized by warming of surface water, reaching a maximum of 12°C, while at the bottom the water remained around 4°C. This season was also characterized by an increase in abundance of *Calanus glacialis* and *Calanus hyperboreus*, the appearance of Echinodermata larvae between June and July, and Bivalvia larvae in August and September. During this season *M. pusillus* abundance increased considerably.

- The autumn season (AW in Paper I) from September to January, characterized by decreasing water temperatures, leading to a homogenously mixed water column with temperatures around 5°C in January. This season was also characterized by a steep increase in the abundance of small copepods such as *O. similis*, *Acartia longiremis*, and *Pseudocalanus* spp., a relatively high abundance of *Metridia longa*, *Paraeuchaeta norvegica*, gastropod larvae and Chaetognatha. This season was also marked by the occurrence of boreal/Atlantic species such as *C. helgolandicus*, *Pseudocalanus elongatus*, *Pseudocalanus minutus*, *Temora longicornis* and *Themisto abyssorum* and *Evadne normanni* indicating an influx of Atlantic water in the fjord. The presence of those species was limited to the autumn season. An autumn phytoplankton bloom occurred in late September and October.
- Winter (PS in Paper I) between February and March, characterized by a cold homogenously mixed water column, an overall low abundance of zooplankton, a high abundance of copepod nauplii, and a relatively high contribution of nudibranch larvae and *Triconia* sp.

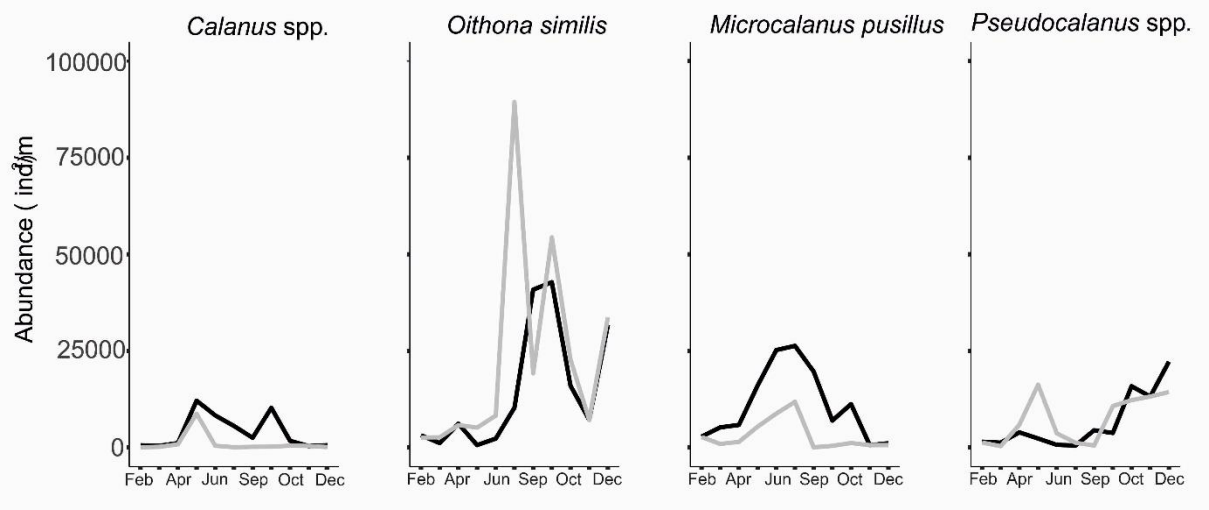

Figure 6: Seasonal changes in abundance of the most abundant species of copepods in Ramfjord. The light grey line is the abundance in the surface layer (50-0 m) and the black line shows the abundance in the deep layer (115-50 m).

3.2 Mesozooplankton community diel vertical migration behaviour

The active acoustics showed evidence of DVM throughout the study at all three frequencies (Paper III and Figure 7). Three patterns were observed:

- ➔ classical DVM, a migration from the deep-water during day time to the surface water during night time, was observed in spring (April).
- ➔ asynchronous DVM was observed in summer (from May to July).
- ➔ Multiple DVM behaviour with classical DVM behaviour and small scale DVM at depth (August-December), with the time spent in the upper layer changing proportionally to the length of the night.

The change of DVM behaviour corresponded well with the seasonal change in light, highlighting the role played by light in DVM behaviour.

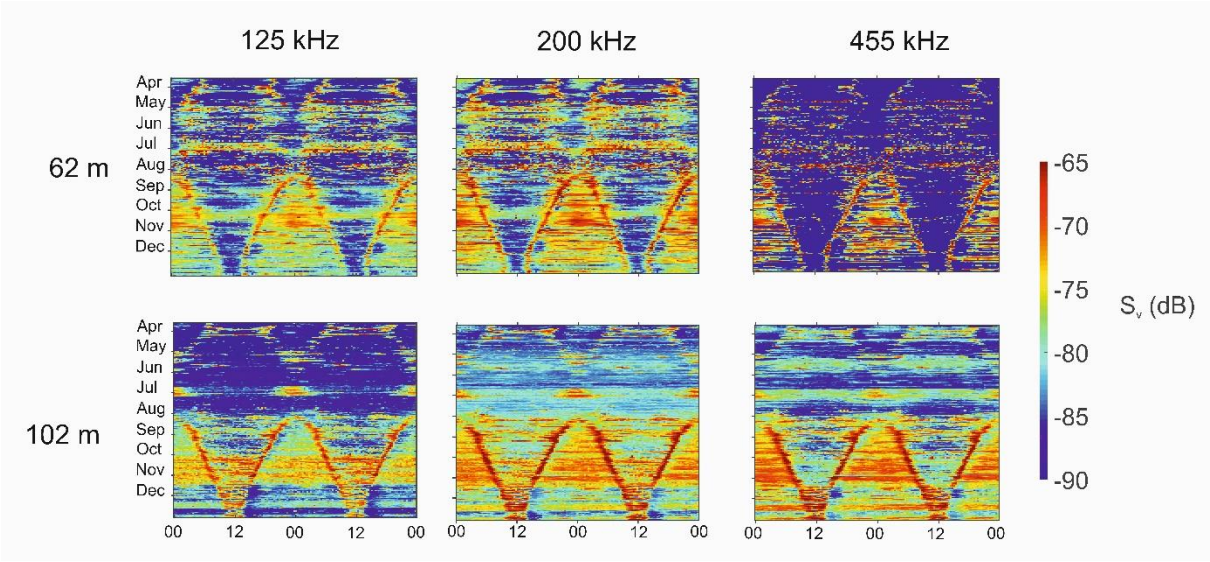

Figure 7: Actograms presenting acoustic backscatter from 62 m (top) and 102 m (bottom) at 125 kHz (left), 200 kHz (middle) and 455 kHz (right). The same patterns of DVM behaviour are observed at each frequency.

The seasonal changes in swimming activity of *Calanus spp*. observed during the lab experiments (Paper III) corresponded systematically with the seasonal changes in DVM behaviour based on the acoustic analysis (Paper III, Figure 2). The seasonal variation in rhythm strength, indicating the synchronicity between organisms, was similar for the swimming activity and the acoustic data. These shared characteristics between the laboratory behaviour and the behaviour observed in the natural environment, demonstrate that the swimming behaviour under artificial conditions responds to the same mechanisms as DVM behaviour, and can consequently be use as a proxy of DVM behaviour.

3.3 Calanus life cycle and overwintering behaviour (Paper I and Paper II)

In Ramfjord, the *Calanus* complex is composed of four species. *Calanus finmarchicus* and *C. glacialis* are present year-round and largely dominate this genus complex, while *C. helgolandicus* and *C. hyperboreus* were only occasionally observed in low abundance. The developmental stage composition indicates that the main reproductive period of *Calanus* spp. was between January and March, prior to the spring bloom. A second reproductive event was detected in autumn around the autumn bloom. The seasonal variation in the prosome length distribution of CVs, and particularly the decrease in mean prosome length over the mid-overwinter period in *C. finmarchicus*, indicates that this second reproductive event was likely due to reproduction in *C. finmarchicus*, and not *C. glacialis*, which did not vary in size over the study period. Based on all the data in this thesis, I suggest that the overwintering period in *Calanus* spp. in Ramfjord was between August and December. In Ramfjord, *C. finmarchicus* remains active during this period and enters a winter resting phase, rather than a diapause as usually encountered in *C. finmarchicus* populations inhabiting deeper environment (Hirche, 1996). The environmental conditions in Ramfjord (temperature, extreme photoperiod and short productive period) caused lower lipid accumulation and consequently lower activity levels than in more favourable environments, such as Loch Etive.

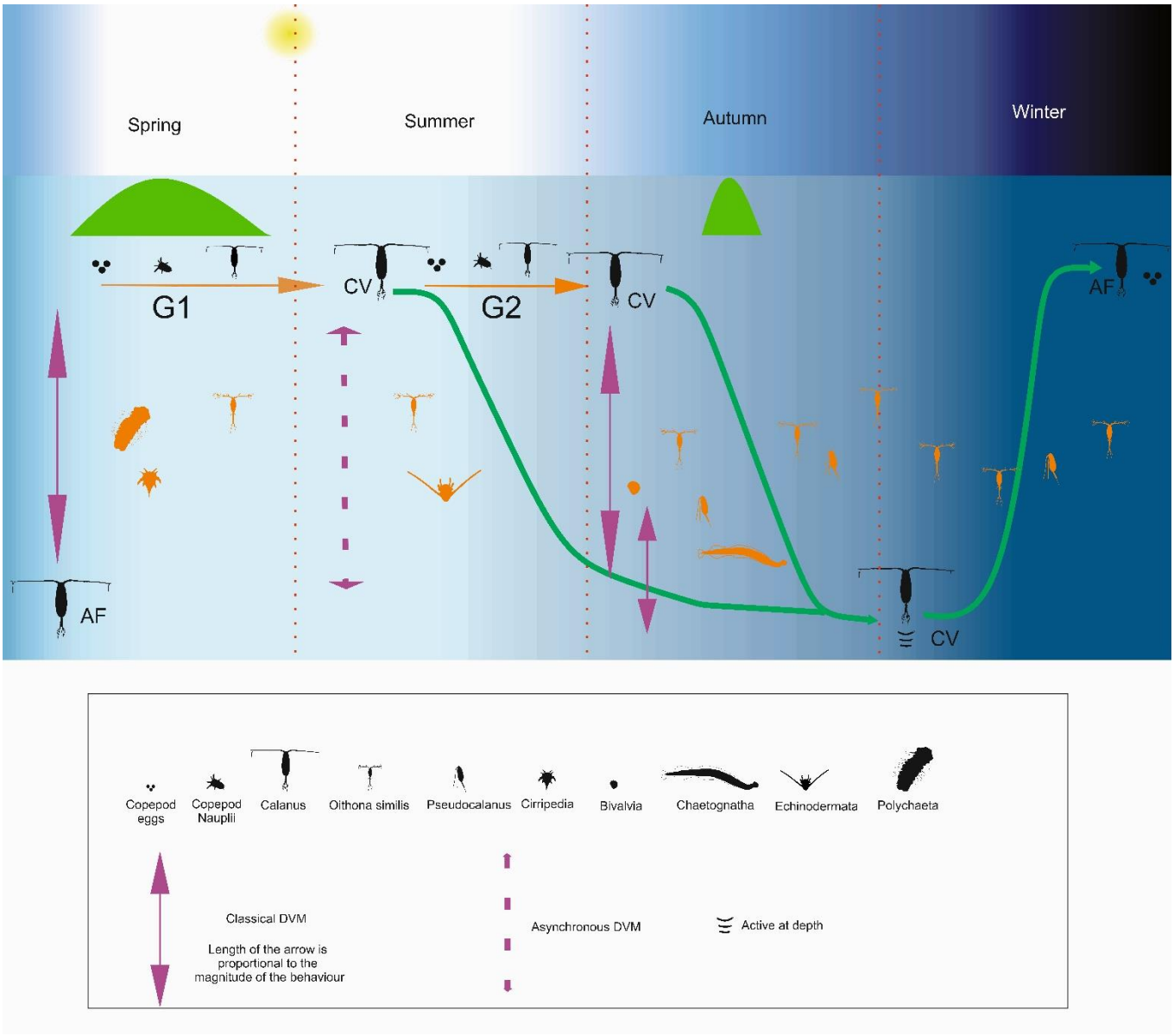

Figure 8: Conceptual drawing summarising seasonality in the mesozooplankton community in Ramfjord. The orange organisms show the community composition, and the number of those organisms indicates changes in their abundance throughout the study period. The orange arrows indicate Calanus spp. life cycle. The green arrows illustrate seasonal vertical migration of Calanus spp. The purple arrow illustrates diel vertical migration behaviour of mesozooplankton. The colour code of the arrow refers back to the colour code used for the different objectives illustrated in Figure 3

4 Discussion

4.1 A new methodological approach to zooplankton seasonality

Most mesozooplankton studies from high latitudes have focused on the larger members of the mesozooplankton community (in particular the calanoid copepods) (Pasternak et al., 2008). However the more prominent small copepods (e.g., *O. similis, Microcalanus pusillus, Pseudocalanus* spp...), and to some extent meroplankton also got some attention (Pasternak et al., 2008; Svensen et al., 2011; Michelsen et al., 2017; Descôteaux, 2022). The dominance of *Calanus* species in terms of biomass at high latitudes, associated to the difficulties in collecting and identifying small organisms properly, explains the low attention paid to the smaller mesozooplankton fraction (Pasternak et al., 2008; Silberberger et al., 2021). However, to fully understand ecosystems functioning, it is essential to both examine the ecological significance of the small mesozooplankton as well as integrate all components of the zooplankton community simultaneously. By using a number of different methods and especially by implementing newly developed methods (i.e., metabarcoding as a quantification method and the monitoring of copepods swimming activity with an infrared beam) that are more time and cost efficient, this thesis provides one of the most complete pictures of the mesozooplankton seasonality at high latitudes, exploring simultaneously several aspects of zooplankton seasonality, such as diversity, community structure, populations dynamics, and behaviour.

4.1.1 Metabarcoding

Metabarcoding has become a well-established method to detect the hidden diversity in zooplankton community (Lindeque et al., 2013; Bucklin et al., 2016). Using metabarcoding an exhaustive list of species was established for Ramfjord, and the seasonal changes in species composition were revealed (Paper I). The addition of this method by far extended our insight into the zooplankton diversity compared to what would have been possible using only morphological identification, a technique that is particularly time-consuming and requires a high level of taxonomical knowledge. Often, only a few well-known and distinct species can be identified to high taxonomic level, while cryptic and sibling species can only be identified to lower taxonomic levels. However, visual identification enables the identification of the life stage of specific species as well as measurements of the sizes of single individuals and, more

importantly, quantifying each species in terms of abundance as well as species specific biomass. Abundance and biomass quantification is essential in zooplankton investigations, as it determines the community structure. The definition of stage composition allows for population dynamics determination. Metabarcoding does not offer such possibilities, as the sample is ground when prepared for metabarcoding and becomes unusable for other analysis. The absence of quantification in any way is a major disadvantage of molecular methods, such as metabarcoding. If this shortcoming could be resolved, metabarcoding would become an even more valuable method as it would make zooplankton studies more time and cost efficient as well as also more detailed and precise than currently possible (Silberberger et al., 2021). Ershova (2021b) designed a method to estimate the biomass of each identified species based on the proportion of sequence reads and the dry weight of a replicate sample. In paper I, I applied this method for the first time in an ecological study in combination to morphological identification. The species-specific estimation of the biomass with a molecular tool is a non-negligible progress in the zooplankton ecology field. This method revealed the domination of the small copepods year-round in terms of biomass, a high biomass of meroplankton during the spring bloom, and the dynamics between sibling species such as *Pseudocalanus* and *Calanus* species. However, my study also revealed some shortcomings in the application of Ershova et al., 2021 that need to be resolved to make this method a true alternative for future studies. I found that the estimation of biomass of the largest species, such as *Calanus hyperboreus*, in particular did not correspond between the molecular method and the visual observation. Bases on the BWSR estimation, *C. hyperboreus* appeared as the dominant *Calanus* species in August, but only three individuals of older copepodite stages and one young stage were enumerated. Overall, I only observed 16 *C. hyperboreus* individuals in the entire sample, a relatively low number compared to the other *Calanus* species with an estimation of 1400 individuals in the sample. This suggests an overestimation of the *C. hyperboreus* population by the metabarcoding method or an underestimation with the visual identification method. A misidentification of *C. hyperboreus* in favour of the other species could have resulted in a such underestimation of the *C. hyperboreus* population, but this seems unlikely as the dominant developmental stages of *Calanus* at that time were CIV and CV, stages easily identified as *C. hyperboreus* due to a spine on the last segment of the prosome. I assumed that the large size of *C. hyperboreus* led to a higher proportion of DNA within the samples and consequently to an overestimation of *C. hyperboreus* using metabarcoding and BWSR. An improvement in the detection of some species is also necessary, especially for the detection of bivalves, which were

barely detected with metabarcoding but were identified visually. The issue has previously been reported by Pereira et al.,(2011). The inability of the metabarcoding to detect an entire class of meroplankton is a major disadvantage in the description of the mesozooplankton community. Finally, this method would benefit from a widely utilisation to improve the protocol efficiency for zooplankton. Indeed, in Paper I, the change of DNA extraction kit has impacted substantially the efficiency of species richness determination. Furthermore, molecular identification, such as metabarcoding, always benefits from the increase of sequence indexing in the gene banks by the worldwide scientific community.

4.1.2 Swimming activity

Using LAMs to monitor the behaviour has advantages over other methods commonly used, such as complex video set-up often involving infrared lamp or camera (Mahjoub et al., 2011; Miljeteig et al., 2014; Båtnes et al., 2015). In comparison to these methods, the LAM set up is relatively simple and the data processing is less time-consuming. The simplicity of the LAM method enables an increase of the number of individuals studied, and thus the statistical significance of the data. This simplicity, also make this method more reproductible than other systems relaying on complex set up which vary with the model. Furthermore, the LAMs are relatively flexible, the number and position of the infrared beams in the chamber can easily be adapted to increase the precision on the position of the copepod in the tube. This method enables many kinds of possibilities of investigations in terms of environmental condition or origin of the organisms. Due to its small size, the LAM, is easily transportable and can be used in both ship or land-based field campaigns to investigate the swimming behaviour of organisms in open ocean or coastal area (Grigor et al., 2022). In this thesis, I confirmed the utility of this method to study *Calanus* spp. overwintering (Paper II) and I also demonstrated the use of the LAM method in the investigation of DVM behaviour (Paper III). While the LAM set up itself worked as intended, the light set up could be improved in future studies. In particular, one should strive to achieve a more realistic light climate in terms of reflecting the light environment at a given water depth more accurately, and by including a gradual transition between light and dark exposure to reproduce dusk and dawn. A period of constant darkness prior to the light/dark cycle exposure could also improve the interpretation of the behaviour by measuring the direct response to light and bypassing eventual endogenous response. Nevertheless, the LAM method

can be regarded as a reliable method to monitor swimming activity of copepods under laboratory conditions.

4.1.3 Active acoustic

Active acoustics have been used extensively to monitor DVM behaviour in all kinds of environments (e.g., (Kaartvedt et al., 2009; Berge et al., 2014; Klevjer et al., 2016; Liu et al., 2022)), especially in remote areas, such as the Arctic. It has proven to provide new insights into seasonal changes of DVM behaviour and its complexity (Cottier et al., 2006; Wallace et al., 2010; Berge et al., 2014). The ability to use it on autonomous platforms allows for measurements with high temporal resolutions, but with a low taxonomic resolution. The similarities between the community DVM behaviour and *Calanus* swimming activity has enabled me to establish *Calanus* DVM behaviour (Paper III), despite the dominance of small copepods and the interference of other species in the acoustic signal, such as krill (Berge et al., 2014). However, a more detailed picture of the complexity of the DVM behaviour of the zooplankton was theoretically possible with the data set used for this thesis. The combination of active acoustic data with net data usually provides a good basis to identify the organisms that may cause the backscatter signal by providing community composition and vertical distribution (Berge et al., 2014; Darnis et al., 2017a). For this thesis, a detailed community composition was available, but only for the mesozooplankton. Larger zooplankton, such as euphausiids and chaetognaths, were not sampled. However, these organisms are considered to be responsible for the majority of the acoustic signal at 200 and 125 kHz frequency, respectively. This thesis would have benefited the sampling of the larger zooplankton community to establish the complexity of the DVM pattern, but also to integrate the prey predator interaction in the interpretation of the data, and particularly *Calanus* behaviour. The absence of the population dynamics of the larger zooplankton is the biggest limitation of this thesis. However, even without net data for the large zooplankton, the DVM behaviour can theoretically be segregated between the chaetognath, the euphausiid, and the copepods using an algorithm defined by Darnis(2017b) but a problem with the calibration of the 455 kHz transducer prevented the use of this method. Despite the high quality of the data set, the interpretation was therefore limited to describe the general trend in the community. This method should be reproduced adding net sampling of larger zooplankton and swimming activity experiments of other copepod species,

such as *Oithona similis* and *Pseudocalanus* spp., in order to characterize the DVM behaviour of the main species of copepods and thus obtain a more detailed view of the complexity of the DVM behaviour in Ramfjord.

4.2 A complex ecosystem

4.2.1 Small copepods dominance

At high latitudes, the zooplankton community is described to be largely dominated by *Calanus* genus in spring and summer in terms of biomass, while the small copepods are usually the most abundant (Arashkevich et al., 2002 4366; Mueter et al., 2009; Søreide et al., 2022). In Ramfjord, the small copepods were dominating in terms of abundance and biomass year-round, and consequently the biomass measured in Ramfjord was overall rather low. At its maximum the biomass in summer in Ramfjord was 2 to 5 times lower than the biomass reported in other high latitudes fjords in summer (Ormańczyk et al., 2017; Hop et al., 2019). Ramfjord is rather shallow, which may explain the low abundance of the *Calanus* population. I showed that during the overwintering period, *Calanus finmarchicus* remains active (Paper II), and that *Calanus spp*. perform DVM on a small scale (Paper III) due to the shallow depth and the continuous exposure to a day/night cycle. *Calanus* spp. life strategy to cope with the strong seasonality at high latitudes, and in particular the non-productive period, is to overwinter at depth in a state of diapause and to accumulate a large amount of lipid to sustain the organisms during this period (Hirche, 1996; Varpe, 2017). However, high mortality has been observed during the polar night, and is attributed to lipid depletion in some of the individuals (Daase et al., 2014; Ejsmond et al., 2018; Daase and Søreide, 2021). I suggest that the winter resting stage adopted by *Calanus* spp. in Ramfjord amplifies the lipid metabolism during this period and leads to an increase in non-predatory mortality in *Calanus* spp. The same strategy was observed in Loch Etive, however more lipid were consumed in Ramfjord than Loch Etive, suggesting that the high latitude environmental conditions (i.e., less food and lower temperature leading to larger body size) probably result in a higher mortality than at boreal latitude. Furthermore, by overwintering in a dark deep environment, *Calanus* spp. reduces the visual predation risk. However, due to the shallow depth, the predatory mortality is possibly higher in Ramfjord than in deeper environments. This mortality during the non-productive period may explain the low biomass of *Calanus*. The high dominance of small copepod and the large proportion of meroplankton is

also essential to understand the functioning of Ramfjord ecosystem. Studies have demonstrated the role of the small mesozooplankton, by highlighting the carbon flux and grazing pressure the small mesozooplankton generate in an ecosystem, as well as their role as prey for fish larvae (Pasternak et al., 2008; Silberberger et al., 2021). A deeper investigation on these points in an ecosystem such as Ramfjord are important to fully understand fully the ecosystem functioning.

4.2.2 Autumn complexity

The seasonal changes in community structure and behaviour of mesozooplankton in Ramfjord were directly and indirectly influenced by the underwater light. The seasonal changes in the community structure were mostly influenced by temperature and the phytoplankton phenology, thus indirectly by the seasonal changes in incoming solar radiation (Paper I). The seasonal changes in DVM behaviour were directly influenced by the underwater light environment, with seasonal changes in behaviour corresponding to the seasonal change in photoperiod (Paper II, Paper III). Consequently, both aspects showed a similar seasonality, which can be summarized as four seasons: the winter, or non-productive period, which was marked by the moulting and ascent of *Calanus* species to prepare the reproduction; the spring with a high phytoplankton availability and a "normal" day/night cycle favouring a classical DVM behaviour in the community, the reproduction of *Calanus* species and benthic species, resulting in the presence of meroplankton. The summer is characterized by constant illumination and a reduced availability of phytoplankton, favouring asynchronous DVM behaviour and triggering SVM in some zooplankton species, such as *Calanus* spp. The light transition from midnight sun to polar night, and the occurrence of an autumn phytoplankton bloom, affect the fjord community in autumn. During this season, the zooplankton community was highly complex similarly to the complexity reported in Kongsfjord in Autumn by Berge et al.,(2014) in Kongsfjorden. We find a high diversity of life history strategies between the different taxa, reflecting the different adaptation available to cope with the extreme seasonality at high latitude. These include the accumulation of high amounts of lipids and the descent to overwinter at depth, strategies typically described to be present in *Calanus* species (Paper I, Paper II and Paper II). Another life history strategy depends on the window of favourable conditions for the reproduction, but does not include specific adaptation to survive the non-productive period. Such strategy is found in euphausiids and *Metridia longa* (Båmstedt et al., 1985; Huenerlage et al., 2015), and

is represented in our study by the maintenance of a classical DVM behaviour during the non-productive period (Paper III and Figure 7). Finally, life history strategies of some taxa seem to be unaffected by high latitude seasonality. These are characterized by having a life cycle that is independent of the window of favourable conditions. For example, *O. similis* can reproduce year-round (Lischka and Hagen, 2005), has a flexible feeding strategy with the ability to utilize both phytoplankton and heterotrophic food sources (Castellani et al., 2005; Zamora-Terol et al., 2013), and remains in the upper water layers year-round (Lischka and Hagen, 2005). A small scale SVM has been reported for this species, with an ascent in the surface water during autumn or during the non-productive period (Søreide et al., 2022), i.e., opposite of the classical SVM behaviour, and is often associated with the peak of reproduction (Paper I). This strategy is recognised as a way to reduce competition, as the large calanoid copepod have descended in the deep water (Hansen et al., 1999). But in Ramfjord, the small copepods with this flexible strategy peaked in autumn, at the same time that diversity was highest due to the advection of boreal species into the fjord, not supporting a reduction of competition as a motivation for this strategy.

5 Conclusion

This thesis has demonstrated the complexity of the mesozooplankton community in Ramfjord by providing the most complete species composition and establishing that, unlike the classical ecosystem at high latitude, in Ramfjord the small copepods are dominating in terms of abundance and biomass. This highlights the need to ensure that the small mesozooplankton are taken into account when studying pelagic ecosystems. The role of these organisms in the ecosystem has been underestimated, and an increase of consideration for these species would enable a better understanding of marine ecosystem. However, this will not be possible without the development of methods that enable efficient sampling and identification of these species. Metabarcoding as an identification and quantification method appears promising in these respects. In addition, species-specific investigations are needed in terms of behaviour to properly define the role of each species and their impact on the energy budget of the ecosystem. This thesis focused on *Calanus* behaviour and demonstrated the tight coupling between the behaviour and the light seasonality, but also established the LAM as a method that can easily be used to investigate species-specific behaviour in response to environmental conditions.

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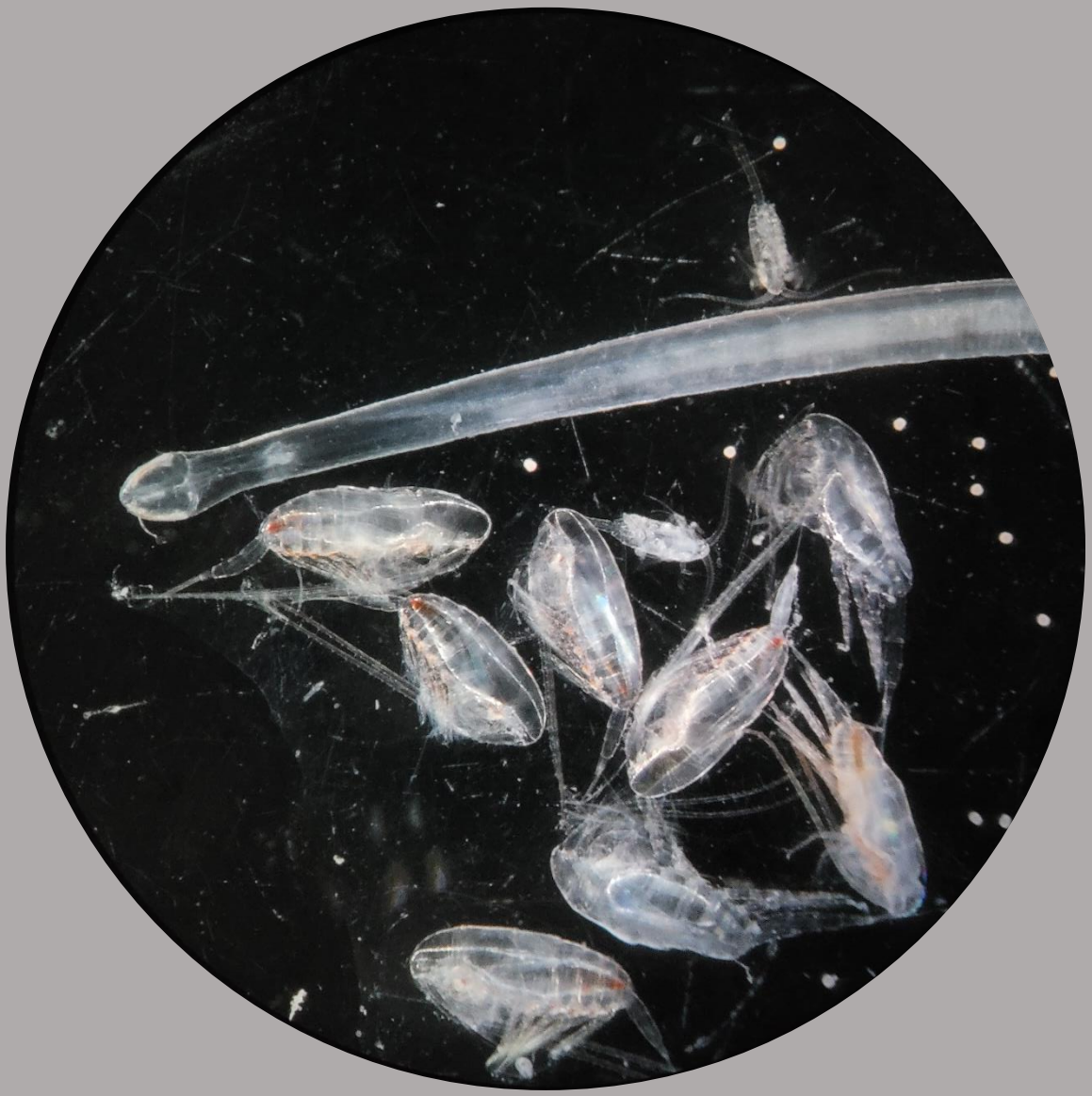
Paper I

Seasonal Variability in the Zooplankton Community Structure in a Sub-Arctic Fjord as Revealed by Morphological and Molecular Approaches



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Seasonal Variability in the Zooplankton Community Structure in a Sub-Arctic Fjord as Revealed by Morphological and Molecular Approaches

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Phyto- and zooplankton in Arctic and sub-Arctic seas show very strong seasonal changes in diversity and biomass. Here we document the seasonal variability in the mesozooplankton community structure in a sub-Arctic fjord in Northern Norway based on monthly sampling between November 2018 and February 2020. We combined traditional morphological zooplankton identification with DNA metabarcoding of a 313 base pair fragment of the COI gene. This approach allowed us to provide the most detailed mesozooplankton species list known for this region across an entire year, including both holo- and meroplankton. The zooplankton community was dominated by small copepods throughout the sampling period both in terms of abundance and relative sequence counts. However, meroplankton was the most diverse group, especially within the phylum polychaeta. We identified four distinct periods based on the seasonal analysis of the zooplankton community composition. The pre-spring bloom period (February–March) was characterized by low abundance and biomass of zooplankton. The spring bloom (April) was characterized by the presence of Calanus young stages, cirripedia and krill eggs. The spring-summer period (May–August) was characterized by a succession of meroplankton and a relatively high abundance of copepods of the genus *Calanus* spp. Finally, the autumn-winter period (September–December) was characterized by a high copepod diversity and a peak in abundance of small copepods (e.g., *Oithona similis*, *Acartia longiremis*, *Pseudocalanus acuspes*, *Pseudocalanus elongatus*, *Pseudocalanus moultoni*, *Pseudocalanus minutus*). During this period, we also observed an influx of boreal warm-water species which were notably absent during the rest of the year. Both the traditional community analysis and metabarcoding were highly complementary and with a few exceptions showed similar trends in the seasonal changes of the zooplankton community structure.

Keywords: metabarcoding, sub-arctic, seasonal community dynamics, zooplankton, meroplankton, copepods

INTRODUCTION

Marine ecosystems in Arctic and sub-Arctic regions are governed by strong seasonality in incoming solar radiation, leading to distinct seasonal peaks in primary production. Additionally, nutrient availability, relevant to algal growth, is governed by stratification and mixing of water masses, which again are affected by the seasonality in snow and ice melt, river run-off, wind mixing and solar radiation, in addition to algal nutrient uptake dynamics. Herbivorous zooplankton, which plays an essential role in marine ecosystems at high latitudes, tunes their life cycle to the seasonality in primary production, often using lipid stores to survive the non-productive period. The relatively short and intense growing season necessitates a good synchronization between life history events in zooplankton grazers (such as reproduction and growth) and the productive periods of the primary producers. This synchronization allows the acquisition and accumulation of energy and an efficient energy transfer to higher trophic levels. The dark winter season is particularly poorly studied for activity of phyto- and zooplankton, although recent research demonstrated that this season is by no means a period of inactivity, and several trophic levels remain active and complete important parts of the life cycles in the dark season (Berge et al., 2020; Johnsen et al., 2020).

Mesozooplankton is a key link in the energy transfer between primary producers and higher trophic levels (Steele, 1974; Arnkvaern et al., 2005). They include animals that permanently live in the water column (holoplankton) and those who only spend their larval stage as plankton (meroplankton). Zooplankton abundance, diversity and distribution are considered good indicators of the state of marine ecosystems (Hughes, 2000; Taylor et al., 2002; Hays et al., 2005). They are strongly influenced by hydrography and currents, with advection being an important mode of transport and dispersal, and any changes in the hydrographic regime may affect their distribution and fitness dramatically. Most zooplankton species are not commercially harvested, so changes in distribution and abundance reflect changes in fitness due to environmental forcing (e.g., changes in food availability, predator pressure, and abiotic factors) rather than exploitation, although they can also be influenced by eutrophication and pollution. Furthermore, planktonic life cycles are often short and their population dynamics are not affected by the individual's ability to persist over many years, so plankton populations rapidly respond to environmental changes (Hays et al., 2005).

Fjords are semi enclosed systems that are a characteristic feature of the Norwegian coastline (Stone, 1980; Cottier et al., 2010). Despite being coastal locations, they can have depths of 200–2000 m and provide habitats for deep-sea communities. Fjords that are separated from the open ocean by a shallow sill are more influenced by local processes, as advection from outside the fjord is reduced. Northern Norwegian fjords can be highly affected by freshwater inflow from either glacial or river discharges and snow melt, causing periods of partial coverage of sea ice. Many northern Norwegian fjords, however, are characterized by weak stratification and are also often influenced by Atlantic water masses (Eilertsen et al., 1981a; Reigstad and Wassmann, 1996) allowing them to remain mostly ice free. Due to their unique properties and often easy accessibility by small vessels, fjords provide excellent long-term sites to study seasonality in community composition and population structure of marine fauna. Most studies on fjord populations have, however, focused on either only single seasons or on specific groups or species, such as *Calanus* spp., or krill (e.g., Matthews et al., 1978; Bagoien et al., 2000; Niehoff and Hirche, 2005; Skreslet et al., 2015), and there are surprisingly few studies describing the seasonal variability in the zooplankton communities of Norwegian fjords or sub-Arctic fjords elsewhere.

The pelagic ecosystem of Balsfjord is one of the best studied among northern Norwegian fjords (Hopkins et al., 1989) mostly due to numerous studies conducted there in the 1980s (e.g., Eilertsen et al., 1981a; Falk-Petersen and Hopkins, 1981; Tande and Hopkins, 1981; Hopkins et al., 1984). Although located above the Arctic Circle, Balsfjord is not influenced by Arctic water masses and can be regarded as a sub-Arctic fjord. However Balsfjord is one of the coldest fjords in northern Norway due to the presence of a sill at the mouth of the fjord that limits the exchange of deep water (Oug and Høisœter, 2000). Since the 1990's, seasonal studies of zooplankton in this region have been limited (Wexels Riser et al., 2010; Svensen et al., 2018; Barth-Jensen et al., 2020; Trudnowska et al., 2020; Ershova et al., In revision). The mesozooplankton community in Balsfjord has been defined as poor in diversity but high in biomass (Hopkins, 1981; Hopkins et al., 1989). It was found to be dominated by copepods in terms of species numbers and abundance (Hopkins, 1981), but euphausiids can also be abundant, forming large sound scattering layers (Hopkins et al., 1978) and play an important role in the vertical carbon flux (Wexels Riser et al., 2010). However, despite being a relatively well studied fjord system, seasonal zooplankton investigations in Balsfjord, as elsewhere, have been significantly biased toward a few organisms that are easily identified, i.e., large copepods and euphausiids. For example, the seasonal variability in the population structure, sex-ratio and gonad maturation, body weight, carbon and nitrogen content, and enzyme activities have been well studied for *Calanus finmarchicus* (Tande and Hopkins, 1981; Tande and Slagstad, 1982; Tande, 1982; Tande and Gronvik, 1983) and *Metridia longa* (Tande and Gronvik, 1983; Grønvik and Hopkins, 1984; Hopkins et al., 1984; Båmstedt et al., 1985), as have the seasonal changes in lipid composition (Falk-Petersen, 1981; Sargent and Falk-Petersen, 1981; Falk-Petersen et al., 1982; Falk-Petersen, 1985) and population dynamics (Falk-Petersen and Hopkins, 1981) in euphausiids in Balsfjord. However, only a few studies have focused on other parts of the zooplankton community in Balsfjord, such as population dynamics and overwintering strategies in small copepod species (Norrbin et al., 1990; Barthel et al., 1995; Svensen et al., 2018; Barth-Jensen et al., 2020), or the role of zooplankton in the vertical carbon flux (Reigstad and Wassmann, 1996; Pasternak et al., 2000). Even less is known about the meroplankton community (Falk-Petersen, 1982), as the benthic community, and especially their larval stages, are generally much less studied (Oug, 1977; Michelsen et al., 2017).

One challenge of working with mesozooplankton is the complexity of accurate identification to species level.

Zooplankton species identification is time consuming and requires specialist taxonomic expertise (Pan et al., 2008). In addition, the presence of cryptic species, and difficulties to morphologically identify even the most common copepod species complexes, such as *Calanus* or *Pseudocalanus* (Gabrielsen et al., 2012; Choquet, 2017; Choquet et al., 2017, 2018), severely limit our ability to document zooplankton biodiversity or identify species-specific life history strategies. For example, recent advances using molecular tools have revealed that the *Calanus* communities in Northern Norwegian fjords are not, as previously assumed, largely to exclusively dominated by *C. finmarchicus* (Choquet et al., 2017) but by a mix of *C. finmarchicus* and *Calanus glacialis*, demonstrating our lack of understanding of the *Calanus* species complex in this region. This also raises the question in how far previous studies on population structure and reproductive strategies of *C. finmarchicus* in Balsfjord (and elsewhere) (Tande and Hopkins, 1981; Tande and Slagstad, 1982; Tande, 1982; Tande and Gronvik, 1983) are biased by the undetected presence of other *Calanus* species in the fjord. Morphological species identification, of meroplankton in particular is almost impossible due to the small size and lack of species-specific morphological differences between many larval and juvenile stages. Meroplanktonic organisms are therefore often only identified to phylum and little is known about species-, or even family-, specific seasonal variability within the meroplankton community (Michelsen et al., 2017).

Over the last decades, advances in high-throughput DNA sequencing technology have improved our ability to identify the "hidden" diversity in plankton communities (Lindeque et al., 2013). Metabarcoding allows for a large-scale taxonomic identification of community samples by analysis of one or more barcode regions (Lindeque et al., 2013; Bucklin et al., 2016). Barcoded sequences are grouped in molecular operational taxonomic units (MOTU) and can be identified to the species level when compared to sequences stored in genetic libraries. Metabarcoding has the advantage of being faster than sorting samples under the microscope and is rapidly becoming more cost-effective compared to traditional morphological approaches. It can provide more detailed assessment of species diversity (Lindeque et al., 2013; Gran-Stadniczeñko et al., 2019), including groups that do not retain their morphological features in preservatives or lack them altogether, like most larval forms. Although the quantitative value of metabarcoding is still disputed (Bucklin et al., 2016), protocols are emerging that allow to use the numbers of sequence reads as a semi-quantitative proxy of organism's biomass (Ershova et al., In revision). This semi-quantitative approach is particularly useful for seasonal studies, allowing to document the succession and seasonal changes in the contribution of different species of both holo- and meroplankton. However, metabarcoding cannot provide details on the developmental stage composition of a population, or the size composition within a zooplankton community. Thus, it appears essential to combine traditional microscopic analysis with metabarcoding to understand the variability in species diversity and zooplankton community structure in relation to seasonal changes in hydrography.

Here we used the combination of both approaches to (1) provide a complete species zooplankton inventory, (2) describe the seasonal variability in zooplankton community structure in relation to seasonal changes in hydrography and the availability of microalgae, and (3) described the population dynamics of sibling species of common copepods species that are difficult to identify based on morphology alone (i.e., *Calanus* spp. and *Pseudocalanus* spp.) in Ramfjord, a side arm of Balsfjord (**Figure 1**). A sill at 30 m at Balsfjord mouth separates the Balsfjord system from the open sea limiting the deep-water exchange and enabling us to observe seasonal patterns in the zooplankton community relatively undisturbed by exchange of water and organisms between the Balsfjord system and the open sea.

MATERIALS AND METHODS

Study Area

The study was conducted in Ramfjord (**Figure 1**), northern Norway, a 13 km-long and 1 km-wide fjord, which consists of two basins. At their deepest, the innermost and the outermost basins are 50 and 130 m deep, respectively. The fjord can be partly ice-covered as the inner part of the fjord is heavily affected by freshwater inflow between October and April. The sampling station (125 m water depth; location 69°31′49.9N, 19°02′11.9E) was located close to the deepest point of the outer basin, which was ice-free for the entire sampling period.

Field Sampling

Hydrography

Monthly sampling was conducted between November 2018 and February 2020 (**Table 1**) on board of R/V *Hyas*. Vertical temperature and salinity profiles were measured during each sampling event with a conductivity-temperature-depth (CTD) profiler (CTD SBE 19plus). In addition, temperature, salinity and *in situ* chlorophyll fluorescence (relative values not comparable with fluorescence value from other studies) were measured continuously at hourly intervals from 18th March 2019 to 11th June 2020 from a moored underwater observatory (69°32.005′N, 19°02.904′E,115 m water depth) which included a CTD (Seabird Electronics SBE 16) at 18 m depth and 10 temperature loggers (SBE 65) at 10–15 m intervals between 17 and 107 m water depth along the mooring cable.

Chlorophyll *a* Concentration and Phytoplankton Community

Chlorophyll *a* (Chl *a*) concentration was measured at 13 sampling events (**Table 1**, note missing data in August, September 2019 and February 2020) from water samples taken with a 5 L Niskin bottle at 5 and 30 m. About 250 mL triplicate samples were filtered onto GF/F filters (Whatman plc, Maidstone, United Kingdom) in the dark and frozen at −20°C until processing. Chl *a* was extracted in 96% Ethanol for about 24 h at 4°C. The extracts were measured on a Turner Trilogy AU-10 fluorometer (Turner Designs, 2019) before and after acidification with 5% HCl. Chl *a* and phaeophytin concentrations were calculated based on calibrations done with a Chl *a* standard (Sigma S6144).

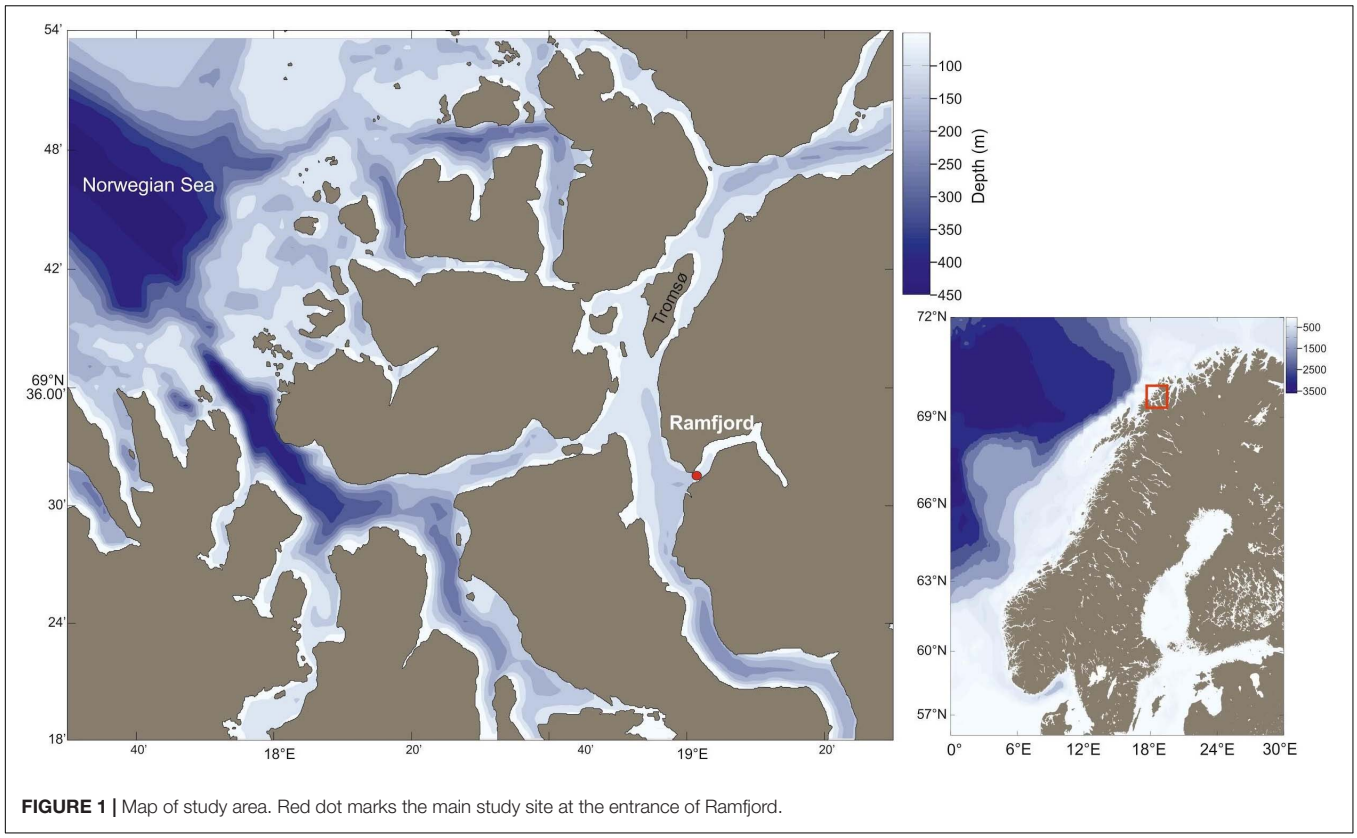

FIGURE 1 | Map of study area. Red dot marks the main study site at the entrance of Ramfjord.

TABLE 1 | Overview of sampling date, method used and data available for each sampling point.

Date	Net meshsize (μm)	Taxonomy	Metabarcoding run	Extraction kit	Chl *a* conc.	Algae taxonomy	CTD
18.11.2018	64	x	x	E.Z.N.A. Mollusc	x	NA	x
11.12.2018	64	x	x	E.Z.N.A. Mollusc	x	X	x
09.01.2019	64	NA	x	E.Z.N.A. Mollusc	x	NA	x
06.02.2019	64	x	x	E.Z.N.A. Mollusc	x	X	x
13.03.2019	64	x	x	E.Z.N.A. Mollusc	x	X	x
01.04.2019	180	x	x	E.Z.N.A. Mollusc	x	X	x
14.04.2019	180	x	NA	E.Z.N.A. Mollusc	x	X	NA
14.05.2019	180	x	x	E.Z.N.A. Mollusc	x	NA	x
13.06.2019	180	x	x	E.Z.N.A. Mollusc	x	X	x
20.08.2019	180	x	x	E.Z.N.A. Mollusc	NA	NA	x
03.09.2019	180	x	x	E.Z.N.A. Mollusc	NA	NA	x
25.09.2019	180	x	x	E.Z.N.A. Mollusc	x	X	x
14.10.2019	180	X	x	PowerSoil	x	X	x
14.11.2019	180	X	x	PowerSoil	x	NA	x
03.12.2019	180	X	x	PowerSoil	x	NA	x
04.02.2020	180	X	x	PowerSoil	NA	X	x

x indicate that data are available, whiles NA indicate that the data are not available.

Nine phytoplankton samples (**Table 1**) were taken with a phytoplankton net (KC Denmark) with 10 μm mesh size from 35 to 0 m depth. The samples were fixed in 2% (final concentration) neutral Lugol and stored in a dark borosilicate glass bottle until counting. Phytoplankton taxa were identified in a 2 mL well plate using an inverted microscope (Zeiss Primovert, Carl Zeiss AG, Germany) and taxa were identified using Throndsen et al. (2007).

Zooplankton Sampling

A WP2 net (Hydro-bios, Kiel, Germany) with a closing mechanism and an opening of 0.25 m^2 was used to sample zooplankton. Between November 2018 and March 2019, a net with a mesh size of 64 μm was used and from April 2019 on the mesh size was changed to 180 μm. Three net hauls were taken at each of the 15 sampling events (**Table 1**). One sample

was taken from 115 to 50 m and one from 50 to 0 m. Both were preserved in 4% formaldehyde-in-seawater solution. These samples were used to analyze the community composition based on morphology. The third tow was taken from 115 to 0 m and immediately preserved in 96% ethanol. Every net haul was taken with a towing speed ranging between 0.4 and 0.5 m s^{-1}.

Zooplankton Sample Analyses

Morphological Mesozooplankton Community Analysis

For each formalin-preserved sample, the community composition was determined under a Zeiss Discovery V20 stereo microscope (Zeiss, Oberkochen, Germany). First, large (>5 mm) and conspicuous organisms were picked out from the entire sample using fine forceps, identified and counted. Then, the remaining sample was diluted to a known volume and 5 ml subsamples were taken with an automatic pipette with the pipette tip cut at 5 mm diameter to allow free collection of mesozooplankton. The number of subsamples were determined to count at least 100 *Calanus* spp. copepodites which usually corresponded to more than 100 counted individuals of the most common genera in the same sample (*Oithona similis*, *Microcalanus pusillus*, *Pseudocalanus* spp., *Acartia longiremis*). Copepods were identified to the lowest taxonomic level possible based on morphological traits. The developmental stages were determined for *Calanus* spp. individuals. Non-copepods were identified to phylum. Abundance (individuals m^{-2}) was estimated by dividing the number of species per sample with the mouth opening area assuming 100% net filtration efficiency.

For further analyses, the abundance from the two depth layers was combined to one depth integrated abundance (115–0 m).

The copepods were classified into two groups, according to their adult prosome length, with copepods with an adult size < 1.5 mm being classified as "small copepods" while the rest were classified as "large copepods" (**Table 2**).

Dry Weight and Biomass

After being analyzed, each sample was split in two parts using a Motoda box splitter. One half was archived. The other half was used to determine the biomass in terms of dry weight (DW) by removing excess water using a 180 μm sieve, washing the sample with fresh water, drying it for at least 24 h at 50°C and then weighing it with a microbalance (Sartorius BP 615; precision 0.1 mg).

Metabarcoding

The ethanol-preserved sample was split into two parts using Motoda box splitter. One split was homogenized for 30–60 s using a 1000 W blender and allowed to settle for 3–4 h. Excess ethanol was removed by centrifugation and three replicates (±0.3 g) of the homogenized sample were transferred to 2 ml microcentrifuge tubes. DNA was extracted from each replicate using the E.Z.N.A. Mollusc DNA Kit (Omega-Pro) (samples from November 2018 to September 2019) or the PowerSoil DNA Extraction Kit (Qiagen, October 2019–February 2020) (**Table 1**) following the manufacturer's protocols. Leray-XT primers containing sample tags (Wangensteen et al., 2018), including the forward primer mlCOIintF-XT 5′-GGWACWRGWTGRACWITITAYCCYCC-3′ and reverse primer jgHCO2198 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′ (Geller et al., 2013) were used to amplify a 313 base pair (b.p.) region of the mitochondrial cytochrome *c* oxidase (COI) gene. The PCR protocol was 10 min at 95°C, followed by 35 cycles of: 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min, and a final extension of 5 min at 72°C. The tagged PCR products were pooled into a single library and cleaned using Minelute PCR purification columns[1]. The Illumina library was prepared from the DNA pool using the NextFlex PCR-free library preparation kit (Perkin-Elmer), quantified using the NEBNext Library Quant Kit for Illumina (New England BioLabs) and was sequenced on an Illumina MiSeq using a V3 2 × 250 bp kit.

Bioinformatics

Initial quality filtering of the sequencing data was conducted using OBITools v1.01.22 (Boyer et al., 2016). *Illuminapairedend* was used for aligning paired end sequences and filtering out those with an alignment score < 40. *ngsfilter* was used for demultiplexing and removal of primer sequences. Reads with a length of 299–300 b.p. were selected using *obigrep* and dereplicated using *obiuniq*. Chimeric sequences were then removed using the *uchime_denovo* algorithm (Edgar et al., 2011) in vsearch v1.10.1 (Rognes et al., 2016). Step-by-step clustering was performed in SWARM 2.1.13 (Mahé et al., 2015) using a distance value of d = 13 (Antich et al., 2021) to cluster individual sequences into Molecular Operational Taxonomic Units (MOTUs). After removing singletons (MOTUs with abundance of 1 read), taxonomic assignment of the representative sequence of remaining MOTUs was then performed using *ecotag* (Boyer et al., 2016) against DUFA-Leray v.2020-06-10, a custom reference database (publicly available from github.com/uit-metabarcoding/DUFA), which includes Leray fragment sequences extracted from BOLD and Genbank, completed with in-house generated sequences. Putative pseudogene sequences in the resulting dataset were then removed using LULU (Frøslev et al., 2017). MOTUs assigned to Prokaryotes and clearly non-planktonic organisms (e.g., insects, mammals) were removed, and a second taxonomy check of the remaining MOTUs was conducted using BOLD (Barcode of Life Database[2]). A species level identification was assigned with a minimum of 97% similarity. Finally, only MOTU's observed in a minimum of two sample replicates and accounting for at least 0.01% of the total reads of any sample were kept in the final dataset.

Diversity Index

Specific richness was defined as the number of taxa identified by metabarcoding. The specific richness was calculated using the entire metabarcoding data set (total specific richness) as well as using only the data set excluding phytoplankton and fish species (zooplankton specific richness) and the data set including only the copepod species (copepod specific richness).

[1]www.qiagen.com

[2]www.boldsystems.org

TABLE 2 | List of all species identified using metabarcoding, how they were categorized, and if or to what level they were identified in the visual inspection.

Phylum/Subphylum	Class/Order	Species	Category	Visually identified as
Bryozoa	Gymnolaemata	*Membranipora membranacea*	Meroplankton	Bryozoa larvae
Chaetognatha	Sagittoidea	*Eukrohnia hamata*	Non-copepod holoplankton	*Eukrohnia hamata*
Chaetognatha	Sagittoidea	*Parasagitta elegans*	Non-copepod holoplankton	*Parasagitta elegans*
Chordata	Ascidiacea	Ascidiacea indet	Meroplankton	Ascidia larvae
Chordata	Ascidiacea	*Ascidiella aspersa*	Meroplankton	Ascidia larvae
Cnidaria	Anthozoa	Actiniaria indet.	Meroplankton	Not observed
Cnidaria	Anthozoa	*Urticina felina*	Meroplankton	Not observed
Cnidaria	Hydrozoa	*Clytia hemisphaerica*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Corymorpha sp*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Euphysa aurata*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Lizzia blondina*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Melicertum octocostatum*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Mitrocomella polydiademata*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Nanomia cara*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Obelia geniculata*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Obelia longissima*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Plotocnide borealis*	Non-copepod holoplankton	Not observed
Cnidaria	Hydrozoa	*Rathkea octopunctata*	Non-copepod holoplankton	Not observed
Cnidaria	Scyphozoa	*Aurelia aurita*	Non-copepod holoplankton	Not observed
Cnidaria	Scyphozoa	*Cyanea* sp. RUYNKAR	Non-copepod holoplankton	Not observed
Crustacea	Amphipoda	*Themisto abyssorum*	Non-copepod holoplankton	Not observed
Crustacea	Cirripedia	Akentrogonida indet	Meroplankton	Cirripedia nauplii
Crustacea	Cirripedia	*Balanus balanus*	Meroplankton	Cirripedia nauplii
Crustacea	Cirripedia	*Balanus* sp.	Meroplankton	Cirripedia nauplii
Crustacea	Cirripedia	*Semibalanus balanoides*	Meroplankton	Cirripedia nauplii
Crustacea	Cirripedia	*Verruca stroemia*	Meroplankton	Cirripedia nauplii
Crustacea	Cladocera	*Evadne nordmanni*	Non-copepod holoplankton	Not observed
Crustacea	Cladocera	*Podon leuckartii*	Non-copepod holoplankton	*Podon leuckartii*
Crustacea	Copepoda	*Acartia longiremis*	Small copepod	*Acartia longiremis*
Crustacea	Copepoda	*Calanus finmarchicus*	Large copepod	*Calanus* spp.
Crustacea	Copepoda	*Calanus glacialis*	Large copepod	*Calanus* spp.
Crustacea	Copepoda	*Calanus helgolandicus*	Large copepod	Not observed
Crustacea	Copepoda	*Calanus hyperboreus*	Large copepod	*Calanus hyperboreus*
Crustacea	Copepoda	*Candacia armata*	Small copepod	Not observed
Crustacea	Copepoda	*Centropages hamatus*	Small copepod	Not observed
Crustacea	Copepoda	*Centropages typicus*	Small copepod	*Centropages typicus*
Crustacea	Copepoda	Cyclopoida indet.	Small copepod	Cyclopoida indet.
Crustacea	Copepoda	*Diaixis hibernica*	Small copepod	Not observed
Crustacea	Copepoda	Harpacticoida indet.	Small copepod	Harpacticoida indet.
Crustacea	Copepoda	*Longipedia coronata*	Small copepod	Not observed
Crustacea	Copepoda	*Longipedia* sp.	Small copepod	Not observed
Crustacea	Copepoda	*Metridia longa*	Large copepod	*Metridia* spp.
Crustacea	Copepoda	*Metridia lucens*	Large copepod	*Metridia* spp.
Crustacea	Copepoda	*Microcalanus pusillus*	Small copepod	*Microcalanus pusillus*
Crustacea	Copepoda	*Microsetella norvegica*	Small copepod	*Microsetella norvegica*
Crustacea	Copepoda	*Oithona similis*	Small copepod	*Oithona similis*
Crustacea	Copepoda	*Triconia borealis*	Small copepod	*Oncaea borealis*
Crustacea	Copepoda	*Paracalanus parvus*	Small copepod	Not observed
Crustacea	Copepoda	*Paraeuchaeta norvegica*	Large copepod	*Paraeuchaeta norvegica*
Crustacea	Copepoda	*Pseudocalanus acuspes*	Small copepod	*Pseudocalanus* spp.
Crustacea	Copepoda	*Pseudocalanus elongatus*	Small copepod	*Pseudocalanus* spp.
Crustacea	Copepoda	*Pseudocalanus mimus*	Small copepod	*Pseudocalanus* spp.
Crustacea	Copepoda	*Pseudocalanus minutus*	Small copepod	*Pseudocalanus* spp.
Crustacea	Copepoda	*Pseudocalanus moultoni*	Small copepod	*Pseudocalanus* spp.

(Continued)

TABLE 2 | Continued

Phylum/Subphylum	Class/Order	Species	Category	Visually identified as
Crustacea	Copepoda	*Temora longicornis*	Small copepod	*Temora longicornis*
Crustacea	Decapoda		Meroplankton	Decapod larvae
Crustacea	Decapoda	*Eualus pusiolus*	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Hyas coarctatus*	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Munida sarsi*	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Pagurus pubescens*	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Pandalus borealis*	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Pandalus* sp.	Meroplankton	Decapod larvae
Crustacea	Decapoda	*Sabinea septemcarinata*	Meroplankton	Decapod larvae
Crustacea	Euphausiacea	*Meganyctiphanes norvegica*	Non-copepod holoplankton	Euphausiacea
Crustacea	Euphausiacea	*Thysanoessa inermis*	Non-copepod holoplankton	Euphausiacea
Crustacea	Euphausiacea	*Thysanoessa raschii*	Non-copepod holoplankton	Euphausiacea
Crustacea	Isopoda		Non-copepod holoplankton	Isopoda indet.
Ctenophora	Ctenophora	Ctenophora indet.	Non-copepod holoplankton	Not observed
Echinodermata	Asteroidea	*Asterias rubens*	Meroplankton	Echinoderm larvae
Echinodermata	Asteroidea	*Ctenodiscus australis*	Meroplankton	Echinoderm larvae
Echinodermata	Asteroidea	*Solaster endeca*	Meroplankton	Echinoderm larvae
Echinodermata	Echinoidea	*Echinocardium cordatum*	Meroplankton	Echinoderm larvae
Echinodermata	Echinoidea	*Echinus esculentus*	Meroplankton	Echinoderm larvae
Echinodermata	Echinoidea	*Strongylocentrotus droebachiensis*	Meroplankton	Echinoderm larvae
Echinodermata	Echinoidea	*Strongylocentrotus pallidus*	Meroplankton	Echinoderm larvae
Echinodermata	Holothuroidea	*Cucumaria frondosa*	Meroplankton	Echinoderm larvae
Echinodermata	Holothuroidea	*Labidoplax buskii*	Meroplankton	Echinoderm larvae
Echinodermata	Holothuroidea	*Thyonidium drummondii*	Meroplankton	Echinoderm larvae
Echinodermata	Ophiuroidea	*Ophiocten affinis*	Meroplankton	Echinoderm larvae
Echinodermata	Ophiuroidea	*Ophiopholis aculeata*	Meroplankton	Echinoderm larvae
Echinodermata	Ophiuroidea	*Ophiura albida*	Meroplankton	Echinoderm larvae
Echinodermata	Ophiuroidea	*Ophiura robusta*	Meroplankton	Echinoderm larvae
Mollusca	Bivalvia	*Hiatella* sp.	Meroplankton	Bivalve larvae
Mollusca	Gastropoda	*Aporrhais pespelecani*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Eubranchus exiguus*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Gastropoda*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Lacuna vincta*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Limapontia capitata*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Microchlamylla gracilis*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Oenopota* sp.	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Placida dendritica*	Meroplankton	Gastropod larvae
Mollusca	Gastropoda	*Velutina velutina*	Meroplankton	Gastropod larvae
Mollusca	Polyplacophora	*Tonicella marmorea*	Meroplankton	Not observed
Nematoda			Non-copepod holoplankton	Not observed
Nemertea		*Malacobdella grossa*	Meroplankton	Not observed
Nemertea		*Micrura varicolor*	Meroplankton	Not observed
Platyhelminthes			Non-copepod holoplankton	Not observed
Polychaeta	Amphinomida	*Paramphinome jeffreysii*	Neroplankton	Polychaete larvae
Polychaeta	Capitellida	*Capitella capitata*	Meroplankton	Polychaete larvae
Polychaeta	Capitellida		Meroplankton	Polychaete larvae
Polychaeta	Eunicida indet.		Meroplankton	Polychaete larvae
Polychaeta	Eunicida	Dorvilleidae indet.	Meroplankton	Polychaete larvae
Polychaeta	Eunicida	*Nothria conchylega* CMC02	Meroplankton	Polychaete larvae
Polychaeta	Maldanidae	*Euclymene zonalis*	Meroplankton	Polychaete larvae
Polychaeta	Maldanidae	*Maldane sarsi*	Meroplankton	Polychaete larvae
Polychaeta	Orbiniidae	*Scoloplos armiger*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida		Meroplankton	Polychaete larvae

(Continued)

TABLE 2 | Continued

Phylum/Subphylum	Class/Order	Species	Category	Visually identified as
Polychaeta	Phyllodocida	*Aglaophamus malmgreni*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Antinoella finmarchica*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Bylgides sarsi*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Eunoe oerstedi*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Gyptis mackiei*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Harmothoe sarsi*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Harmothoe* sp. CMC01	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Lepidonotus squamatus*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Nereimyra punctata*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Pholoe baltica*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Phyllodoce grenlandica*	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Phyllodoce* sp.	Meroplankton	Polychaete larvae
Polychaeta	Phyllodocida	*Tomopteris* sp.	Non-copepod holoplankton	*Tomopteris* sp.
Polychaeta	Sabellida	*Hydroides elegans*	Meroplankton	Polychaete larvae
Polychaeta	Sabellida		Meroplankton	Polychaete larvae
Polychaeta	Scalibregmatidae	*Scalibregma inflatum*	Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Spionidae indet.*	Meroplankton	Polychaete larvae
Polychaeta	Spionida		Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Laonice cirrata*	Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Scolelepis* sp.	Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Spio* sp.	Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Spiophanes kroyeri*	Meroplankton	Polychaete larvae
Polychaeta	Spionida	*Spiophanes sp.*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Ampharete finmarchica*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Amphitrite cirrata*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Chaetozone setosa*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Flabelligera affinis*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Lanassa venusta*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Melinna elisabethae*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Neoamphitrite grayi*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Pectinaria koreni*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Pista maculata*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Polycirrus medusa*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Polycirrus* sp.	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Terebellidae indet.*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida	*Thelepus cincinnatus*	Meroplankton	Polychaete larvae
Polychaeta	Terebellida		Meroplankton	Polychaete larvae
Rotifera	Ploima		Non-copepod holoplankton	Not observed
Pisces	Gadiformes	*Melanogrammus aeglefinus*	Fish	Not observed
Pisces	Pleuronectiformes	*Hippoglossoides platessoides*	Fish	Not observed
Pisces	Pleuronectiformes	*Microstomus kitt*	Fish	Not observed
Chlorophyta	Mamiellales	*Bathycoccus prasinos*	Phytoplankton	NA
Haptophyta	Prymnesiophyceae	*Phaeocystis* spp.	Phytoplankton	NA
Ochrophyta	Bacillariophyceae	*Chaetoceros* spp.	Phytoplankton	NA
Ochrophyta	Bacillariophyceae		Phytoplankton	NA
Pyrrophycophyta	Dinophyceae indet.		Phytoplankton	NA
Pyrrophycophyta			Phytoplankton	NA

The iNEXT R package (Hsieh et al., 2016) was used to ensure that the richness saturation plateau was reached for all samples (**Supplementary Figure 3**).

Data Analysis

We used the metabarcoding data as a semi-quantitative estimate of relative biomass of zooplankton taxa

(Ershova et al., In revision) by multiplying the total zooplankton biomass with the proportion of the sequence reads for each species for each corresponding month to calculate biomass-weighted sequence reads (BWSR, mg DW m^{-2}).

Multivariate analyses of the community composition were performed on morphological identification data (abundance). An estimation of the abundance of *C. finmarchicus*, *C. glacialis*, *C. helgolandicus*, *P. acuspes*, *P. elongatus*, *P. minutus,* and *P. moultoni* was calculated based on the relative composition obtained with metabarcoding, by multiplying the genus abundance by the proportion of the targeted species, and add to the data set used for the multivariate analysis. As metabarcoding data were missing in mid-April, the abundances estimation at this sampling point was estimated as the average of estimated abundance over the entire period. Abundance data were fourth-root transformed in order to reduce the impact of super abundant and rare species. Copepod nauplii, *Microsetella norvegica* and rare copepods species were excluded from the analyses since abundance estimates of these taxa were likely biased due to the change of the net mesh size over the study period. Chi-squared distances were calculated and used to perform hierarchical cluster analysis.

To elucidate the relationships between zooplankton community structure across seasons and environmental parameters, a Canonical Correspondence Analysis (CCA) was performed using the previously described data set. Explanatory variables included average water column temperature salinity and algal fluorescence obtained from the CTD profile as well as the Chl *a* concentrations from water samples. The significance of the overall model and individual terms was calculated using permutation tests [ANOVA function in the R package vegan; Oksanen et al., 2020)] at a significance level of $p < 0.05$ and only significant constraining factors were retained. Missing CTD data in mid-April were assumed to be similar to the ones obtained 14 days before in early April. Missing measurements of Chl *a* concentrations in August, early September and February 2020 were replaced by the average Chl *a* concentration over the study period. All analyses were preformed using R (version 4.0.1) (R Core Team, 2020) and the package vegan (Oksanen et al., 2020).

RESULTS

Hydrography

The water column was cold, and well mixed from January to mid-May. The lowest temperatures were observed between March and May (0–2°C) (**Figure 2A**). The surface started to warm in mid-May, and from mid-May to August, the water column was stratified with warm water (5–10°C) in the uppermost 40 m and colder water (around 4°C) below 70 m. By September, the entire water column had warmed to >6°C and highest surface temperatures (12°C) were observed in early September. The water column started to cool down in November, and for the rest of the year the water was well mixed. From mid-December the water temperature was between 2 and 4°C (**Figure 2A**). Salinity varied between 32 and 33.5 throughout the year in most samples (**Figure 2B**). A relative fresh surface layer (salinity < 25) was observed starting in mid-May, coinciding with the onset of snowmelt on land resulting in increased freshwater runoff.

Chlorophyll and Phytoplankton Community

In situ concentrations of Chl *a* were low from November to March (**Figure 2C**). The abundance of microalgae was low, and the community consisted mainly of pennate diatoms and dinoflagellates during the polar night and in February 2019, while flagellates dominated in February 2020 (**Figure 2D**). Chl *a* concentration and fluorescence increased strongly in early April (10.2 mg m^{-3} at 5 m) and the algae community was dominated by centric diatoms (mainly *Chaetoceros socialis;* **Figure 2D**). By mid-April, the prymnesiophyte *Phaeocystis pouchetii* dominated, but Chl *a* concentration and fluorescence were low. Fluorescence at 18 m depth peaked in early May (∼20 mg m^{-3}), coinciding with the onset of stratification, and in July (∼50 mg m^{-3}). However, no Chl *a* and phytoplankton data are available in those periods. In June, flagellates, mainly *Chrysochromulina* sp. dominated the phytoplankton community, and the fluorescence and Chl *a* concentrations (3 mg m^{-3}) were relatively low (**Figures 2C,D**). Very high Chl *a* concentrations at the end of September (22 mg m^{-3}) and in October (29 mg m^{-3}) at both depth, indicate the occurrence of an intense autumn phytoplankton bloom dominated by centric diatoms (*Chaetoceros* sp.). Interestingly, this signal was not caught by the fluorescence sensors on the mooring in 18 m depth. From late November 2019 the fluorescence sensor did not provide reliable readings due to biofouling and *in situ* Chl *a* concentrations were low.

Seasonal Variability in the Zooplankton Community

Mesozooplankton Diversity

Thirty-five unique taxa were identified morphologically over the study period. Of these, 25 belonged to holoplankton, including 13 species of copepods: nine species or genera of small copepods and four species or genera of large copepods (**Table 2**). Ten taxonomic groups (class or phylum) of meroplankton were identified. The highest specific richness was observed in late April, and the lowest in February (2020). Only five copepod taxa were present throughout the entire study (i.e., *Calanus* spp., *Pseudocalanus* spp., *Microcalanus pusillus*, *Oithona similis*, *Acartia longiremis*), while all other taxa were absent during some months.

Metabarcoding revealed a total of 490 MOTUs, which corresponded to 154 unique taxa (**Table 2**). Hundred and fourteen of these were identified to species level, 17 to genus, and 22 to family or broader. Of the 154 unique identified taxa, 121 were present in more than four samples (**Supplementary Table 1**). Twenty-six species were present during every month, but only two of them (*Pseudocalanus acuspes* and *M. pusillus)* represented more than 1% of the sequence reads every month. Seven species represented at least 1% of the sequence reads during most of the months. These included *A. longiremis, Calanus finmarchicus, C. glacialis, O. similis, Pseudocalanus moultoni, Nanomia cara,* and *Parasagitta elegans*.

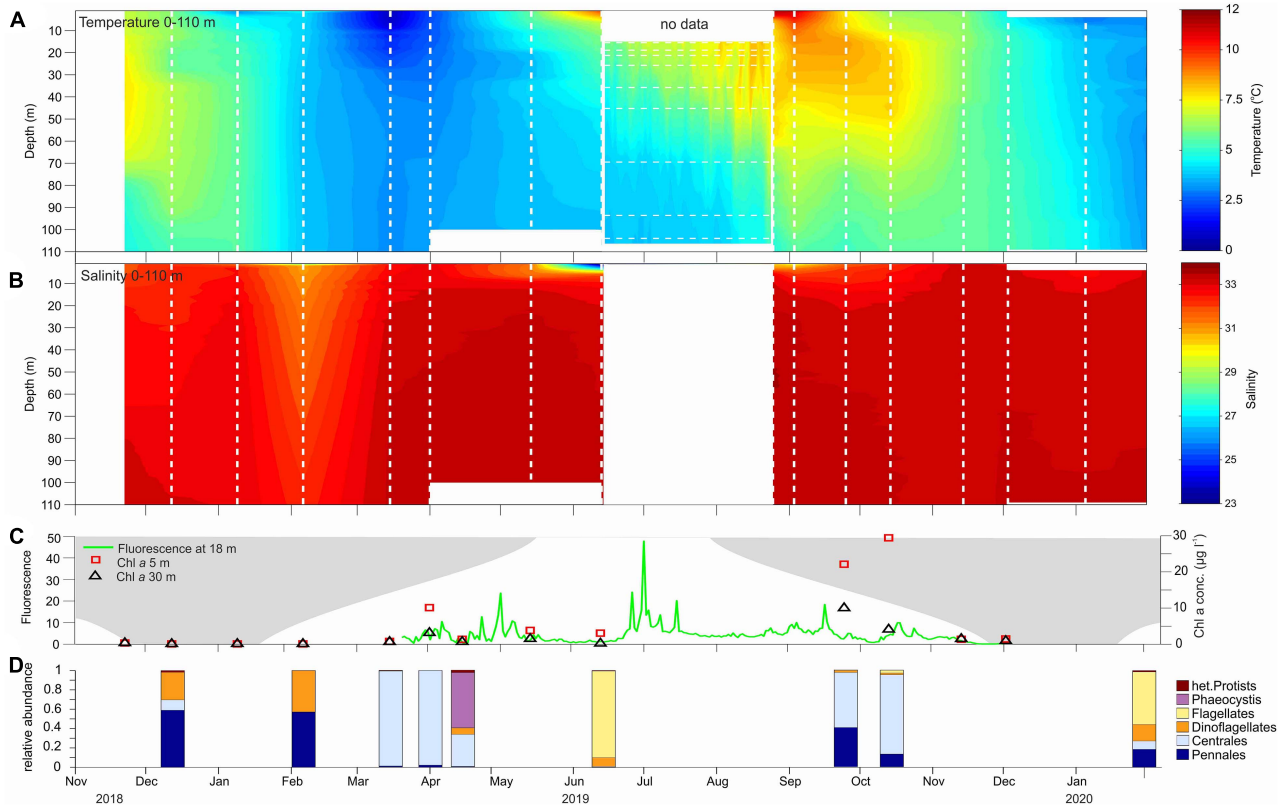

FIGURE 2 | Seasonal variation (November 2018–January 2020) in temperature **(A)** and salinity **(B)** based on CTD profiles taken at each sampling event (dates indicated by white vertical lines), fluorescence at 18 m and Chl *a* concentration at 5 and 30 m **(C)**, and taxonomic composition of phytoplankton community **(D)**. No CTD data available in **(A,B)** from 15 June to 18 August. Gap in **(A)** is filled with temperature measurements from mooring located close to the sampling site (with horizontal lines indicating depth of sensors along the mooring line). Fluorescence data available starting 15 March 2019; no fluorescence data after mid November 2019 due to biofouling of sensor. Seasonal changes in light climate is indicated in **(C)** with dark shaded areas corresponding to number of hours of darkness each day.

Of the 154 identified taxa, 36% (55) belonged to the holoplankton, while 58% (90) are known as benthic species and were therefore categorized as meroplankton. The remaining nine belong to fish and phytoplankton groups. Polychaeta presented the highest species richness with 46 taxa identified. Copepods were second in terms of number of taxa with 27 taxa identified over the study period (**Table 2**). Cnidaria and echinodermata were well represented as well, with respectively 15 and 14 taxa identified (**Table 2**). However, half of the identified cnidarians were present during only 1–4 months. Other groups such as bivalvia, amphipoda, ctenophora or chitonida were only detected as single taxa, generally identified at taxonomic levels above species (**Table 2**). Metabarcoding indicated that species richness was highest between December 2018 and February 2019 with a maximum number of species (110) in February (**Table 3**). It is noteworthy that during this period, sampling was conducted using a smaller mesh size. Between April and late September, the species richness varied between 80 and 104. Species richness was lowest between October 2019 and February 2020, when a different DNA extraction kit was used (**Table 1** and **Supplementary Figure 2B**), with around 50 taxa identified, while between 80 and 110 taxa were identified in the other months (**Table 3**). The use of a different DNA extraction kit reduced the diversity that we were able to identified (**Supplementary Figure 2**), However, it did not impacted the diversity of Copepoda taxa that we were able to detect (**Supplementary Figure 2B**). A maximum Copepoda species richness of 26–28 was reached between November 2018 and February 2019 (**Table 3**) when we used the smallest net mesh size (**Table 1**). However, it reached the same number in September (**Table 3**) when the largest net mesh size was used (**Table 1**).

Mesozooplankton Biomass, Abundance, and Community Structure

Total mesozooplankton abundance and biomass in Ramfjord varied between 1.2×10^4 to 23×10^4 ind m^{-2} and 174 to 2609 mg DW m^{-2}, respectively. Lowest abundance and biomass were measured between December and March during both winter seasons, while the highest values were recorded in summer/early autumn between August and October (**Figures 3A,B**). The mesozooplankton community was dominated in terms of abundance and biomass by small copepods (adult size < 1.5 mm) all year-round (**Figures 3C,D**). Small copepods represented up to 97% of the community in terms of abundance and up to

TABLE 3 | Species richness in Ramfjord based on metabarcoding analysis.

Date	Total species richness	Zooplankton species richness	Copepod species richness
18.11.2018	89	80	26
11.12.2018	108	99	27
09.01.2019	107	98	28
06.02.2019	109	100	26
13.03.2019	94	85	23
01.04.2019	103	94	24
14.04.2019	NA	NA	NA
14.05.2019	92	83	24
13.06.2019	84	75	24
20.08.2019	79	90	21
03.09.2019	90	81	26
25.09.2019	100	91	25
14.10.2019	52	43	22
14.11.2019	57	48	22
03.12.2019	55	46	20
04.02.2020	47	38	19

The total species richness included all species or taxa identified. For the zooplankton species richness phytoplankton and fish species were removed.

94% in terms of sequence read (**Figures 3C,D**). Non-copepod holoplankton (**Figure 3** and **Table 2**), consisting mainly of chaetognaths and krill, dominated in terms of sequence reads in November and December 2018, although their abundance was negligible (<1%). In late April, a high number of krill eggs was observed (64920 ind m^{-2}). The estimated BWSR of non-copepods varied between 0.44 mg DW m^{-2} in November 2019 and 690.96 mg DW m^{-2} in late September, which accounts for <0.1% to 55% of the total biomass (**Figure 3D**).

Large copepods (>2 mm at adult stage) were present year around, with highest abundance observed between April and June (**Figure 3C**). In terms of abundance, they represented a maximum of 27% of the zooplankton community in May, but only 2% in early September (**Figure 3C**). High abundances of copepod nauplii were recorded in February and March, accounting for up to 49% of the total abundance (**Figure 3C**). The highest BWSR of large copepods was observed in June (253 mg DW m^{-2}), and their contribution to the sequence reads varied between 1 and 25%, in November 2019 and June, respectively (**Figure 3D**).

Small copepods

The lowest proportion of small copepods, in terms of abundance, was observed in late April when only 20% of the community consisted of small copepods, while they represented between 50 and 90% of the community during the rest of the year (**Figure 3C**). The BWSR of small copepods varied between 0.35 mg DW m^{-2} in early April and 1359 mg DW m^{-2} in late September. The small copepods community had a relatively high diversity with nine species identified morphologically and 24 species detected using metabarcoding (**Table 2**). *Acartia longiremis*, *M. pusillus*, *O. similis* and four species of *Pseudocalanus* (*P. acuspes*, *P. elongatus*, *P. minutus*, *P. moultoni*) were present in Ramfjord year-round (**Supplementary Table 1**). These species represented more than 1% of all the sequence reads, together with *Paracalanus parvus* and *Temora longicornis.* We combined these species as the main representatives of the small copepod community (**Figure 4**). *Paracalanus parvus* was not observed visually, likely due to its morphological similarity to *Pseudocalanus* spp. at juvenile stages (**Table 2** and **Figure 4**).

The abundance of small copepods was relatively low between November and May (<3.10^4 ind m^2) (**Figure 4**). Only

FIGURE 3 | Seasonal changes in the total zooplankton biomass **(A)**, total zooplankton abundance **(B)**, proportions of main taxonomic groups based on morphological analysis **(C)**, and metabarcoding **(D)** in Ramfjord during November 2018–February 2020.

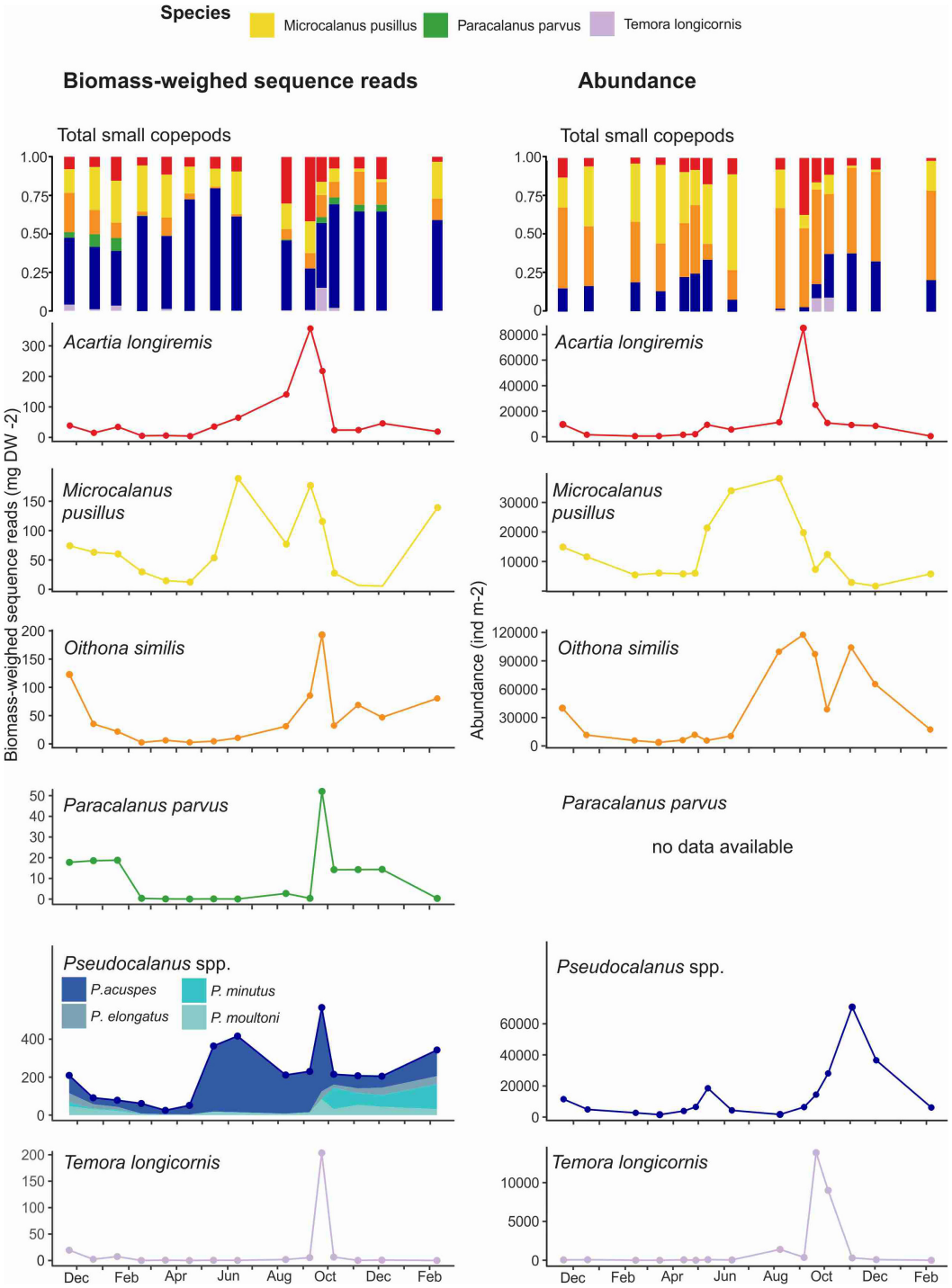

FIGURE 4 | Seasonal changes in small copepod BWSR (mg DW m^{-2}, **left column**) and abundance (individuals m^{-2}, **right column**) in Ramfjord between November 2018 and February 2020. The first row represents the relative proportion of the main small copepod species to the small copepod community remaining rows represent the seasonal variation of the main small copepod species.

Microsetella norvegica had a peak in abundance during this time, dominating the community in terms of abundance in February and March 2019 with up to 39 × 10^3 ind m^2. However, the number of sequence reads of *M. norvegica* was negligible (less than 1% of the total sequence reads) during the entire study period.

From May, the abundance of small copepods increased and reached its maximum in September (23 × 10^4 ind m^{-2}).

O. similis dominated the small copepod community throughout most of the study period in terms of abundance, contributing up to 65% and with a maximum abundance of 11×10^4 ind m^{-2} in September (**Figure 4**). In terms of sequence reads, *O. similis* represented a low portion of the small copepod community, with a maximum of 20% in November 2019 (**Figure 4**), and its BWSR varied from 1 mg DW m^{-2} to 192 mg DW m^{-2}.

Between November 2018 and January 2019, the small copepod community as estimated by BWSR was represented by all the main species depicted in **Figure 4**. The contribution of *O. similis* and *M. pusillus* BWSR was slightly higher, contributing 24 and 27%, respectively, while the other species contributed 1–15% to the small copepod BWSR (**Figure 4**). Between February and August *P. acuspes* dominated the community in terms of BWSR (up to 73%), which varied between 33 mg DW m^{-2} and 441 mg DW m^{-2} (**Figure 4**). The rest of the BWSR was composed of *A. longiremis* (between 5 and 41%) and *M. pusillus* (between 1.7 and 28%) (**Figure 4**). From late September to the end of the study, copepods of the genus *Pseudocalanus* dominated the small copepod community in terms of the BWSR (**Figure 4**). *P. minutus* had the highest proportion of sequence reads, up to 35% in October, with a BWSR varying from 1.94 mg DW m^{-2} to 113 mg DW m^{-2}. *Pseudocalanus elongatus* contributed least to the *Pseudocalanus* biomass, representing only between 2 and 12% of the small copepod BWSR between late September and December 2019 (**Figure 4**).

Meroplankton community

Eight groups of meroplankton, the six presented in **Figure 3** plus Bryozoa and Decapoda, were identified during the entire study period.

Meroplankton accounted for only 0.5–12% of the total zooplankton abundance between November 2018 and August 2019, and the abundance was negligible during the study period except in April when Chl *a* concentrations were high (**Figure 3C**). However, meroplankton BWSR varied between 1.81 mg DW m^{-2} in late September and 312 mg DW m^{-2} in March, which represented respectively 3.1 and 46% of the BWSR. The highest proportion of meroplankton, in terms of BWSR, was observed in early April when it accounted for 86% of the total BWSR (**Figure 3C**).

The composition of the meroplankton community estimated by metabarcoding followed the overall trends provided by morphological analysis, but with a higher taxonomic resolution. Polychaeta larvae and juveniles were present year-round in Ramfjord (**Figure 5**) and made up a high proportion of the meroplankton community in terms of abundance, contributing between 9% in October and 82% in December 2019 (**Figure 5**). The highest abundance of polychaeta larvae was observed in mid-April (4360 ind m^{-2}) (**Figure 5**). They dominated the meroplankton community in terms of BWSR between November 2018 and March 2019 and between October and December 2019 when they accounted for 14–80% of the meroplankton BWSR (**Figure 5**). Cirripedia larvae dominated the meroplankton community in terms of abundance and BWSR in April with a peak in abundance (19×10^3 ind m^{-2}) in mid-April (**Figure 5**). In June, echinoderm larvae accounted for 89% of the meroplankton abundance and 70% of the meroplankton BWSR (**Figure 5**). Their maximum abundance, 8.2×10^3 ind m^{-2}, was also observed in June (**Figure 5**).

Juveniles bivalves dominated the meroplankton community between November 2018 and February 2019 (23–68% of meroplankton) (**Figure 5**), and between August and November 2019 (up to 70%; **Figure 5**), with a peak in abundance in August (62×10^2 ind m^{-2}). However, their BWSR was low or negligible over the entire study period (**Figure 5**). Likewise, the BWSR of gastropod larvae was low, never contributing more than 7% to the meroplankton community (**Figure 5**). However, in terms of abundance they represented up to 43% of the community in February 2019 (**Figure 5**).

Calanus spp. species and stage composition

Based on morphological characteristics, only *Calanus hyperboreus* copepodite stage III (CIII)-adults could be identified with confidence and their abundance was overall very low (maximum 80 ind. m^{-2} in May). We did not identify other *Calanus* individuals to species morphologically and defined them as *Calanus* spp. Abundance of *Calanus* spp. was relatively low between November and early April (around 1000 ind. m^{-2}) and reached a maximum of 20 820 ind. m^{-2} in May (**Figure 6A**). In March and April, adult stages dominated the *Calanus* spp. population, with females contributing 77% in March and 66% in early April (**Figure 6B**). In mid- April, males were the dominating stage, representing 50% of the population (**Figure 6B**). Young copepodite stages (CI, CII, and CIII) started to appear in March, and were dominating the population in May (**Figure 6B**). Older copepodite stages (CIV and CV) were present in later winter (February–March), reappeared in higher proportions in May and were dominating the population from June on for the rest of the year (**Figure 6B**). CVs contributed up to 93% of the population between June and October (**Figure 6B**). A second peak in *Calanus* spp. abundance (10428 ind. m^{-2}) was observed in late September (**Figure 6A**). In October and November, CIIIs were detected again, representing 20 and 40% of the population, respectively (**Figure 6B**).

Metabarcoding detected the presence of four species of the genus *Calanus: C. hyperboreus*, *C. finmarchicus*, *C. glacialis,* and *C. helgolandicus* (**Figure 6C**). *C. finmarchicus* and *C. glacialis* dominated the *Calanus* community in Ramfjord for most part of the year, contributing equally in September, while *C. finmarchicus* dominated in February and March, and from October to December, and *C. glacialis* in January, and from April to June. In contrast to the low abundance observed, *C. hyperboreus* BWSR was relatively high between November 2018 and January 2019 and from April to August, when they represented between 0.3 and 64% of the *Calanus* sequence reads (**Figure 6C**). The contribution of *C. helgolandicus* to the *Calanus* community was low year-round (from 0 to 19%), with higher proportion observed during autumn-winter months (between 0.1 and 19%) (**Figure 6C**).

Seasonality of zooplankton community in Ramfjord

Hierarchical cluster analysis of abundance data of the entire zooplankton community, identified four main assemblages:

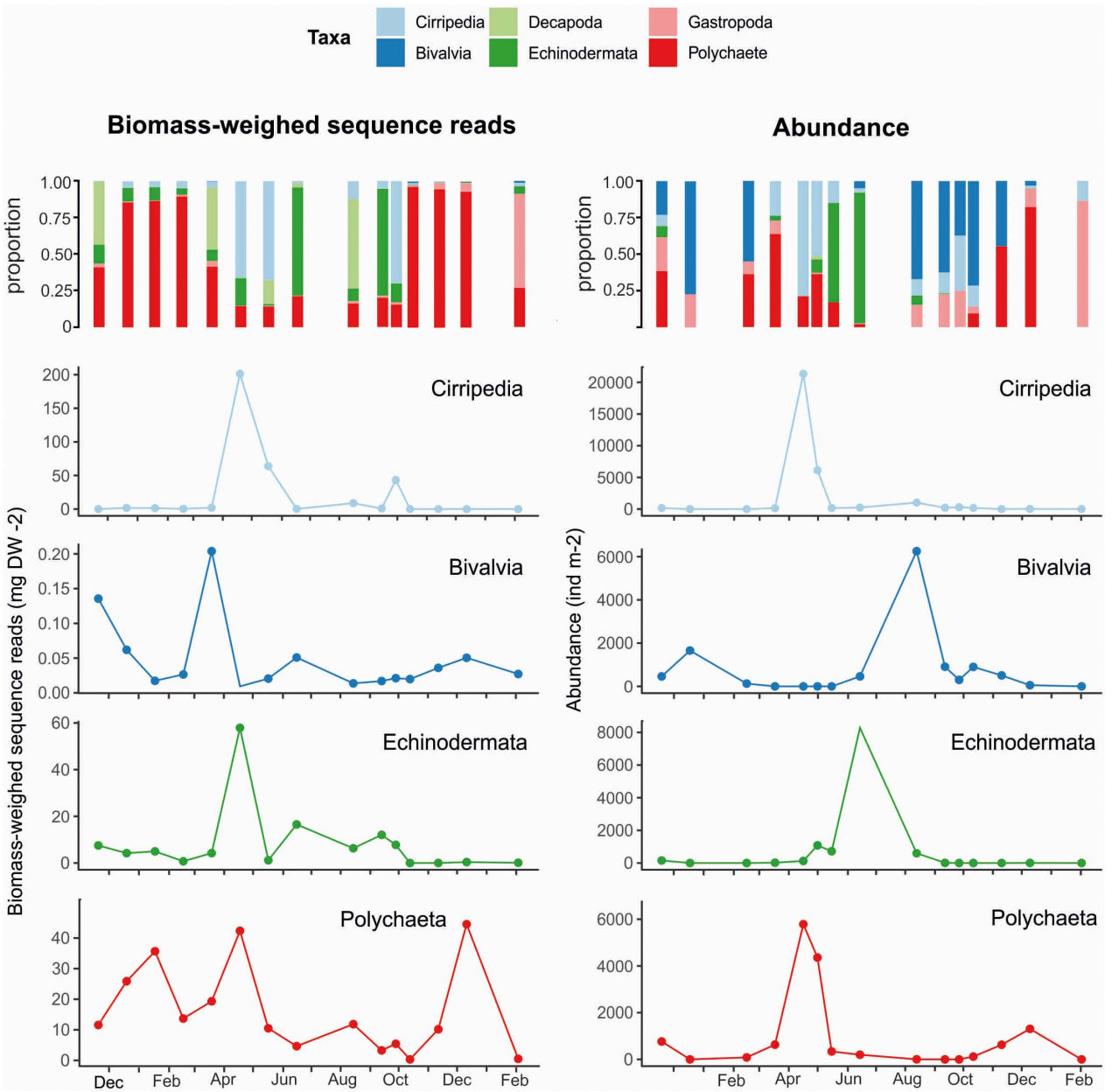

FIGURE 5 | Seasonal changes in the meroplankton BWSR (mg DW m^{-2}, **left column**) and abundance (individuals m^{-2}, **right column**) in Ramfjord between November 2018 and February 2020. The first row represents the relative proportions of the main meroplankton groups to the meroplankton community; the remaining rows represent the seasonal variation of the main meroplankton groups.

spring bloom (SB), spring/summer (SS), Autumn/winter (AW), and pre-spring bloom (PS) (**Supplementary Figure 1**).

The CCA models was significant ($p < 0.05$) with temperature and fluorescence as significant constraining factors ($p < 0.05$), while salinity and Chl *a* were insignificant. The resulting CCA model for abundance explained 29% of the total inertia (variance) in the abundance data, with 21% accounted for by the first axis (**Figure 7**). The results of the CCA were consistent with the cluster analysis, showing a clear separation of the samples based on season. The two April samples formed their own group (SB), being distinguished by the presence of cirripedia nauplii, euphausiid larvae and decapod larvae (**Figure 7**), and a high abundance of krill eggs (1333 ind m^{-2}) The SB and PSB assemblages were most distinct on the ordination, while SS and AW grouped closer together (**Figure 7**). The SS group was distinguished by relatively high fluorescence and water temperatures (**Figure 7**), and a higher abundance of *Calanus* spp. (**Figure 7**), particularly *C. hyperboreus* and *C. glacialis* (**Figure 7**), as well as echinoderm larvae and *A. longiremis*. The AW group was characterized by a wide range of water temperatures and low

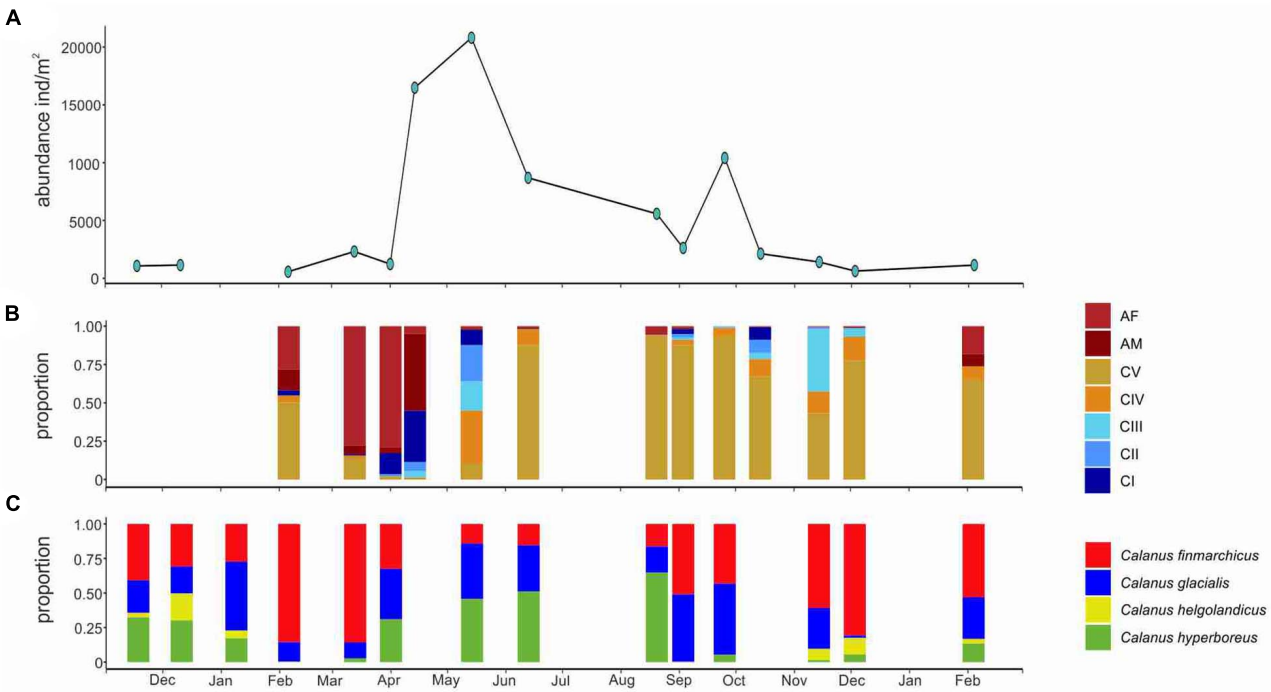

FIGURE 6 | Seasonal changes in *Calanus* spp. Abundance **(A)**, stage composition **(B)**, and species composition (based on metabarcoding) **(C)** in Ramfjord November 2018–February 2020.

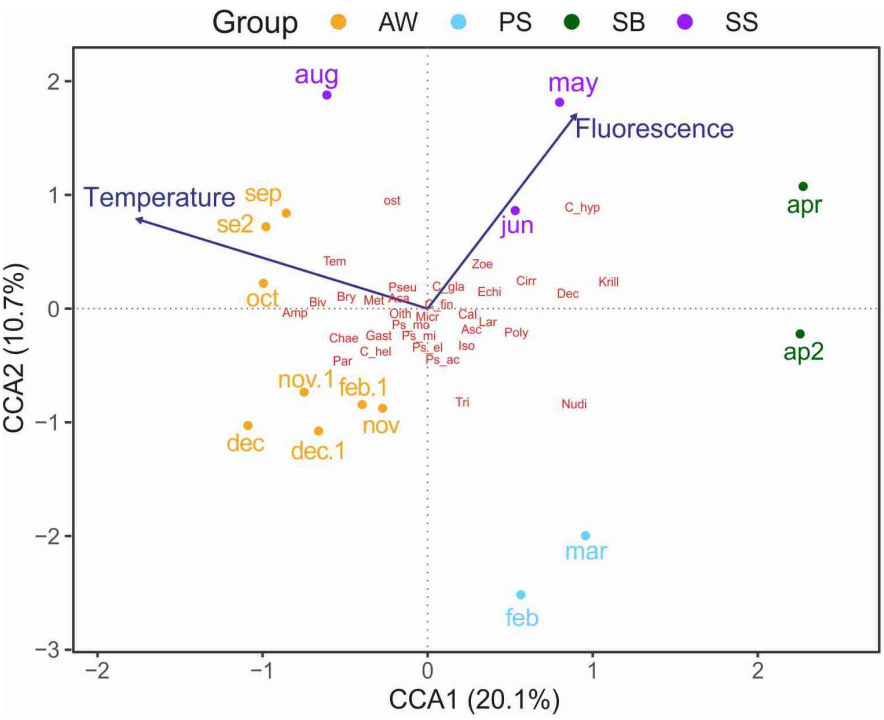

FIGURE 7 | Canonical correspondence analysis (CCA) of fourth-root transformed abundance data. The eigenvalues as percentages are provided for dimensions I and II with a total of 30.8% and variance explained. Colors correspond to the assemblages obtained by the hierarchical cluster analysis (**Supplementary Figure 1**). The abbreviations used in this analysis are explained in **Table 4**.

fluorescence. Furthermore, high abundance of small copepods (e.g., *O. similis*, *Pseudocalanus* spp., *M. pusillus*), *Metridia longa*, *Paraeuchaeta norvegica*, gastropoda larvae, chaetognatha and a relatively low abundance of *Calanus* spp. were characteristic for AW. The PS group, which included February and March samples, was characterized by low temperatures and fluorescence. Zooplankton abundance was overall low within this group, with a higher relative contribution of nudibranch larvae and *Triconia* sp. Although they were not included in the multivariate analyses, during this season we also registered a high abundance of copepod nauplii (23348 ind m^{-2} in February and 53908 ind m^{-2} in March).

TABLE 4 | List of abbreviation used in the CCAs and their meaning.

Abbreviation	Meaning
C_fin	*Calanus finmarchicus*
C_gla	*Calanus glacialis*
C_hel	*Calanus helgolandicus*
Oith	*Oithona similis*
Micr	*Microcalanus pusillus*
Pseu	*Pseudocalansu* spp.
Ps_el	*Pseudocalnaus elongatus*
Ps_mi	*Pseudocalanus minutus*
Ps_mo	*Pseudocalanus moultoni*
Ps_ac	*Pseudocalanus acuspes*
Tri	Triconia sp.
Met	*Metridia* spp.
Aca	*Acartia longicornis*
Par	*Paraeuchaeta* spp.
Tem	*Temora longiremis*
C_hyp	*Calanus hyperboreus*
Amp	Amphipoda
Lar	Appemdicularia
Asc	Ascidia
Biv	Bivalves juvenille
Bry	Bryozoa
Chae	Chaetognatha
Cirr	Cirripedia
Ost	Ostracoda
Dec	Decapod
Echi	Echinodermata
Gast	Gastropoda
Iso	Isopoda
Krill	Krill larvae
Nudi	Nudibranche
Poly	Polychaeta larvae
Zoe	Zoea
nov	November 2018
dec	December 2018
jan	January 2019
feb	February 2019
mar	March 2019
apr	Early April 2019
ap2	Mid-April 2019
may	May 2019
jun	June 2019
aug	August 2019
sep	Early September 2019
se2	Late September 2019
oct	October 2019
nov.1	November 2019
dec.1	December 2019
feb.1	February 2020

DISCUSSION

The present study is one of only a few studies combining metabarcoding and morphological identification to study the seasonal variability of a zooplankton community with relatively high temporal resolution (Gran-Stadniczeñko et al., 2019; Schroeder et al., 2020). This approach enabled us to describe the seasonal variability of the zooplankton community in Ramfjord with high quantitative and taxonomical accuracy, while at the same time also linking the detected changes to environmental variables.

The Zooplankton Community Diversity

Using metabarcoding, we identified 154 taxa in Ramfjord over the study period, which was four times as many taxa as using the morphological identification alone. The mesozooplankton diversity in this fjord system has previously been described as low (Hopkins, 1981) although the authors did not specify compared to what this assessment was made. Mesozooplankton diversity is rarely fully described, but compared to other well-studied high latitude fjords, such as Kongsfjorden (97 taxa over 20-year-long time series, Hop et al., 2019b), Rijpfjorden (42 taxa over 8 months) (Weydmann et al., 2013) and Dolgaya Bay (33 taxa in July, Dvoretsky and Dvoretsky, 2010), the zooplankton community in Ramfjord does not appear to be particularly low in diversity. However, the majority of taxa identified in our study can be categorized as meroplankton (i.e., larvae and juveniles of 90 benthic adult taxa), which are usually not identified to lower taxonomic level. Of the 55 holoplanktonic taxa, copepods accounted for 27, which is low compared to Kongsfjorden (52 species), but diverse compared to Rijpfjorden and Dolgoya Bay as well as Hudson Bay (Estrada et al., 2012) (13, 15, and 13 species, respectively). The lower number of copepod species from studies relying on visual identification are, however, only comparable to the number of copepod species we identified morphologically (13), strongly supporting the concept of combining metabarcoding with morphological identification.

Meroplankton diversity in Ramfjord exceeded estimates from other northern Norwegian locations (including Balsfjord) that were based on morphology. Here, meroplankton species richness varied between 37 (Porsangerfjord) and 65 taxa (Vesterålen) (Falk-Petersen, 1982; Andersen, 1984; Fetzer and Arntz, 2008; Silberberger et al., 2016; Michelsen et al., 2017). Meroplankton diversity was also higher than the diversity of benthic organisms previously assessed in Ramfjord (Oug, 1977), particularly with regard to polychaetes and echinoderms. However, the benthic communities of Ramfjord and Balsfjord are poorly studied

(Oug and Høisœter, 2000), which makes it difficult to conclude whether the meroplankton species identified in Ramfjord were part of local populations or present due to advection. Among meroplankton, polychaetes were the most diverse group. This is not surprising, since polychaetes are the most diverse group of benthic organisms in other Arctic, sub-Arctic and Norwegian fjords (Holte, 1998; Ellingsen and Gray, 2002; Bluhm et al., 2011). Oug and Høisœter (2000) found that polychaetes represented 97% of all species of the soft-bottom macrofauna community in Balsfjord (of a total of 78 species). Little is known about the biodiversity of taxa usually associated with hard bottom communities in our study area. The presence of larval stages of cirripedia and ascidia has been reported previously in this fjord system (Falk-Petersen, 1982). Sandnes and Gulliksen (1980) identified the sea urchins *Strongylocentrotus droebachiensis* and *S. pallidus* as key species in the system, controlling the abundance of sessile organisms such as the barnacle *Semibalanus balanoides* and the limpet *Testudinalia testudinalis*. Larval stages of these species were present in our study (**Table 2** and **Supplementary Table 1**) while the gastropods and bivalves mentioned by Sandnes and Gulliksen (1980) did not show up in our species inventory. Bivalvia were the only group whose diversity of larval stages was lower than the diversity of adult forms previously described in the study area (Oug, 1977; Sandnes and Gulliksen, 1980; Vahl, 1980; Drent, 2002) with only one MOTU of bivalve identified. For juvenile bivalves we also found the largest difference between the visual analysis and metabarcoding. While high abundance was observed in summer, bivalves hardly featured in the BWSR. This underestimation of bivalve diversity can be explained by a lack of relevant data in the reference database, PCR bias using our chosen primer set, or problems with DNA extraction. Molluscs are notoriously hard to extract DNA from Pereira et al. (2011) presumably due to the presence of polysaccharides that inhibit DNA polymerase, and although we used an extraction kit tailored for molluscs during most of our study period, this demonstrates the ongoing challenges for this taxonomic group.

Most of the holoplankton taxa identified using metabarcoding (**Table 2** and **Supplementary Table 1**) are species common to boreal and Arctic zooplankton communities (e.g., Dvoretsky and Dvoretsky, 2010; Estrada et al., 2012; Weydmann et al., 2013; Hop et al., 2019b). Particularly for *Calanus* and *Pseudocalanus*, metabarcoding provided a more detailed insight into the species composition than previous studies. Since life cycles and life strategies may differ even in closely related species within the same genus (McLaren et al., 1989; Lischka and Hagen, 2005; Ershova et al., 2021), correct species identification is crucial for describing species specific life history strategies and for documenting changes in population dynamics (see below). *Pseudocalanus* lacks easily distinguishable morphological features that would aid species identification, particularly when it comes to the early life stages, and they are therefore often reported as *Pseudocalanus* spp. Only two species of *Pseudocalanus*, i.e., *P. acuspes* and *P. minutus* (Norrbin, 1993, 1994) and one species of *Calanus*, i.e., *Calanus finmarchicus* (Hopkins et al., 1989) have previously been reported from Ramfjord/Balsfjord, while we revealed the presence of four species of each genus coexisting in Ramfjord, (i.e., *C. finmarchicus*, *C. glacialis*, *Calanus helgolandicus*, *Calanus hyperboreus*, *Pseudocalanus acuspes*, *Pseudocalanus elongatus*, *Pseudocalanus minutus*, *Pseudocalanus moultoni*, **Table 2** and **Supplementary Table 1**).

While morphological features such as size (e.g., Daase and Eiane, 2007) or coloration (Nielsen et al., 2014) may enable species identification of *Calanus* spp. with some degree of accuracy in the high Arctic, they are not reliable in populations along the Norwegian coast (Choquet et al., 2017). This can lead to an under representation of *C. glacialis* in particular and explains the lack of records of these species in Norwegian fjords (included Balsfjord) in most previous studies. The Arctic *C. hyperboreus* on the other hand, is easily distinguishable from the other *Calanus* species due to its large size and a clear morphological feature (a spine on the last prosome segment) in the older copepodite stages and in adults. Metabarcoding revealed a relatively high proportion of *C. hyperboreus* in Ramfjord, while only very few individuals were recorded by visual identification. This discrepancy between molecular and visuals tools could be explained by the fact that the number of sequence reads is a proxy of the biomass (Lindeque et al., 2013; Ershova et al., In revision). Because of its large size, *C. hyperboreus* (prosome length up to 5 mm) can represent a substantial part of the biomass, even if the abundance is low. However, we only identified very few *C. hyperboreus* individuals per sample visually (1 or 2 per sample) compared to *Calanus* in the size range of *C. finmarchicus* and *C. glacialis* (2–4 mm, 100-5000 per sample), thus even in terms of BWSR *C. hyperboreus* should not contribute to the *Calanus* weighted sequence reads in such high proportion. If *C. hyperboreus* was present in high numbers as young copepodite stages (CI -II) they could have been misidentified, as size differences of young stages are less pronounced between the different *Calanus* species. However, the sequence reads indicate highest relative contribution of *C. hyperboreus* in June and August when the *Calanus* population was dominated by late copepodite stages and young stages were rare. Thus, further studies are needed, e.g., using mock samples, to investigate the relationship between *C. hyperboreus* biomass, abundance and relative sequence reads.

Seasonality in the Zooplankton Community in Ramfjord

Long term data on hydrographic conditions are not available for the Ramfjord/Balsfjord system. However, the hydrographic conditions we observed in Ramfjord during our study were similar to observations from Balsfjord in 1976–1977 (Eilertsen et al., 1981a) and 2013–2014 (Svensen et al., 2018), with temperature peaking between 8 and 12°C in the surface during summer, and otherwise varying between 2 and 4°C at depth during summer and throughout the water column during winter and spring. We also observed salinity in a similar range to observations from Balsfjord (32 -33), with lower salinity in surface waters during snow melt and heavy snow fall (Eilertsen et al., 1981a; Svensen et al., 2018). We concluded that no strong differences in hydrographic settings was evident between the current and historical data.

A tight coupling of seasonal changes in zooplankton community structure and the strong seasonality in phytoplankton biomass and production, driven by the light regime, is typical for polar and sub-polar areas (e.g., Pertsova and Kosobokova, 2003; Weydmann et al., 2013; McKinstry and Campbell, 2018). In Ramfjord, the seasonal variability in the zooplankton community structure was manifested in four distinct periods (**Figure 8**), characterized by differences in overall abundance, presence of meroplankton, and shifts in diversity.

The pre-spring bloom (PS) period (February–March) was characterized by increasing day length but low water temperatures and an overall low phytoplankton and zooplankton abundance. However, the high abundance of copepod nauplii indicates that reproduction had started and given the low Chl *a* concentration, most of this effort was likely fueled by internal energy reserves, indicating a dominance of capital breeders among the reproducing copepods (Varpe, 2009) (see below).

April was initiated with a peak in Chl *a* concentration, marking the start of the spring bloom (SB). A succession from a dominance of diatoms such as *Chaetoceros socialis* and *Thalassiosira* spp. to a dominance of *Phaeocystis pouchetii* has previously been observed in Ramfjord and other areas including, e.g., the Barents Sea and the marginal ice zone in the Greenland sea (Eilertsen et al., 1981b; Tande and Bamstedt, 1987; Gradinger and Baumann, 1991; Orkney et al., 2020). We did not observe *P. pouchetii* beyond late April and thus lack data to assess how long it dominated the spring bloom in Ramfjord and its importance during the rest of the year as we may have underestimated the presence of the single cell stage of *P. pouchetii* for most parts of the year using a 10 µm phytoplankton net, which may only collect larger colonies that form during periods of high abundance.

The spring bloom fueled reproduction and development in the zooplankton and benthos community, as indicated by an increasing dominance of nauplii and juvenile stages. Adults and early developmental stage of *Calanus* spp. were abundant as well, as were krill nauplii and eggs, and within the meroplankton community, cirripedia nauplii and polychaete larvae reached their peak abundance. The SS period, starting in May, was characterized by low Chl *a* concentration and fluorescence, indicating a post bloom situation, dominated by flagellates as previously observed by Gaarder (1938) and Eilertsen et al. (1981b). We unfortunately lack data on Chl *a* and microalgae taxonomy from July to September to properly describe seasonal changes in the phytoplankton community throughout summer. The fluorescence measurements at 18 m depth indicate the

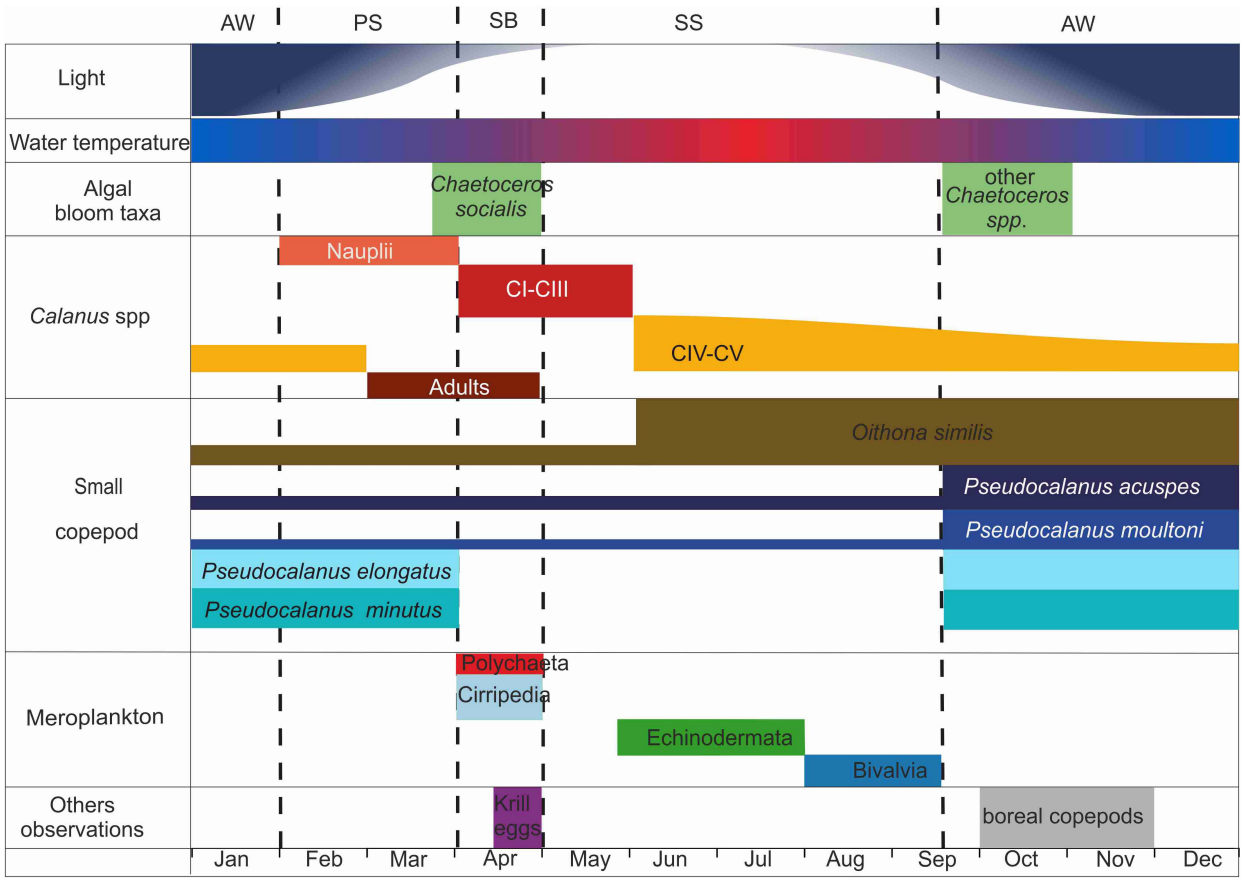

FIGURE 8 | Conceptual understanding of the seasonality variability in the zooplankton community in Ramfjord. The size of the boxes is proportional to the abundance of the organisms, but size of boxes is not comparable between the different compartments.

presence of microalgae throughout summer with a potential bloom event in July. Thus, algal food was available throughout summer, and a succession of different meroplankton taxa characterized the SS period. Cirripedia nauplii and polychaete larvae had disappeared from the water column by June, while the abundance of echinoderm larvae increased in June followed by an increase of juvenile bivalves in August. A similar succession in meroplankton has previously been described in Ramfjord (Falk-Petersen, 1982) and corresponds to observations from Arctic fjords in Svalbard (Kuklinski et al., 2013; Kwasniewski et al., 2013; Stübner et al., 2016) and Greenland (Nielsen et al., 2007).

Algal bloom dynamics in boreal and sub-Arctic regions typically include at least two seasonal peaks, one in spring and one in autumn (Eilertsen et al., 1981b; Eilertsen and Frantzen, 2007), and the AW period (AW, mid-September–January) in Ramfjord commenced with an autumn bloom dominated by diatoms (a mix of *Chaetoceros* species) similar to observations by Eilertsen et al. (1981b). Particularly high Chl *a* concentration measured in October far exceeded those during the spring bloom. This autumn bloom coincided with highest copepod diversity and peak in zooplankton abundance but also a steep decline in zooplankton biomass from September to October, largely driven by the decrease in abundance of the large *Calanus* species. Small copepods thus became the main contributors to the zooplankton community not only in terms of abundance but also in terms of BWSR, and biomass remained relatively constant throughout the AW period, even when temperature and light decreased, and Chl *a* concentration remained low. Hansen et al. (1999) hypothesized that the descent to overwintering depth of the large *Calanus* species creates a free niche in upper water layers that benefits small copepods, such as *Oithona similis*, *Microcalanus pusillus*, *Acartia longiremis,* and *Pseudocalanus* spp. These predominately omnivorous species (Saiz and Kiørboe, 1995; Castellani et al., 2005; Cleary et al., 2016) thus become the dominating functional group in the zooplankton community during AW.

The occurrence of species regarded as Atlantic/boreal, such as the copepods *C. helgolandicus*, *P. elongatus*, *Temora longicornis*, *P. minutus,* and *P. elongatus* and other species such as *Themisto abyssorum* and *Evadne normanni,* was restricted to AW when strong south-west winds often prevail in the Tromsø area (Eilertsen et al., 1981a), forcing water of Atlantic origin into the fjord system. Those species were likely advected into the fjord system and do not have established populations in the fjord.

Life History Strategies of Common Copepods

Except for *Calanus* spp., we did not identify copepodite stages of most common copepod species, thus we lack data to discuss the population dynamics of these species. However, changes in the total and relative abundance throughout the seasons nevertheless allow us to draw some conclusions about their life history strategies in Ramfjord. As observed in Disko Bay, Greenland (Madsen et al., 2008), the small copepods peak in Autumn in Ramfjord. This abundance peak of small copepod was prior or simultaneously to the second phytoplankton bloom, while it occurred after the algal bloom in Disko Bay (Madsen et al., 2008). The ubiquitous *O. similis* is known to actively feed and reproduce all year-round (Svensen et al., 2011; Zamora-Terol et al., 2014). Similar to observations from Kongsfjorden (Lischka and Hagen, 2005; Hop et al., 2019b), Greenland (Digby, 1954; Madsen et al., 2008), Malangen (Falkenhaug et al., 1997), and Kola Bay (Dvoretsky and Dvoretsky, 2009), we observed the highest abundance between August and December with peaks in September and November, thus coinciding with periods of relative warm water temperature that may affect recruitment and development rates positively (Svensen et al., 2011; Zamora-Terol et al., 2014). The life cycles and life history strategies of *A. longiremis*, *T. longicornis,* and *M. pusillus* are overall not well studied. *A. longiremis* is a neritic species commonly observed in Arctic and sub-arctic zooplankton communities (e.g., Hopcroft et al., 2010; Estrada et al., 2012; Ershova and Kosobokova, 2019; Hop et al., 2019a). In Ramfjord, *A. longiremis* was present year-round, indicating that it has established a population here, although its abundance sharply peaked during October. Falkenhaug et al. (1997) also observed a peak of *Acartia* spp. at the same period in Malangen, however the authors did not discuss this increase of abundance. We suggest a possible advective source as well. *T. longicornis,* a temperate, brackish water weakly selective herbivorous species whose reproduction is coupled to the phytoplankton spring bloom (Peters et al., 2013), appeared only during the autumn bloom, suggesting that the population was not established in Ramfjord but was the result of advection. *M. pusillus* represented a substantial proportion of the small copepod community particularly in spring and summer, with a peak of abundance in August. This is similar to *Microcalanus pygmaeus* in west Greenland (Digby, 1954) but in contrast to observations from Kongsfjorden (Hop et al., 2019b), east Greenland (Ussing, 1938) and the Amundsen Gulf (Darnis and Fortier, 2014), where *Microcalanus* spp. abundance peaked in later autumn and winter, indicating different strategies in the sub-Arctic *Microcalanus* populations compared to the high Arctic.

Pseudocalanus Species Complex

The peak in *Pseudocalanus* abundance in October coincided with a change in the *Pseudocalanus* species composition, although we cannot discount the possibility of a bias related to a change in DNA extraction kit coinciding with this change. While *P. acuspes* dominated in spring/summer and only *P. moultoni* was found in addition, *P. minutus* and *P. elongatus* appeared in autumn when all four species were found in more or less equal proportions. A similar succession has been recently observed in Svalbard (Ershova et al., 2021) indicating species specific differences in life history strategies. The pronounced annual cycle in abundance in *P. acuspes*, peaking during summer and declining in winter, indicates a dependence on the spring bloom for growth and development. This is in agreement with recent observations from Svalbard (Ershova et al., 2021) and previous studies in the Baltic sea (Peters et al., 2006; Renz and Hirche, 2006), Balsfjord (Norrbin, 1991) and Nova Scotia (McLaren et al., 1989). *P. acuspes* life cycle is described as mostly annual, but the *P. acuspes* population can also produce a second generation (McLaren et al., 1989; Norrbin, 1991; Peters et al., 2006) and such a second reproductive effort could explain the second peak

in BWSR in autumn. It has been suggested that *P. moultoni* has a more boreal distribution where it reproduces year-round (McLaren et al., 1989), but little is known of *P. moultoni* life cycle in Arctic and sub-Arctic waters as it has been misidentified for a long time (Aarbakke et al., 2017; Hop et al., 2019b). Our study confirmed that this species is established in Ramfjord year-round, and thus likely can reproduce successfully in sub-Arctic fjords. *P. minutus* the largest of the observed species, has a strictly annual life cycle in other parts of the world, spending most of its life as C4–C5 copepodites and relying on lipid stores more than its sibling species (McLaren et al., 1989). The distribution of *P. minutus* is generally restricted to ice-covered Arctic shelf seas (Melnikov et al., 2005; Persson et al., 2012; Ershova and Kosobokova, 2019) or the deep Atlantic Ocean (Wiborg, 1955; Aarbakke et al., 2017). In Isfjorden, a Svalbard fjord heavily influenced by Atlantic advection and lacking a seasonal ice cover, *P. minutus* failed to complete its life cycle and was only advected into the fjord during the summer months (Ershova et al., 2021). Similarly, we observed *P. minutus* in Ramfjord only between September and March, together with other non-resident species, suggesting an Atlantic origin. Likewise, *P. elongatus* is a warm water boreal species (Unal et al., 2006) and was present in Ramfjord only during the fall and the winter, likely due to advection.

Calanus Life Cycle

The co-existence of several *Calanus species* is not unusual for Arctic and sub-Arctic locations where sympatric populations of *C. glacialis*, *C. finmarchicus* and *C. hyperboreus* are common (e.g., Madsen et al., 2001; Arnkvaern et al., 2005; Darnis and Fortier, 2014; Choquet et al., 2017). These species differ in life history strategies such as timing of reproduction, life cycle duration and overwintering stages (Tande et al., 1985; Arnkvaern et al., 2005; Falk-Petersen et al., 2009) even when living in the same habitat. Our data do not allow us to distinguish life history strategies of the different *Calanus* species in Ramfjord. Higher sampling resolution around the spawning event as well as genetic identification of nauplii and the young copepodite stages are needed to describe the life cycles of the different *Calanus* species in Ramfjord more precisely.

Development from egg to nauplii and CV takes around 60 days at temperatures observed in Ramfjord (McLaren, 1978; Campbell et al., 2001; Daase et al., 2011). Since we observed high abundance of copepod nauplii in February and March, and CI were recorded in high abundance in early April, spawning of *Calanus* spp. probably started in February and thus before the phytoplankton spring bloom. Spawning prior to the spring bloom is common in *C. glacialis* (Daase et al., 2013), while *C. finmarchicus* is often described to follow an income breeding strategy (i.e., being dependent of the spring bloom for gonad maturation and reproduction (Varpe et al., 2009). Previous studies from Balsfjord described a close synchronization of spawning with the phytoplankton bloom in *C. finmarchicus* (Tande and Hopkins, 1981; Tande, 1982; Grønvik and Hopkins, 1984; Hopkins et al., 1984). However, Hirche et al. (2001) observed CI and CII *C. finmarchicus* in the water column before the spring bloom in the Norwegian Sea, indicating that *C. finmarchicus* is able to start reproduction ahead of the bloom, and we suggest that nauplii and young copepodites likely consisted of both *C. glacialis* and *C. finmarchicus* in Ramfjord.

An increase in abundance of young copepodites prior to the autumn bloom in October suggested a second spawning event, most likely by *C. finmarchicus* who can produce a second generation as previously described in the Scotian shelf (McLaren et al., 2001) and the Norwegian Sea (Wiborg, 1954; Marshall and Orr, 1955; Matthews et al., 1978; Strand et al., 2020).

We suggest that the *C. finmarchicus*/*glacialis* population in Ramfjord has a 1-year life cycle as the population was dominated by CV for large parts of the year, a common overwintering stage of both *C. finmarchicus* and *C. glacialis* (Tande, 1982; Arnkvaern et al., 2005). For *C. glacialis*, a 1–2 years life cycle (e.g., Kosobokova, 1999; Arnkvaern et al., 2005; Daase et al., 2013) and for *C. finmarchicus* a 1-year life cycle is commonly described for Svalbard fjords (Kwasniewski et al., 2003; Arnkvaern et al., 2005), the Barents Sea (Tande et al., 1985), as well as in sub-Arctic locations (Wiborg, 1954; Marshall and Orr, 1955; McLaren, 1978; Gislason and Astthorsson, 1998; Astthorsson and Gislason, 2003) including Balsfjord (Tande, 1982). In Ramfjord, *Calanus* spp. had disappeared from the upper 50 m by August (data not shown) except for the young stages observed in October and November 2019. The generally low abundance of *Calanus* throughout the water column in autumn and winter suggests that the population either suffered high mortality already at the start of the overwintering period, or that they do not overwinter in Ramfjord but seek refuge elsewhere. Relatively low abundance and high mortality of *C. finmarchicus* have been observed in other Norwegian fjords during winter (Bagoien et al., 2001; Skreslet et al., 2015), although deep fjords can be suitable for overwintering (Hirche, 1983; Espinasse et al., 2016). Due to its shallowness, Ramfjord is likely not a good overwintering habitat for *Calanus* spp., whose overwintering population is generally found at greater depth, particular in oceanic populations of *C. finmarchicus* and *C. hyperboreus* (600–2000 m, Hirche, 1991; Heath et al., 2004). These deep habitats provide not only refuge from predation and physiological advantages (lower metabolic cost in cold water), but also affect the buoyancy of lipid rich copepods with implications on their energy budget during diapause. Changes in wax ester chemistry reduces the buoyancy when descending below 500 m (Pond and Tarling, 2011), thus affecting buoyancy control during overwintering. *Calanus* spp. in shallow waters such as Ramfjord cannot reach a depth where they are neutrally buoyant, thus may have to work actively to remain at depth, which can be energetically demanding and reduce fitness.

Methodical Considerations

While metabarcoding provides a much more detailed species inventory than visual inspection, how the proportion of frequency reads relates to the actual proportion of species in the community and how sequences reads can be used to quantify species biomass is still in debate. A meta-analyses of 22 studies looking at a wide variety of biological communities ranging from land plants to fish investigated to which degree metabarcoding is quantitative (Lindeque et al., 2013; Lamb et al., 2019) and showed a weak correlation between the number of reads and

biomass with a large degree of uncertainties. However, only two of those examined studies looked at marine zooplankton, and neither of those used the primer set that we employed in this work. The quantitative value of our approach is described in detail in Ershova et al. (In revision) and has shown robust correlations between relative biomass and sequence read counts in high latitude marine zooplankton communities, although with biases toward certain taxonomic groups. The higher quantitative value of this method is obtained by the application of universal primers with a high level of degeneracy and the absence of a second PCR step at the library preparation stage. However, it is important to highlight that the BWSR measure that we use in this study remains semi-quantitative, meaning that it is useful in the context of seasonal comparisons of taxa-specific biomass in a single study, but likely not for comparing biomass estimates using other methods. It is noteworthy that in our study taxonomy and metabarcoding generally showed similar trends in the seasonal changes of the zooplankton community structure. BWSR captured the same seasonal peaks in *O. similis, Microcalanus* spp., *A. longiremis,* and *T. longicornis* as the abundance estimates. Only for *Pseudocalanus* and *M. norvegica* did the two methods show different patterns. The high BWSR of *P. acuspes* between May and August contradicted the low abundance of *Pseudocalanus* spp. in that period, although this could have been biased by the presence of nauplii which also peak during this time period (Vazyulya et al., 2001) and were not identified to genus. Low abundance but high BWSR would indicate a dominance of older stages and adults (few individuals but high individual biomass), but given *P. acuspes* life history (see above) the population should have been composed mainly of young stages with low individual biomass during that time period, and high abundance of those would have been recognized during the visual inspection. *Microsetella norvegica* is not sampled effectively by traditional zooplankton nets (Svensen et al., 2018) and was likely underestimated, especially after the change in mesh size when abundance decreased markedly. Despite its low individual biomass, we would have expected a higher proportion of sequence reads at the early part of the study when we used the smaller mesh size, but the number of sequence reads of *M. norvegica* were almost negligible year-round.

The increase in mesh size from April did not only result in an underestimation of *M. norvegica* but likely also other smaller organisms, such as small meroplankton and young copepodite stages, particularly those of small copepods (Vinogradov and Shushkina, 1987; Nielsen and Andersen, 2002; Tseng et al., 2011). Consequently, their abundances and the species richness between November 2018 and April are not directly comparable with the rest of the study. There is however little evidence that larger copepods and older life stage of smaller copepods (including copepodites stage CIII-adults of Calanus and Pseudocalanus) are caught less efficiently with a mesh size of 64 μm compared to 180 μm (e.g., Nichols and Thompson, 1991; Di Mauro et al., 2009; Altukhov et al., 2015; Chen et al., 2016). Thus, we are confident that the seasonal patterns we observed in the morphological data are not severely biased by the change in mesh size, especially when we take the change of mesh size into consideration in our data interpretation. Furthermore, despite the use of the 180 μm mesh, we did observe a strong increase of the abundance of most of the small copepod. Even if their abundance is underestimated, our data provide clear evidence of seasonal changes, such as a strong increase in abundance in autumn. Finally, the application of two different DNA extraction kits during the course of the study highlights the biases that can be introduced at this stage of the analysis. The EZNA Mollusc DNA Kit recovered, on average, 44% higher diversity than the Qiagen PowerSoil Kit, especially among the meroplankton and non-crustacean taxa (**Supplementary Figure 2**). Unfortunately, the lack of a temporal overlap in the application of the two kits precludes a more concrete analysis of the taxonomic biases of either and remains to be resolved in future studies.

One of the main concerns of the effect of climate warming on plankton communities is the potentially negative effect of changes in the algal bloom phenology related to zooplankton life history strategies. These changes may alter the energy transfer through the pelagic food web and potentially also impact benthic invertebrates through their pelagic early life stages. Furthermore, biogeographical distributional shifts may change community composition with repercussions on energy transfer and ecosystem structure (Beaugrand et al., 2009; Chust et al., 2013). In order to document changes and to be able to distinguish between natural seasonal variability and climate change impacts on ecosystems structure and functioning, we need to establish baselines, such as detailed species inventories and how community composition varies seasonally. Species specific changes in life histories can only be observed if species are correctly identified, as even closely related species may vary in their annual routines and their role in the ecosystem structure. Our study demonstrates that the combination of both morphological and metabarcoding approaches is providing the necessary quantitative and qualitative detail to document seasonal changes in community composition and population structure. While our study focused on mesozooplankton, future studies are needed to fully describe the community composition of the microplankton and macrozooplankton community.

CONCLUSION

There are few studies from sub-Arctic locations describing the seasonal variability in the zooplankton community structure. The combination of traditional methods of identification and state-of-the-art molecular tools allowed us to provide high-resolution data on seasonal variability in zooplankton abundance and diversity at a much higher taxonomic resolution. Both methods were highly complementary, with metabarcoding providing the most extensive species list of mesozooplankton from a Norwegian fjord to date, particular in terms of the meroplankton which are rarely identified to species in most zooplankton studies. 154 unique taxa were identified in Ramfjord over the study period, 58% were meroplankton organisms. Seasonality in the zooplankton community structure was driven by the seasonal changes in temperature and algae biomass and was manifested not only by changes in abundance and biomass but also by changes in diversity, although methodological shortcomings

limits our ability to identify seasonal changes in diversity to some extent. The succession of meroplankton was an important factor driving the seasonal changes in the mesozooplankton community over summer. An assessment of the diversity of the benthic community is needed to determine the role of advection and local production of the meroplankton community in the Ramfjord/Balsfjord system, and how seasonal changes in meroplankton composition and abundance are linked to difference in the life history strategies of the various species.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.gbif.org/dataset/b4804f19-8a8a-49e7-8dc2-79b528635696. The raw sequencing datasets presented in this study are publicly 882 available in the Sequence Read Archive (SRA) repository of NCBI. Bioproject ID: PRJNA742507. 883 https://www.ncbi.nlm.nih.gov/bioproject/742507.

AUTHOR CONTRIBUTIONS

EE, MD, RG, TV, and EC designed the study. EC, TV, and EE realized the sampling. EE and OW did the metabarcoding analysis and the bioinformatic. EC, MD, and EE did the zooplankton visual identification and wrote the first draft of the manuscript. TV did the phytoplankton cell count and the measure of the cholorophyll *a* concentration. MD, EC, and EE did the data analysis and data visualization. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.705042/full#supplementary-material

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Paper 2

Photoperiodism and overwintering in boreal and sub-Arctic *Calanus finmarchicus* populations

Photoperiodism and overwintering in boreal and sub-Arctic *Calanus finmarchicus* populations

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The copepod *Calanus finmarchicus*, a key species in the North Atlantic, generally spends the non-productive season by descending into deep waters and entering diapause, a physiological state characterized by reduced metabolism and arrested development. In the open ocean, overwintering depths are below 600 m where temperature and light conditions are favourable to initiate diapause. However, *C. finmarchicus* has also been reported diapausing in areas with shallow water depth such as fjords, coastal waters and shelf seas. In these environments, the temperature and light conditions are different, and it has been hypothesized that under such conditions *C. finmarchicus* may remain active throughout winter. Here, we investigated changes in the swimming activity of *C. finmarchicus* from shallow fjords in the eastern North

Atlantic during overwintering in response to ambient photoperiod. We conducted monthly experiments with populations from two fjords from different latitudes (sub-Arctic Ramfjord, 69°N and boreal Loch Etive, 56°N), measuring the locomotor activity of individual *C. finmarchicus* CVs exposed to natural light: dark cycle. At both locations, peaks in activity in response to the light cycle were observed to shift from nocturnal during the early overwintering phase to diurnal during mid and late overwintering phase, with a minimal intensity observed during the mid-overwintering phase. In Ramfjord, activity and rhythmicity were generally lower than in Loch Etive. We conclude that *C. finmarchicus* remains active throughout its overwintering period when in shallow (< 200 m) locations but down-regulates its locomotor activity during the main overwintering phase, which we describe as a winter resting state as distinct from classical diapause.

1 Introduction

The primarily herbivorous copepod *Calanus finmarchicus* is a key species in the North Atlantic marine ecosystem. It plays a major role in the transfer of energy from phytoplankton to higher trophic levels due to its ability to accumulate large amounts of lipids (Falk-Petersen et al., 2009). *Calanus finmarchicus* also contributes to the biogeochemical carbon cycle by releasing organic matter at depth through its diel and seasonal vertical migration (SVM). The lipid storage and SVM behaviour not only confer *C. finmarchicus* an essential role in the ecosystem, but ensure survival through the non-productive period, i.e., when food availability is reduced, and thus ultimately improve reproductive success (Maps et al., 2011). SVM is observed in a number of pelagic organisms and describes the seasonal migration from the upper pelagic to deeper waters at the end of the productive season, and an ascent to surface layers in late winter/ early spring. When at depth, *C. finmarchicus* enters a physiological state, called diapause (hereafter referred to as classical diapause), characterized by reduced metabolism, as well as reduced enzymatic, feeding and swimming activity (Hirche, 1983, Marshall and Orr,

1955, Hirche, 1996). During this time, development is arrested to a late developmental stage, which in this species is most commonly the copepodite stage V (CV) (Hirche, 1983, Marshall and Orr, 1955, Hirche, 1996). This overwintering strategy enables *C. finmarchicus* to reduce its metabolic costs and risk of being preyed upon by visual predators during the non-productive period, due to cooler temperatures and darkness of the deep-water environment (Marshall and Orr, 1955, Hirche, 1996).

Diapause is a well-known behaviour in *C. finmarchicus*, but the initiation of diapause is poorly understood. Several factors are commonly recognized as involved in the initiation of diapause, including exogenous seasonal cues such as photoperiod, temperature, food availability, and endogenous cues such as lipid thresholds and circadian clocks (Irigoien et al., 2004, Häfker et al., 2017). But diapause initiation appears to be more complex than a simple response to one of these factors. It has been suggested that a combination of factors may trigger diapause and that these factors may change geographically. Baumgartner and Tarrant (2017) suggested that cues may differ between animals inhabiting environments with shallow water depth and those inhabiting environments with deep water depth and polar environments. They contend that in locations with shallow water depth, diapause is triggered by a combination of factors such as photoperiod (Sømme, 1934, Fiksen, 2000), temperature (Corkett and McLaren, 1979), food abundance (Rey-Rassat et al., 2002) or predation pressure (Ji, 2011), while for organisms living in the deep oceanic basins and in polar environments a single seasonal cue such as photoperiod or temperature may be enough to induce diapause. For the latter, organisms inhabiting environments with shallow depth at high latitudes should respond similarly to those from deep environments.

The distribution of *C. finmarchicus* extends from 40° N to 80° N (Hirche and Kosobokova, 2007, Choquet et al., 2017, Grieve et al., 2017). Along this distributional range, it is exposed to a large gradient of environmental conditions including seasonal changes in day length,

temperature, and prey availability. *Calanus finmarchicus* has evolved its life history strategy to take advantage of a relatively predictable and extended productive period, timing its reproduction to match with the phytoplankton bloom (Miller et al., 1991). However, there is high plasticity in the timing of overwintering and reproduction within its distributional range (Johnson et al., 2007). In boreal latitudes, the overwintering period is generally shorter, lasting for a couple of months, and up to three generations per year can be produced (Saumweber and Durbin, 2006, Durbin et al., 1997, McLaren and Corkett, 1986, Michaud and Taggart, 2007, , McLaren et al., 2001). However, populations with one-year life cycles have been described as far south as the Gulf of St Larwences and across most of the Canadian Atlantic (Plourde et al., 2001, Plourde et al 2009). At the northern border of the species distributional range, *C. finmarchicus* has a one-year life cycle (Daase et al., 2021) and the overwintering phase can last for up to 10 months (Hirche, 1996).

Overwintering depth also varies substantially across the distributional range of *C. finmarchicus*. In the deep basins of the North Atlantic, diapause occurs in the mesopelagic layer > 600 m (Heath et al., 2000). However, successful overwintering of *C. finmarchicus* populations has also been observed in shallower environments such as fjords and shelf seas with bottom depth typically < 300m (Durbin et al., 1995, Hirche, 1991, Tande, 1982) which have a very different light climate and temperature regime. Can we, therefore, expect differences in behaviour and physiology between populations overwintering in environments of shallow and deep bottom depth? Recent observations from the Fram Strait showed that *C. finmarchicus* residing in the epipelagic at the beginning of the non-productive period were more active than those residing in the mesopelagic layers (Grigor et al., 2022). Furthermore, active feeding has been observed during the non-productive period in *C. finmarchicus* populations overwintering in shallow environments (Butler et al., 1970, Hirche, 1996) as well as during periods of limited food availability such as mid-summer in the Gulf of St. Lawrence

(Ohman and Runge, 1994). These feeding activities during peridos of low food availability are usually characterized by a dietary shift from herbivorous to omnivorous, carnivorous and/or detritivorous. These lines of evidence suggest that populations residing in environments that are depth-constrained remain more active during the non-productive period. An number of factors such as temperature, lipid structure and food availability likely play a significant role causing the differences in behaviour between individuals inhabiting shallow and deep environments (Pond and Tarling, 2011, Pond, 2012, Pond et al., 2014). We suggest that one of the factors preventing *C. finmachicus* to enter a classical diapause in environments with shallow water depth is the exposure to a changing photoperiod, as they cannot migrate into mesopelagic layers where the absence of a changing photoperiod would enable them to enter diapause.

Our aim was to determine locomotor activity during the overwintering period of *C. finmarchicus* in shallow environments under ambient photoperiods at two latitudes: the boreal Loch Etive (56°N, west Scotland) and the sub-Arctic Ramfjord (69°N, northern Norway). The main objectives of our study were to: (1) determine if *C. finmarchicus* overwintering in shallow environments enter a period of classical diapause, and (2) investigate patterns of rhythmicity in swimming activity over the diel cycle at two latitudes with contrasting photoperiod. Concurrently with these objectives, we characterized morphological differences between boreal and sub-Arctic *C. finmarchicus* populations to determine if differences in overwintering activities between these locations correlated with differences in body size and energy reserves.

2 Method:

2.1 Study area:

The study was conducted in Loch Etive, Scotland (UK), and in Ramfjord, near Tromsø (Norway). Loch Etive is located at a boreal latitude (56°45′N, 5°18′W) (Figure 1a), where day

length (i.e., from sunrise to sunset) varies from ca. 7 hours in December to ca. 17 hours in June (Figure 1b). Ramfjord is located north of the Arctic circle (69°31N, 19°02E) (Figure 1a). Ramfjord has an Arctic light regime with the day length increasing from 0 h between the end of November to the end of January (polar night) to a maximum of 24 h between the end of May to end of July (midnight sun) (Figure 1b). But Ramfjord is characterized as a sub-Arctic fjord since it is not directly influenced by Arctic water masses. In both locations, the sampling station was located near the deepest point of the fjord, at 145 m and 130 m in Loch Etive and Ramfjord, respectively.

2.2 Sampling

Monthly sampling was conducted from August 2017 to March 2018 in Loch Etive and from January 2019 to December 2019 in Ramfjord (Table 1). At each sampling event, a CTD profile was taken (SBE 19 in Etive, Seabird SBE 19 plus in Ramfjord, Sea-Bird electronics, Bellevue) (Table 1). In Loch Etive, additional CTD profiles were taken occasionally outside of the main sampling event (Table 1). *Calanus finmarchicus* was collected with a WP2 net with a closing system (Hydro-Bios, Kiel) and a maximum mesh size of 200 µm. At both locations, samples were taken from a few meters above the seafloor to 50 m deep and were carefully transferred to the local laboratory by keeping the organisms at stable temperatures and minimizing light exposure by keeping the sampled organisms in a dark cooler. Animals were transferred to a temperature-controlled room set to ambient seawater temperature at the collection site and kept in these conditions overnight (about 18 h) before setting up the experiments.

2.3 Locomotor activity

2.3.1 Experimental settings

For each location and time point, individuals of copepodite stage five (CV) of *Calanus* spp. were carefully picked using a stereomicroscope and soft forceps (see section 2.5 for species identification) (Table1). Individual copepods (copepodite stage CV) were placed in clear

157 acrylic tubes (volume 5 ml; diameter 10 mm diameter and length 64 mm) filled with filtered
158 seawater. The sorting was performed in a temperature-controlled room in darkness but the
159 organism were exposed to light from the stereomicroscope while being sorted. To monitor the
160 locomotor activity of individual CVs, the test tubes were placed in modified LAM10 activity
161 monitors (TriKinetics, Waltham, USA). These are infrared light (IR) beam arrays which detect
162 the movement of an individual copepod in each test tube. IR beams were located at 30 mm
163 above the bottom of each tube i.e., the middle of the filled tubes. Beam breaks were registered
164 every 30 seconds on a laptop computer using proprietary TriKinetics software (filescan.exe).
165 The monitors were placed in a temperature-controlled room with a controlled light environment
166 where the local ambient photoperiod was reproduced (Table 1). In Ramfjord, the individuals
167 were exposed to a day length equivalent to the time when the sun is above the horizon (real
168 day length) plus the civil twilight (the period when the sun is between the horizon and -6 °
169 below the horizon) to simulate a photoperiod as close as possible to the natural light climate.
170 Thus, experiments conducted for Ramfjord during the polar night (December and January)
171 were not maintained under continuous darkness but rather under a short photoperiod (Table 1).
172 Meanwhile, individuals from Loch Etive were exposed to a day length equivalent to the time
173 between sunrise and sunset (Table 1).

174 2.3.2 Data processing

175 At the end of each experiment, the number of beam breaks for each individual was summed
176 over 30 min with the DAMFileScan111 software (TriKinetics, Waltham, USA) and defined as
177 activity intensity (Act_{int}). The average activity intensity over the entire experiment, called
178 activity level (Act_{level}) was calculated for each individual. Individuals which were dead at the
179 end of the experiment (usually less than 10 %, Table 2) were removed from the data set.
180 Individuals with an Act_{level} > 60 breaks per 30 min were assumed to occupy a chamber with a
181 faulty sensor (based on an analysis of outliers) and likewise were removed from the data set.

2.4 Morphology

After the experiment, each individual was photographed using a stereomicroscope. The resulting image was used to measure the prosome length (PL), the prosome area (PA), and the lipid sac area (LA) of each individual using Image J software (Schneider et al., 2012). The lipid sac area to prosome area ratio (LAPA) was calculated for each individual as an index of the amount of lipids stored with each individual independent of body size (, i.e., indicating lipid fullness). Values are presented as means with standard deviation (mean ± sd). Additionally, LA was used to calculate the total lipid content (TL) in mg following Vogedes et al {, 2010 #47}.

$$TL=0.197LA^{1.38} \quad \text{(Eq 1)}$$

The lipid consumption over the overwintering period (%) was calculated by deducting the average LAPA during late overwintering phase from the average LAPA during early diapause.

2.5 Species identification

In Loch Etive, the *Calanus* population consists exclusively of *C. finmarchicus* (Choquet et al., 2017), but in Ramfjord four species of *Calanus* occur sympatrically; *C. finmarchicus, C. glacialis, C. helgolandicus,* and *C. hyperboreus* (Coguiec et al., 2021). *Calanus hyperboreus* was identified visually based on the presence of a spine on the last prosome segment but molecular tools were needed to discriminate between the three other species, as they are morphologically similar and overlap in size. A modified version of the Insertion-Deletion markers (InDels) protocol described by Choquet et al. (2017) was therefore used to identify the individuals used in the experiment in Ramfjord to species level. After the completion of the experiment, DNA was extracted from individual *Calanus* using the HotShot method (Montero-Pau et al., 2008). The Insertion-Deletion (InDel) molecular marker G-150 from Smolina et al. (2014) was amplified by polymerase chain reaction (PCR). The resulting amplicons were loaded on a 2% agarose gel and species identification was carried out based on fragment

lengths. The InDel marker G-150 is diagnostic to discriminate between *C. finmarchicus* and *C. glacialis* but not *C. helgolandicus*. The abundance of *C. helgolandicus* in the fjord is very low compared to other *Calanus* spp. (Coguiec et al., 2021), and in most cases, the marker G-150 does not amplify for that species (see dataset from Choquet et al., 2017), hence we would remove individuals with no amplification from our dataset. As G-150 is not diagnostic between *C. finmarchicus* and *C. helgolandicus*, there is a slight chance for a genotype identical to *C. finmarchicus* to appear in *C. helgolandicus* for that marker. However, due to the above, we consider that the likelihood of having mistakenly included *C. helgolandicus* in our dataset is very low, and henceforth this is not problematic for our findings.

2.6 Data analysis

2.6.1 Processing

For the experiment carried out in Ramfjord, data recorded in March, April and May were excluded from further analysis since not enough *C. finmarchicus* CV were present in net samples (< 2 individuals per sample), which we considered not statistically representative of the population.

A Lomb-Scargle periodogram analysis (R package lomb version 2.0) (Ruf, 1999) was conducted for each individual to determine whether it exhibited a rhythmic pattern in locomotor activity i.e., activity response to the imposed laboratory light/dark cycle. Lomb-Scargle is a particularly robust statistical analysis of rhythmic time-series data, especially for analysis of marine chronobiological data (Chabot et al., 2007, Lambert et al., 2019). Its particular strength lies in the analysis of unequally spaced or missing data (Ruf, 1999) but it is also considered equally powerful in the analysis of continuous data. Lomb-Scargle analysis is derived from the principles of Bayesian probability theory and combines the strength of Fournier analysis with least-square methods (VanderPlas, 2018). The analysis was run for periods < 30 h. All individuals that had a significant period (p-value < 0.05) were classified as rhythmic

232 individuals, while the others were categorized as arrhythmic. From this categorization the
233 proportion of rhythmicity (RH) per experiment was defined as:

234 $$RH = \frac{\#rhythmic\ indiv}{\#\ total\ indiv} \quad \text{(Eq 2)}$$

235 The rhythm strength of each rhythmic individual was defined using the peak normalized power
236 in the periodogram (PNmax) from the Lomb-Scargle analysis (Lambert et al., 2019). A higher
237 value of PNmax indicates a stronger rhythm.

238 The experiments were grouped according to overwintering phases which the animals had
239 theoretically entered at the time of the sampling, based on population dynamics and timing of
240 *C. finmarchicus* overwintering in Loch Etive (Häfker et al., 2018) and Ramfjord (Coguiec et
241 al., 2021). Following Hirche (1996), we defined three overwintering phases: i) the early
242 overwintering ("early" on figures), which defines the period when part of the *C. finmarchicus*
243 CV population had descended to depth (between June and August in both locations) and is
244 equivalent to Hirche's "induction phase"; ii) the mid-overwintering ("mid") defined as the
245 period when the majority of the *C. finmarchicus* population was found at overwintering depth
246 (from September to November in Loch Etive, September to December in Ramfjord); equivalent
247 to Hirche's "refractory phase"; iii) the late overwintering ("late") defined as the time when a
248 part of the population had ascended higher up in the water column to feed and moult prior to
249 reproduction (from December to March in Loch Etive, January and February in Ramfjord), and
250 is equivalent to Hirche's "termination phase".

251 To identify differences in the timing of increased population-level activity over the diel cycle,
252 the population's daily activity was calculated as the average Act_{int} of all individuals per month
253 for each 30 min interval using data from the first three days of each experiment, thereby
254 allowing for robust chronobiological analysis whilst minimizing prolonged laboratory
255 conditions. The data were visualized using a double-plotted actogram. For each experiment,
256 the uniformity of the daily activity over a 24 h period was tested with a Rayleigh test on this

daily activity data. All months in both locations showed non-uniform distributions (Rayleigh test, $p<0.05$). Accordingly, the mean angle (μ), i. e., the time around which the peak of activity is centred, and the mean resultant length (ρ), i. e., the peak activity intensity, were calculated for each experiment. All circular statistics were done in R with the circular package (Agostinelli and Lund, 2022).

2.6.2 Statistics

The normality of prosome length, LAPA, activity level, PNmax and TL were tested with a Shapiro test, but none met the normality conditions (p-value < 0.05). The variance homogeneity for each location between the different diapause phases as well as for each diapause phase between the locations was tested with a Fligner test. The variances were not homogenous (p-value < 0.05), except for prosome length and LAPA between each diapause phase in Loch Etive (p-value > 0.05). Due to the absence of normality and homoscedasticity, only non-parametric tests were applied.

For each location, the variation in prosome length, LAPA, activity level and PNmax over the diapause phases was tested with a Kruskal-Wallis test. If significant ($p < 0.05$), then differences among diapause phases were determined by pairwise Wilcoxon tests with a Holm's correction. If the variables showed a significant difference which was visually difficult to distinguish, the effect size was calculated using Cohen d method and the package effsize on R (Torchiano, 2016).

For each diapause phase, prosome length, LAPA, activity level and PNmax were compared between Loch Etive and Ramfjord with unilateral Wilcoxon tests.

All statistical tests were performed with a 5 % level of significance. All the graphs and statistical tests were performed in R (version 4.01) (R Core Team, 2020).

3 Results

3.1 Hydrography

In Ramfjord, during the mid-overwintering phase (September-December), surface waters cooled from ~12 °C to 6 °C, decreasing gradually with depth to 5 °C at the bottom (Figure 1c). In Loch Etive, during the mid-overwintering phase (September-November) the water column was relatively warm (12 °C) and homogenous (Figure 1d). Between January and May (i.e., late overwintering phase and the active period), the water temperature decreased slightly from 4/5 °C to 1/2 °C. During this period the water column was particularly homogenous in terms of temperature. From May on, the water column started to stratify in terms of temperature with the surface waters becoming warmer than the bottom ones. The water temperature increased between May and September (early overwintering phase). Loch Etive had a stable water column temperature below 50 m depth from July to November.

3.2 Morphology

The average prosome length of *Calanus finmarchicus* CV was higher in Ramfjord (early: 2.6 ± 0.1 mm, mid: 2.4 ± 0.2 mm, late: 2.3 ± 0.2 mm) than in Loch Etive (early: 2.4 ± 0.1 mm, mid: 2.3 ± 0.1 mm, late: 2.3 ± 0.1 mm) during early and mid-overwintering phase (bilateral Wilcoxon p-value < 0.05), while during the late overwintering phase there was no significant difference in average prosome length between Ramfjord and Loch Etive (bilateral Wilcoxon test p-value = 0.18) (Figure 2a). In Ramfjord, the average prosome lengths decreased with each subsequent overwintering phase (Table 2). For Loch Etive, the average prosome length during early overwintering was significantly higher compared to the mid-overwintering phase (Table 2). However, the average prosome length was not significantly different between mid-overwintering and late overwintering and between late and early overwintering (Table 2).

The average lipid sac area to prosome area ratio (LAPA) of CVs was lower in Ramfjord (early: 0.25 ± 0.2, mid: 0.49 ± 0.1, late: 0.34 ± 0.12) than in Loch Etive (early: 0.55 ± 0.09, mid:0.50 ± 0.09, late:0.48 ± 0.08) during each of the overwintering phases (bilateral Wilcoxon p-value < 0.05) (Figure 2b). In both locations, the average LAPA decreased in each subsequent overwintering phase (Table 2) (in Loch Etive Cohen d > 0.6), except for Ramfjord where the average LAPA was lowest in the early overwintering phase. However, during that phase the variation in the LAPA was twice as high as during any other overwintering phase in Ramfjord or Loch Etive. This large variability is caused by a low LAPA in June (the lowest LAPA measured over the study period (0.16±0.15)), while in August (0.47±0.10) the LAPA was higher and relatively close to the values measured during the other overwintering phase (Figure 2c).

Seasonal and spatial variation in the total lipid content (TL) (Figure 2 d), which were reflecting those displayed by the LAPA. The TL was similar in Ramfjord (early: 0.15 mg (without June), mid: 0.14 mg) and Loch Etive (early: 0.16 mg, mid: 0.12 mg) during the early and mid-overwintering phases (bilateral Wilcoxon p-value>0.05), but TL was lower in Ramfjord (0.07 mg) than in Loch Etive (0.12 mg) (unilateral Wilcoxon p-value <0.05) during the late phase. In Ramfjord, the TL during the late overwintering phase was significantly lower than during the early and mid-overwintering phase (Table 2) while in Loch Etive the TL during the early overwintering phase was significantly higher than during the mid and late overwintering phase (Table 2) (Cohen d > 0.8).

3.3 Locomotor activity

The activity level (Act_{level}) was consistently lower in individuals from Ramfjord (early: 8.8 ± 9.7, mid: 7.0 ± 6.0, late: 5.1 ± 6.0) compared to individuals from Loch Etive (early: 14.7 ± 13.0, mid: 11.1 ± 8.4, late: 11.3 ± 9.5) (unilateral Wilcoxon tests p-value < 0.05). The average activity level in Ramfjord was reduced slightly during late overwintering when compared to

328 earlier phases (Figure 3a), with significant differences observed only when compared to mid-
329 overwintering (Kruskal-Wallis p-value = 0.0009, Table 2). For Loch Etive, the activity level
330 was consistent across overwintering phases (Kruskal-Wallis p-value = 0.31).

331 Rhythmic activity with a period in the circadian range (20 to 28 h) was detected in both
332 locations and for each experiment. In Ramfjord, there was high seasonal variability in the
333 percentage of individuals expressing rhythmic swimming activity under ambient temperature
334 and photoperiod conditions, varying from 7 % in January to 82 % in August. On average, 39 ±
335 25 % of the Ramfjord population displayed rhythmic activity. In Loch Etive, the percentage of
336 rhythmic individuals was higher and relatively stable throughout the different experiments,
337 varying from 57 % in January to 83 % in September and averaging to 71 ± 9 % over the entire
338 study (Table3).

339 Rhythm strength (PNmax) did not show a clear seasonal variation in Ramfjord (Figure 3b).
340 The average PNmax differed significantly between the mid and late overwintering phase
341 (pairwise Wilcoxon p-value = 0.049) while the average PNmax was not significantly different
342 between early and mid-overwintering and between late and early overwintering phase. In Loch
343 Etive, PNmax did not vary among overwintering phases (Kruskal-Wallis p-value = 0.08)
344 (Figure 3b).

345 A significant peak in activity over the diel cycle was detected for each experiment for
346 populations from both locations (Rayleigh tests p-value < 0.05). In Ramfjord, daytime peaks
347 in activity were detected between October and February. These peaks were centred over a wide
348 range of time, 12:00 in October, 14:30 in November, 10:00 in December, 14:30 in January and
349 13:00 in February (Figure 4c). From October to December and in January the activity intensity
350 during the peak in activity was relatively low (shortest mean resultant length (ρ)) (Figure 4c).
351 In August and September, the peaks in activity happened during times when individuals were
352 exposed to darkness, with peaks centred around 01:00 in August and 00:00 in both September

experiments (Figure 4a & c). During these months, the activity intensity during the peak was high (longest ρ) (Figure 4c). During the experiment in June, the individuals were exposed to constant illumination and the peak in activity was centred around 20:00 with a relatively high activity intensity over the entire 24 h period and a short mean resultant length (Figure 4a & c). In Loch Etive, the peaks in activity were centred around mid-day (Figure 4d), except for July and November. In July and November, the peaks in activity were observed during night-time around 3:30 and 6:00, respectively (Figure 4b & d). In July, the activity intensity was relatively high throughout the 24 h period. The peaks in activity were more marked during the late diapause in February and March (Figure 4b & d). The shortest mean resultant length was observed in October and November.

4 Discussion

Swimming activity of *Calanus finmarchicus* under seasonal ambient photoperiods was used to characterize behaviour during the overwintering period in shallow fjords at sub-Arctic and boreal latitude. We found that regardless of latitude, *C. finmarchicus* maintains diel patterns of swimming activity consistent with a resting state rather than a torpid state of 'classical' diapause as encountered in the mesopelagic (Hirche, 1996, Kaartvedt, 1996, Grigor et al., 2022). However, at the sub-Arctic latitude the individuals were larger, less fat and less active compared to the boreal latitude. The differences between the two locations and their implications for *C. finmarchicus* metabolism are discussed below.

4.1 *Calanus finmarchicus'* winter resting

In both Loch Etive and Ramfjord, seasonal changes in swimming activity suggest that *C. finmarchicus* displays a different behaviour whilst overwintering in shallow fjordic environments compared to a 'classical' diapause as observed in deep Atlantic/ Arctic off-shelf waters. Here, Grigor et al. (2022) used the LAM methodology to compare the swimming activity of *C. finmarchicus* from epipelagic layers that presumably where not overwintering to

individuals from the mesopelagic that were considered as diapausing. They found that individuals residing in mesopelagic layers in late August maintain very low swimming activity (between 1 and 2 beam breaks 30 min^{-1}). While we detected peaks in activity in each experiment at both locations in the early and late overwintering phases, the peak in activity throughout the mid-overwintering phase was low. Since swimming accounts for 30 to 40 % of the metabolic costs in copepods (Alcaraz and Strickler, 1988), we interpreted the low intensity of the peak in activity during mid-overwintering as a reduction in metabolism. *Calanus finmarchicus* diapause is defined as a physiological state characterized by a reduced metabolism and torpidity; a definition based on observations of animals from deep environments (Hirche, 1996). Conversely, studies from shallow environments on the eastern side of the Atlantic suggest that *C. finmarchicus* may remain active during the overwintering period (Tande, 1982, Hirche, 1991), which is supported by our observations. The activity levels measured during our study (5 to 8 breaks 30 min^{-1} in Ramfjord and 11 to 14 breaks 30 min^{-1} in Loch Etive) are close to those reported for *C. finmarchicus* residing in epipelagic layers (12 to 15 breaks 30 min^{-1}) in the Fram Strait in late August (Grigor et al., 2022). In that study, mesopelagic *C. finmarchicus* displayed considerably lower activity levels (and respiration rates) than epipelagic individuals, suggesting that the mesopelagic copepods were in deep diapause (Grigor et al., 2022). Grigor et al. (2022) also observed higher variance in the swimming activity in the population residing in epipelagic layers (10.8 to 17.9) compared to those at mesopelagic layers (2.11 to 3.63), indicating higher heterogeneity in the activity within the epipelagic population (i.e., some were more active than others). We observed a variance close to what Grigor et al., (2022) observed in the epipelagic for all overwintering phases (Ramfjord: 6 to 9, Loch Etive: 8.4 to 13). Furthermore, Grigor et al. (2022) found that ~5 beam breaks 30 min^{-1} marked the upper limit of mesopelagic activity and the lower limit of epipelagic activity, which corresponds to the lowest limits found in Ramfjord. These observations further

strengthen our interpretation that *C. finmarchicus* in our study never reached a state of diapause comparable to that observed in deep-dwelling copepods by Grigor et al. (2022). It is still not fully understood what regulates the activity of overwintering copepods. Freese et al., (2016) showed that the closely related *Calanus glacialis* overwintering in a shallow Arctic fjord substantially reduce their digestive enzymatic activities in winter, but this reduction seemed to be regulated by food availability rather than being triggered by internal or behavioural factors related to overwintering. The term "active diapause" has previously been used for other copepod species such as *Acartia longiremis* and *Pseudocalanus acuspes* to describe a state where metabolism is reduced but the organisms remain partially active (Corner et al., 1974, Elgmork, 1980, Williams and Conway, 1982, Williams and Conway, 1984, Næss and Nilssen, 1991, Norrbin, 1996, Svetlichny et al., 1998, Grigor et al., 2022). Since diapause is generally described as a phase of dormancy with delayed development and reduced metabolic activity, the term "active" diapause is somewhat contradictory, and instead, we suggest describing the state of overwintering *C. finmarchicus* in shallow environments as entering a "winter resting" state.

During the winter resting phase, rhythmicity in the population in response to ambient photoperiods was overall lower, the rhythms in activity were weaker, and the individuals were less active at the sub-Arctic latitude than at the boreal location. Furthermore, the activity level remained constant during winter resting in Loch Etive, while in Ramfjord the animals became less active during the late overwintering phase. In flies, temperature and photoperiod have been described as factors with an important influence on flying, with high temperatures resulting in more flight and increased rhythmicity while both long and short photoperiods induce lower rhythmicity (Kauranen et al., 2012, Kauranen et al., 2016). It has been suggested that these factors synergically impact the flying activity of flies, with a combination of temperature and photoperiod supporting a more robust rhythm and higher activity level (Kauranen et al., 2012,

Kauranen et al., 2016). We suggest that for *C. finmarchicus* the photoperiod and temperature conditions encountered in Loch Etive facilitate increased and rhythmic swimming behaviour.

4.2 *Calanus finmarchicus'* seasonal response to photoperiod

Photoperiodic responses were characterized by the appearance of peaks in activity coincident with either the light or dark phase of the laboratory diel cycle i.e., day or night. We hypothesize that the response to light is a negative phototactic reaction (Miljeteig et al., 2014) with the individual trying to escape the light and as it moves throughout the tube it increases the number of beam breaks (Miljeteig et al., 2014). However, this hypothesis does not explain the behaviour observed in individuals from Ramfjord during the June experiment, when the individuals were exposed to constant illumination and nevertheless showed a distinct peak in activity in the population. The maintenance of an activity rhythm under constant light conditions in the absence of any other environmental cue is a sign that the internal clock can entrain a rhythm, which has been demonstrated in *C. finmarchicus* (Haefker et al 2017). Such endogenous rhythms are commonly observed in locomotor activity studies (Bregazzi and Naylor, 1972, Sánchez-Vázquez and Tabata, 1998), particularly in flies which can maintain rhythms for weeks under constant environmental conditions (Dubruille and Emery, 2008). However, in terrestrial environments constant illumination is also associated with a decrease or an absence of rhythmicity (Van Oort et al., 2005, Kauranen et al., 2012, Menegazzi et al., 2017, Beauchamp et al., 2018, Bertolini et al., 2019). In Ramfjord, under constant illumination during mid-summer, only 8% of the population was rhythmic, one of the lowest rhythmicity recorded over the entire study. This low percentage of rhythmic individual indicates that a peak in activity is only present in a low proportion of the studied individuals while the majority of the population did not show a peak in activity. It is likely that *C. finmarchicus*, like many marine organisms (Naylor, 2010), cannot maintain rhythmic activity for a long time in absence of environmental cues (Häfker et al., 2017). In its natural environment in Ramfjord, *C.*

finmarchicus is exposed to daily variations in light intensity and spectral quality, despite the presence of midnight sun, due to diel shift in the sun altitude above the horizon and the light attenuation in the water column (Miller et al., 1991, Cohen et al., 2020). Thus, we hypothesize that *C. finmarchicus* did possess a functional biological clock prior to the experiment (Häfker et al., 2017), but the overt absence of environmental cues during the experiment led to the rapid loss of the rhythm. The peak of activity during night in August and September in Ramfjord and July and November in Loch Etive remains unexplained. Under natural conditions, such a behaviour could indicate active feeding during nighttime, which *a priori* differs between summer, when more food is available than during the overwintering period. However, as our experiments were conducted without food supplied, we are not able to conclude if this behaviour reflects feeding activity. Furthermore, physiological difference (such as change in metabolic rate) may also be involved in this behaviour. Based on our experiments we are not able to explain this peak in activity during night time and additional studies are needed testing multiple factors (such as food concentration, temperature changes, predator presences) that could evoke this behaviour.

4.3 *Calanus finmarchicus'* morphology

While we found differences in body size and lipid content between the two study sites, these were most likely related to differences in temperature and food availability, rather than photoperiod. However, seasonal changes in the morphological parameters provide some insights into the seasonal changes in activity. We show that body size (i.e., prosome length) was within ranges previously reported for *C. finmarchicus* CV in similar environments as our sampling locations, e.g., Disko Bay (Madsen et al., 2001) and Georges Bank (Miller et al., 2000). Overall, the individuals from Ramfjord were bigger but had less lipid reserves than the individuals from Loch Etive. We suggest that the temperature dependency of *C. finmarchicus,* where higher temperatures stimulate faster development and shorter generation times resulting

478 in smaller body size (Huntley and Lopez, 1992, Campbell et al., 2001, Møller et al., 2012),
479 may explain these size differences.

480 Since low temperature lead to a longer development time and a higher investment in reserve
481 building it is expected for *C. finmarchicus* in Ramfjord to have more lipid reserve than in Loch
482 Etive (Pepin and Head, 2009). However, we observed a lower LAPA in Ramfjord with an
483 amount of lipid similar to Loch Etive indicating that *C. finmarchicus* did not accumulate more
484 lipid than in Loch Etive as expected. Furthermore, a higher loss of lipid was observed in
485 Ramfjord with a loss of 27 % of the lipid reserves and 12 % in Loch Etive. In Ramfjord, the
486 LAPA and total amount of lipid was lower during the early overwintering phase compared to
487 the other overwintering phase because of the low LAPA and total lipid content in June. We
488 concluded that most CVs individual did not accumulate enough lipid in June and were probably
489 not yet ready to overwinter despite residing at depth. Consequently, if we excluded June from
490 the early overwintering phase when calculating the lipid consumption over the overwintering
491 period, we see a clear decrease in lipid content over the overwintering period (August-
492 February) in both locations. This loss of lipid throughout the overwintering period is in
493 agreement with previous studies showing high lipid consumption during overwintering in
494 shallow environments (e.g., 50 % of the lipid reserve), as compared to the limited lipid
495 consumption during diapause in deeper environments (5 % of the lipid reserve) (Jónasdóttir,
496 1999, Campbell et al., 2004, Clark et al., 2012). An important loss of lipid during the
497 overwintering has also been reported in deep environment on the in the Labrador Sea (Pepin
498 and Head, 2009). We suggest that the more marked lipid decrease in Ramfjord compared to
499 Loch Etive is the result of more active feeding activity during overwintering in Loch Etive
500 compared to Ramfjord and/or a higher consumption of lipid in Ramfjord. The light cycle at
501 high latitudes constrains primary production. At boreal latitudes, light may reduce the primary
502 productivity during autumn and winter, but under favourable nutrient conditions, elevated

primary production is possible year-round (Wood et al., 1973, Brand, 2018). Furthermore, Loch Etive has considerable suspended organic matter (Ansell, 1974, Brand, 2018), which could enhance *C. finmarchicus* feeding activity during overwintering as this species can switch its dietary preferences i.e., to detritivory when preferred food items become scarce (Marshall and Orr, 1958, Butler et al., 1970, Corner et al., 1974, Ohman and Runge, 1994, Hirche, 1996). The larger body sizes observed in Ramfjord are energetically more costly in terms of maintenance (Saumweber and Durbin, 2006) and could explain a higher consumption of lipid than in Loch Etive. However, in Loch Etive the individuals are also subject to high lipid consumption due to the high temperature of their environment. Consequently, it is impossible to establish if lipid consumption is higher is Ramfjord than in Loch Etive with the data collected in this study. A more detailed energetic budget would be necessary to do so. The combination of lipid consumption rate and food availability explain both the counter-intuitive higher lipid fullness at boreal latitudes and the higher activity level. In summary, at boreal latitudes, more energy can be allocated to swimming activity and larger lipid reserve can be accumulated, due to less energy being allocated to metabolism maintenance and better availability of food resources.

Calanus finmarchicus CV body size decreased progressively through the overwintering period in sub-Arctic latitudes but not at boreal latitudes. The production of a second generation has previously been suggested for the population in Ramfjord, with a first generation being produced around the spring bloom (i.e., April/May) and the second one in early autumn (Coguiec et al., 2021). Since *C. finmarchicus* growth is temperature dependent (Huntley and Lopez, 1992, Campbell et al., 2001, Møller et al., 2012), and the surface water is warming from late spring, the ontogenetic development of the second generation is shorter than that of the first generation, which could result in animals with a smaller body size entering the overwintering population later in the season. This could explain the decrease in average

prosome length in Ramfjord, especially between the early and mid-diapause phases. A difference in prosome length between two generations of the same population due to differences in surface water temperature has previously been described for the Newfoundland continental shelf and the Labrador Sea (Pepin and Head, 2009). The advection of the second generation in Ramfjord from Atlantic water can similarly explain the decrease in prosome length. Advection in Ramfjord has previously been reported at the time of the mid-overwintering period (Coguiec et al., 2021). Changes in average prosome length during overwintering can also be explained by early moulting of the bigger individuals to adults. Kosobokova (1998) found that during autumn/winter in the White Sea *Calanus glacialis* males derive from larger CV individuals and appear earlier than females in the population, and such mechanisms would lead to a decrease in prosome length of the CVs' population. Finally, we cannot exclude that a seasonal change in predation pressure on larger individuals may have pushed the population towards smaller body sizes. In Loch Etive, it has been shown that *C. finmarchicus* produces a single generation (Hill, 2009, Clark et al., 2012) but a more recent study found two generations in Loch Etive and suggest that the nutrient enrichment due to fish aquaculture might explain the transition to two-generation a year population dynamics (Häfker et al., 2018). Our prosome length data do not indicate a change in the population size structure that may indicate the appearance of a second generation. However, given the rather stable water surface temperature during spring and winter in Loch Etive, a second generation may not necessarily be characterized by a difference in prosome length and would therefore not be detectable as a change in average prosome length.

4.4 Methodological consideration

A disturbance of the diapause state due to rapid changes in pressure, temperature and light during sampling as well as due to the light exposure during sorting is a concern with the method we used. To keep light exposure at a minimum, the sampled organisms were transfer to a

container immediately after collection which was sealed at once, and the organisms were kept in darkness until the start of the sorting. During the sorting, the room was maintained dark, but organisms were expose to the light emitted by the stereomicroscope. However, Miller et al., (1991) demonstrated that following an exposure that can trigger the diapause exit, a minimum of 10 days were needed to reverse the diapause state. Thus, we find it unlikely that sampling and setting up the experiments would severely disturb a diapause state. We are convinced that our method is suitable to detect diapause/ overwintering, especially since the focus is on a comparison with non-overwintering individuals, either looking at seasonal variability (this study) or spatial variability (Grigor et al., 2022). But swimming activity as an indicator of overwintering and/or diapause is mainly applicable on population level, while characterizing the mechanism of diapause at the individual level remains a challenge. This limitation complicates the investigation of the associated physiological processes, particularly for populations inhabiting shallow environments as the metabolism reduction is not as marked as in deeper environments. The development of molecular markers to identify diapause and winter resting is essential to increase the understanding of the physiological process behind overwintering (Tarrant et al., 2016; Skottene et al., 2019; Lenz et al., 2021).

5 Conclusion

This study helps improve the definition of the overwintering period in *C. finmarchicus* as a gradient in behaviour and physiology. It provides additional evidence towards the adaptability of *C. finmarchicus* to local environments (Tarling et al., 2022a) and may have implications for *C. finmarchicus* in a future warmer world, specifically winter survival, since increased swimming activity may deplete lipid reserves faster (Tarling et al., 2022b). This adaptability may be constrained where and when winter food supplies are restricted. Indeed, high winter mortality in shallow environments in Svalbard in both *C. finmarchicus* and *C. glacialis* have been attributed to insufficient energy stores to sustain activities throughout winter (Daase and Søreide, 2021). However, our observations from Loch Etive suggest that elevated food availability throughout winter allows for sustained activity and less dependence on lipid reserves, supporting a recent model study which showed that exiting diapause early does not lead to poorer fitness as long as food is available (Hobbs et al., 2020). It will now be important to determine how elevated swimming activity during overwintering may affect the energy budget of *C. finmarchicus* in predicting how climate warming may affect their fitness during overwintering. Finally, a gradient in swimming activity of overwintering copepods provides an opportunity for molecular studies to elucidate fundamental processes in diapause by characterizing different overwintering phenotypes in relation to genotype.

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8 Figures

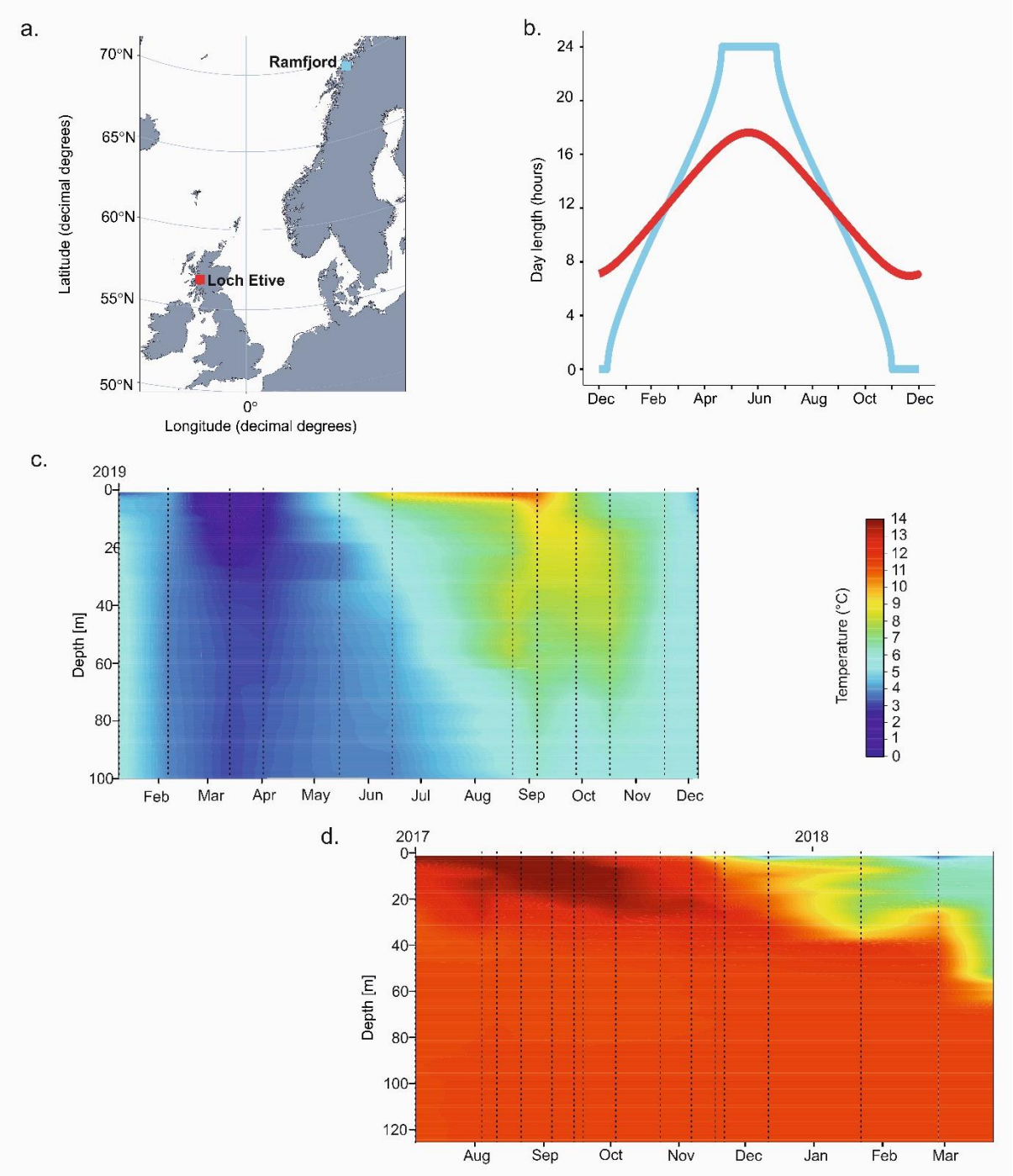

Figure 1: Map of the sampling station locations (a) and day length curve (b), temperature contour plot in Ramfjord (c) from January 2019 to December 2019 and Loch Etive (d.) from July 2017 to March 2018. The black dotted lines in the contour plots are showing the time of the CTD profile collection.

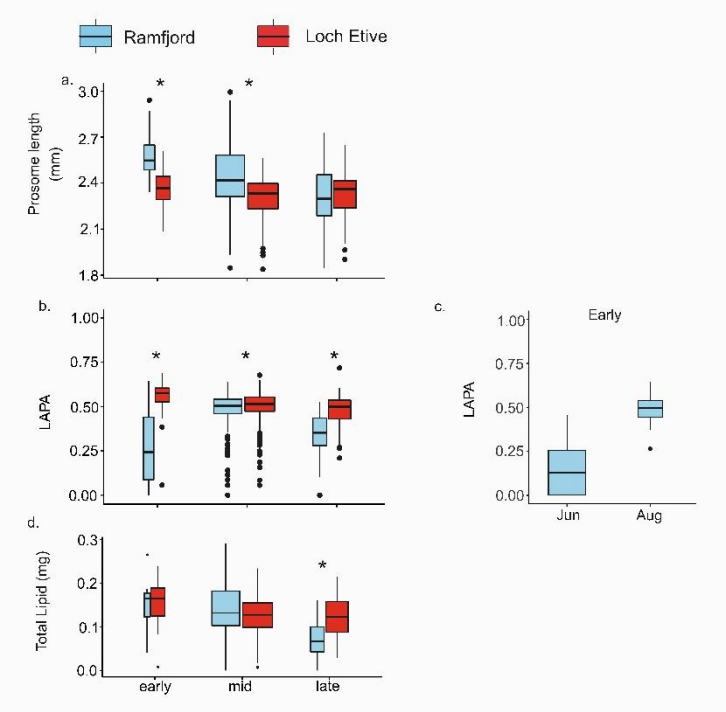

Figure 2: Variations in prosome length (a.), lipid sac area to prosome area ratio (LAPA) (b.) and total amount of lipid in mg (d) of Calanus finmarchicus CV during the different overwintering phases in Ramfjord (blue) and Loch Etive (red), and variation of the LAPA during the early overwintering in Ramfjord (c.). The early overwinter does not include data from June for the Total Lipid but June is included for the prosome length and LAPA. The asterisks indicate when the variable is significantly different in Ramfjord and Loch Etive for a given phase. The width of each box is proportional to the number of individuals used during each diapause phase.

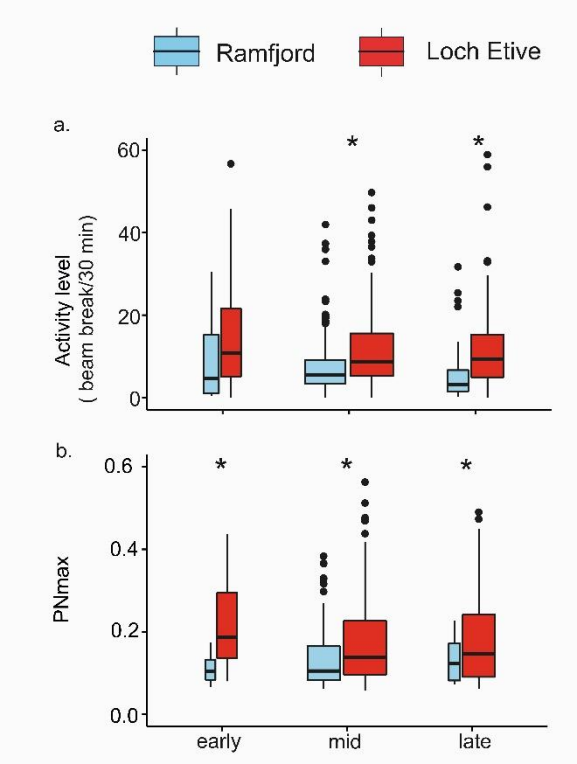

Figure 3: Variations in activity level (beam break/30min) (a.), peak normalized power (PNmax) (b.) of Calanus finmarchicus CV during different overwintering phases in Ramfjord (blue) and Loch Etive (red). The asterisks indicate when the variable is significantly different in Ramfjord and Loch Etive for a given phase. The width of each box is proportional to the number of individuals used during each overwintering phase . The significant p-value are marked in green.

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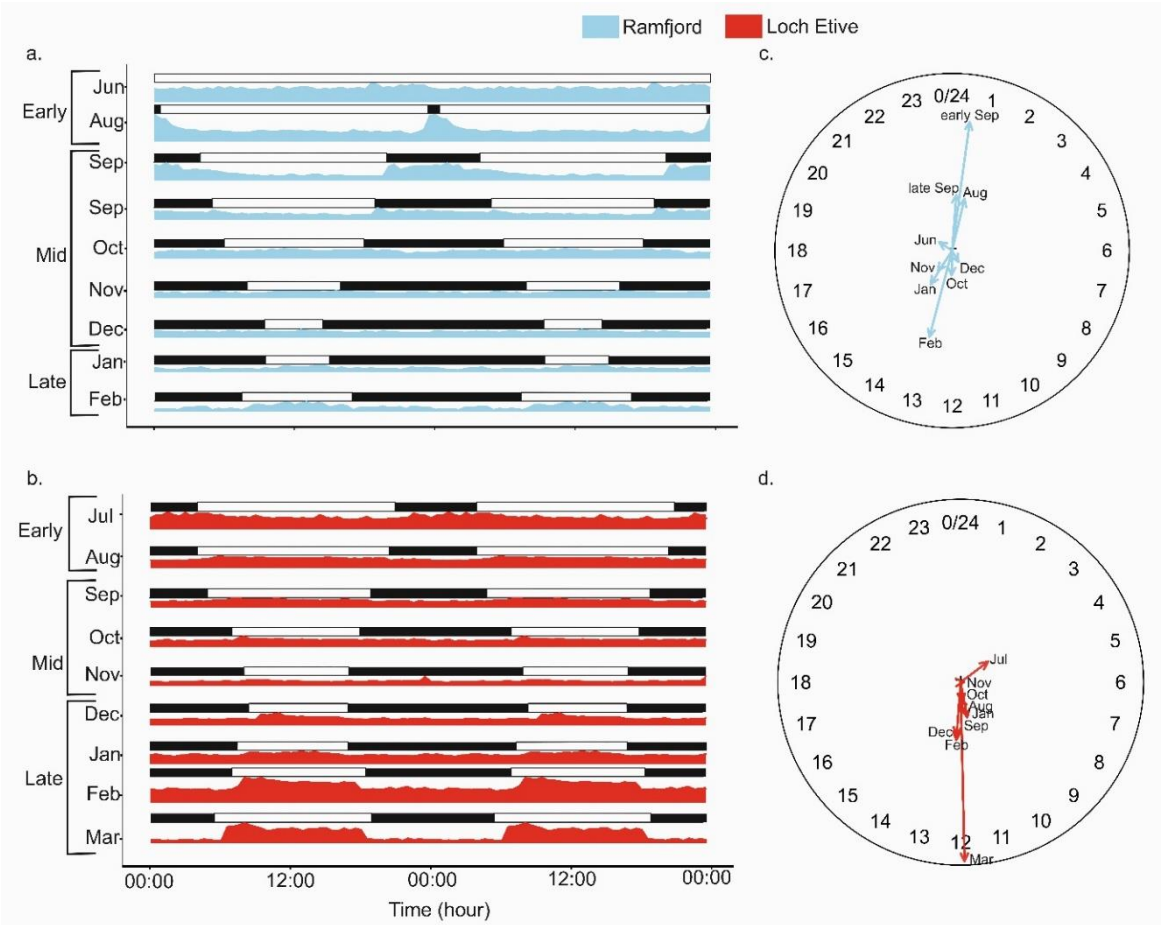


Figure4: Actogram of the daily activity (left panels) and the mean angle (μ) and mean resultant length (ρ) (right panel) of the daily activity over the 3 first days of the experiment obtained by combining all the individual sampled during each month in Ramfjord (a & c) and Loch Etive (b & d). For the actogram, the 24 hours were doubled and joined+ to have better visualisation during the night time. The black segments on top of each actogram, indicate the period when the light was off. The overwintering phases corresponding to the month are indicated on the left side of the y-axis. All months had non-uniform circular distributions (Rayleigh test $p<0.05$). Mean angles correspond to the time of day for peak activity in a given month, with the mean resultant length (plotted here as $\rho*2.5$, for clarity) corresponding to the strength of that activity. Months are labelled for each mean vector.

879 *Table 1: Overview of the sampling events, experimental settings and composition of each experiment. The light cycle format indicates the number of hours of light per day: the number of hours*
880 *of darkness per day. The number of Calanus correspond to the number of individuals used in each experiment, the number of CVs indicates how many of these were stage CV. Number of Calanus*
881 *finmarchicus, Calanus glacialis and unknown is indicated.*

882

location	date	CTD	Nets	experiment duration	light cycle	number Calanus sampled	number Calanus CV	number dead Calanus CV	C. fin	C. gla	unknown
Ramfjord	09/01/2019	x	x	4d	4.5 L : 19.5 D	64	54	5	43	5	1
	06/02/2019	x	x	6d	8.5 L : 15.5 D	64	36	7	27	0	2
	13/03/2019	x	x	6d	13.5 L : 10.5 D	32	1	0	1	0	0
	01/04/2019	x	x	6d	17 L : 7 D	30	4	0	2	2	0
	14/04/2019	x	x	5d	19 L : 5 D	50	2	0	0	2	0
	14/05/2019	x	x	4d	24 L : 0 D	32	10	0	0	10	0
	13/06/2019	x	x	6d	24 L : 0 D	64	52	0	26	25	1
	20/08/2019	x	x	7d	22 L : 2 D	64	50	10	11	28	1
	03/09/2019	x	x	7d	15 L : 9 D	122	92	3	30	24	35
	25/09/2019	x	x	7d	13 L : 11 D	96	78	1	50	23	4
	14/10/2019	x	x	6d	11 L : 13 D	128	117	1	60	51	5
	14/11/2019	x	x	5d	7 L : 17 D	128	108	2	50	56	0
	03/12/2019	x	x	6d	4 L : 20 D	116	100	0	72	23	5
Loch Etive	04/07/2017		x	5d	16 L : 8 D	94	89	23	65	-	-
	11/07/2017	x				-	-	-	-	-	-
	03/08/2017	x				-	-	-	-	-	-
	10/08/2017	x				-	-	-	-	-	-
	21/08/2017	x				-	-	-	-	-	-

	date	CTD	Nets	experiment duration	light cycle	number Calanus sampled	number Calanus CV	number dead Calanus CV	C. fin	C. gla	unknown
Loch Etive	23/08/2017		x	5d	14.5 L : 9.5 D	93	87	3	83	-	-
	04/09/2017	x				-	-	-	-	-	-
	14/09/2017	x				-	-	-	-	-	-
	18/09/2017	x	x	5d	13 L : 11 D	96	80	0	80	-	-
	03/10/2017	x				-	-	-	-	-	-
	23/10/2017	x	x	6d	10 L : 14 D	96	67	1	67	-	-
	06/11/2017	x				-	-	-	-	-	-
	17/11/2017	x				-	-	-	-	-	-
	21/11/2017	x	x	8d	8 L : 16 D	96	79	0	79	-	-
	27/11/2017	x				-	-	-	-	-	-
	11/12/2017	x	x	6d	7.5 L : 16.5 D	96	88	1	87	-	-
	22/01/2018	x	x	6d	8.5 L : 15.5 D	96	95	32	63	-	-
	26/02/2018	x	x	6d	10.5 L : 13.5D	96	19	0	19	-	-
	23/03/2018	x	x	5d	12.5 L : 11.5 D	96	7	0	7	-	-

883

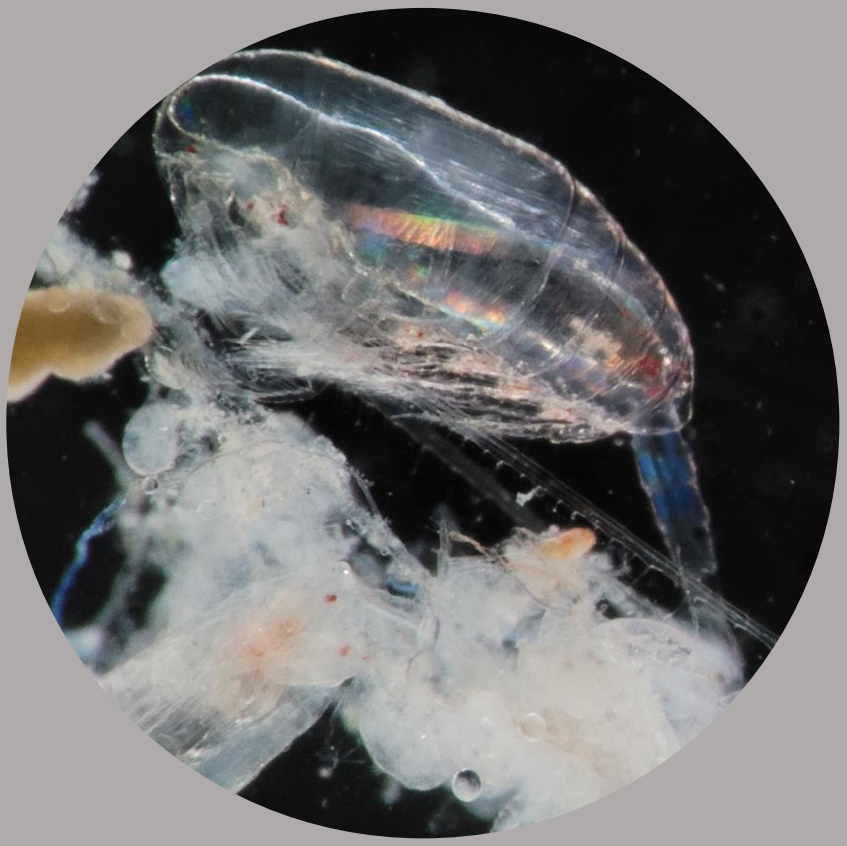
884 *Table 2: Table summarizing the p-value of the pairwise wilcoxon test comparing the variable (prosome length, LAPA, total*
885 *lipid, Activity level and PNmax) over the overwintering phase for each location. The bold and underline numbers indicate a*
886 *significant difference.*

		Ramfjord		Loch Etive	
		early	mid	early	mid
Prosome Length	mid	**0.0001**	-	**0.0059**	-
	late	**1.10E-09**	**4.40E-06**	0.1175	0.1071
LAPA	mid	**1.30E-10**	-	**6.10E-09**	-
	late	**0.043**	**< 2e-16**	**1.90E-11**	**0.0022**
Total Lipid	mid	0.38613	-	**3.60E-07**	-
	late	**0.00019**	**5.40E-15**	**8.60E-07**	0.65
Activtiy level	mid	0.92	-	0.42	-
	late	0.92	**0.0003**	0.42	0.99
PNmax	mid	0.049	-	0.11	-
	late	0.065	0.499	0.11	0.67

887

Paper III

Calanus spp. swimming behaviour under laboratory conditions as a proxy for diel vertical migration in the wild.



Calanus spp. swimming behaviour under laboratory conditions as a proxy for diel vertical migration in the wild.

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Keywords: zooplankton, *Calanus finmarchicus*, behaviour, swimming activity, photoperiod, Diel vertical migration

Abstract

Zooplankton swimming behaviour studies under laboratory conditions are commonly used to infer zooplankton diel vertical migration (DVM). But DVM is a flexible behaviour which changes according to the community composition, predation pressure and light conditions, factors which are difficult to reproduce in laboratories, making the *in-vitro* behaviour less comparable to the natural *in-situ* behaviour. Here, we investigated the relationship between the swimming behaviour of *Calanus* spp. and the DVM behaviour in the zooplankton community in the wild. Swimming activity of *Calanus* spp. from an Arctic fjord (Ramfjord) was monitored monthly on wild individual in the laboratory over a period of nine months. At the same time, we used moored active acoustic instruments to record DVM patterns in Ramfjord. We found that behaviours in the laboratory and the wild are tightly coupled to the ambient seasonality in light (photoperiod) with aligned seasonal variations. We identified three swimming behaviour in *Calanus* spp. over the study period, a negative phototactic response in April, an asynchronous swimming behaviour during constant illumination and long photoperiod exposition and lighter negative phototactic response from late September. In terms of DVM we identified three phase one of classical DVM, one of unsynchronised DVM and one with several classical DVM behaviour identified.

1. Introduction

Interactions between organisms and their environment are complex to investigate, especially in the marine realm due to its vastness, inaccessibility and three-dimensional nature. Zooplankton are particularly difficult to observe under natural conditions given their small size and their transitory presence, caused by active vertical migration or horizontal transportation by currents. Individual behaviour (such as feeding, mating and predator avoidance) is therefore commonly studied under laboratory conditions, usually on low numbers of individuals (Landry and Hassett, 1982, Head, 1986, Li et al., 2001), while behavioural responses of populations, such as vertical migrations, are generally observed *in-situ* using acoustical and optical sensors (Hays, 2003, Benoit-Bird and Lawson, 2015). Recreating the natural conditions of underwater environment in the laboratory i.e., light propagation, pressure changes, currents or predation risk, are difficult or impossible, especially when we consider the combined effect of multiple conditions in parallel. Indeed, it is questionable if laboratory behaviour of zooplankton is even reflective of natural behaviour, and it has been suggested that the optimal approach is to pair laboratory observations with those in the wild (Cohen et al., 2021).

One of the best studied, yet paradoxically least understood marine migrations is that of Diel Vertical Migration (DVM) of zooplankton. DVM usually consists of a migration from surface waters during night time to deeper waters during day time and is largely believed to be a strategy to avoid visual predators strategy (Ringelberg, 2010) where the depth and speed of migrations are regulated by light (Hays, 2003). This so called 'classical' DVM results from a trade-off between feeding in the productive surface waters while also avoiding visual predation by hiding in the dark deep water during daytime (Bandara et al., 2021). DVM behaviour varies seasonally (Wallace et al., 2010), geographically (Hobbs et al., 2018, Wiebe et al., 2022) and with different light regimes (Berge et al., 2020). DVM has been well described using active acoustics but this approach only provides information at the community/population level (Hays, 2003, Benoit-Bird and Lawson, 2015), with little insight into the taxonomic composition of the migrating population or individual behaviour (Berge et al., 2014, Benoit-Bird and Lawson, 2015).

To understand DVM behaviour at the individual level, experimental studies have focused on responses to light (sensitivity to both light quantity and spectral quality, and directionality) and chemical cues, as well as endogenous rhythms in specific species (reviewed in (Cohen and Forward, 2009). With the improvement of methods able to monitor swimming activity and

vertical migration behaviour under constant experimental conditions in the laboratory, there is emerging evidence that endogenous rhythms in swimming activity contribute to DVM (Cohen and Forward, 2009 and references within, Miljeteig et al., 2014, Båtnes et al., 2015, Häfker et al., 2017).

Here we used locomotor activity monitors to document seasonal changes in swimming activity of copepods of the genus *Calanus*. *Calanus* spp. dominate the zooplankton community in Arctic and sub-Arctic seas in term of biomass (up to 80 %) (Niehoff et al., 2002). *Calanus* perform DVM (Bucklin et al., 1995) but the behaviour is highly variable across seasons and geographical regions (Ogi and Rigor, 2013, Berge et al., 2014, Fiksen and Carlotti, 1998, Baumgartner et al., 2011, Falkenhaug et al., 1997a). The aim of this study is to compare seasonal changes in the swimming behaviour observed under experimental laboratory conditions with seasonal changes in natural DVM patterns observed using active acoustics in the wild. We work under the assumption that seasonal changes in the locomotors swimming activity of individuals under laboratory conditions with simulated natural seasonally changing photoperiods reflect seasonal changes in DVM patterns observed in the wild.

2. Method

The study was conducted between April and December 2019 in Ramfjord a shallow (maximum depth: 135 m) and cold (annual average temperature: 5°C) northern Norwegian fjord. Two types of zooplankton behaviour were monitored in this study. *Calanus* spp. swimming behaviour monitored in the laboratory and referred to as *in vitro* behaviour, and the zooplankton community diel vertical migration (DVM) monitored directly in the fjord with acoustic instruments and referred to as the *in-situ* behaviour.

2.1. *In vitro* behaviour

A sampling station (69°31’49.9 N, 19°02’11.9) was established close to the entrance of Ramfjord at a depth of 125 m (Figure 1) and sampled near monthly (Table 1). During each sampling event, zooplankton were collected between 115 and 50m with a WP2 net equipped with a closing mechanism and a mesh size of 180 µm or lower (Hydrobios, Kiel, Germany). The zooplankton were kept in the dark at the ambient environmental temperature in a temperature-controlled room. After one night (max. 18h) in these conditions, copepodites stage V (CV) and adult stage of *Calanus* spp. (Table 2) were carefully picked under a stereomicroscope with soft forceps and individually placed into an acrylic tube (volume 5 ml; diameter 10 mm and length 64 mm) filled with filtered sea water. Each tube was then placed

in a Locomotor Activity Monitor (LAM10, TriKinetics, Waltham, USA). Each monitor has 32 chambers, each equipped with three infrared light beam arrays in its center designed to monitor the movement of an individual organism. A computer recorded the sum of beam breaks over 30 second intervals using the proprietary software (TriKinetics: filesystem.exe). The experiments were run for 5 to 7 days (Table 1) under a natural light/ dark photoperiod cycle (Figure 2 A1). The light cycle was produced with a programmed LED ribbon place above the monitors. The data were processed to provide a measure of activity by summing the number of beam breaks into 30 min intervals with the dedicated software (TriKinetics: filescan.exe). For each experiment, the activity was averaged over all animals for each 30 min interval. The daily activity was calculated by averaging the activity of all the individuals for the month sampled for the first three days of experiment. The daily variation of activity was visualized over time as a double plotted actogram as in Last et al., (2016).

2.2. In situ behaviour

An underwater observatory was moored at close proximity of the sampling station (69°32.005' N, 19°02.904' E) for the duration of the study period (Figure 1). An upward looking Acoustic Zooplankton Fish Profiler (AZFP, ASL Environmental Sciences) was mounted on the observatory at a depth of 108m. Here we use data from the 455 kHz transducer with a ping rate of 20 s to monitor the vertical migration of the zooplankton community. The main acoustic targets at this frequency are copepods. Temperature and salinity were measured by temperature loggers (SBE 65) every 10 to 15 m on the observatory and a Conductivity, Temperature and Depth profiler (CTD; SBE 16) at 18 m depth and a CTD (SBE 19 plus) during each sampling event) were used to calculate sound speed and absorption coefficient for each month (Francois and Garrison, 1982). The AZFP data were cleaned and processed in Echoview (version 12). Echograms were visually scrutinized to remove noise, i.e., from air bubbles. Echoview algorithms were used to remove background transient and impulse noise and attenuate signal following Ryan et al. (2015). The data were integrated over 4 m and 20 min cells to obtain the mean volume backscatter strength (S_v), also referred to as backscatter. S_v data from the deepest layer (102 m) were extracted and plotted as a double plotted actogram as described in Last et al.(2016). The deepest layer was chosen because this represented the full depth of the seasonal migrations as recorded by the AZFP and corresponded to the layer from where the individual for the *in-vitro* experiments were collected. The S_v data were also averaged over weekly intervals (as per Wallace et al. (2010)) and plotted as a 24 h echogram in order to visualise diel

changes in vertical distribution of the backscatter (i.e., DVM behaviour) throughout the entire water column.

2.3. Data analysis

Calanus swimming activity and backscatter (S_v at 102m) were analysed for periodicity, i.e., the presence of a rhythm, with a Lomb-Scargle analysis. For practical reason, the swimming activity was analysed for each individual using R (version 4.0.1) (R Core Team, 2020) with the package lomb (version 2.0) (Ruf, 1999) for the entire experiemental period, while the S_v data were analysed in Matlab (R 2018a) with the package lomb for an interval of 10 days centered around the sampling dates (Table 1). The peak normalized power (PNmax), i.e., rhythm strength, for periods included between 20 and 28 hours, i.e., the circadian range, was extracted and interpreted as an indicator of the synchronicity in the population. For the *in-vitro* behaviour, the PNmax was averaged per sampling event over all studied individuals to obtain the population rhythm strength. Complementary, a Rayleigh test was run on the daily activtiy data to test the uniformity within the data. All experiments showed non-uniform distributions (Rayleigh test, P-value < 0.05). Consequently, the mean angle (μ), i.e. the time around which the peak of activtiy is centered, and the mean resultant length (ρ), i.e., the peak in activtiy intensity in comparaison to the activtiy intensity outside the peak in activtviy, was calculated for each experiment. This analaysis was done in R with the circular package (Agostinelli and Lund, 2022).

3. Results and Discussion

3.1. *In-vitro* behaviour seasonality

Three swimming behaviours were characterized in *Calanus* spp. from Ramfjord *in-vitro*. In April (Phase 1) a strong peak in activity centred around noon (12:00) with a high rhythm strength detected (Figure 2 B1 & Figure 3 C). Increased activity during the photophase was interpreted as a response of negative phototaxis, common in *Calanus* spp. (Miljeteig et al., 2014, Båtnes et al., 2015). This is characterized by downward swimming which, in the confines of the experimental chambers resulted in repeated beam breaks of the activity monitor (confirmed by video observations, unpublished data), and is in agreement with other experiments showing increased swimming during light exposure (Båtnes et al., 2015). While the ultimate effect of DVM is predator avoidance while maximizing food uptake (Lampert, 1989), light is considered as a proximate cue for DVM (Cohen and Forward, 2009) with vertical position in the water being tightly coupled to equal light levels (isolumes) over the course of a

day (Hobbs et al. (2021)). Consequently, the downward migration in a classical DVM behaviour can be regarded as negative phototactic behaviour, i.e., escaping light to hide from visual predators.

In June (Phase 2), the individuals were exposed to constant illumination and the swimming behaviour was characterised by a relatively high and constant swimming activity (Figure 2 A1 and B2). We interpreted this behaviour as an unsynchronised swimming behaviour with increased variability of activity over the day between individual animals supported by the low PNmax values recorded at this time. We suggest that the absence of diel changes in light and consequently the absences of a cue to trigger a synchronised behaviour is causing such behaviour (Dale and Kaartvedt, 2000). Similar, unsynchronised DVM behaviour, has been reported under constant illumination and explained as well by the absence of a light cue to coordinate the vertical migration (Cottier et al., 2006). However, a small peak in activity (i.e., low ρ value) centered around 3:00 am was detected during this phase (Figure 3) which may suggest the expression of an endogenous swimming rhythm (i.e., circadian clock) in some of the animals as reported elsewhere (Häfker et al., 2017). In August (beginning of Phase 3) the same behaviour than in June was observed with reduced overall activity and a peak in activity more important than in June (i.e., ρ value slightly higher) (Figure 2 B1 and Figure 3). The peak in activity was still centered around 03:00 despite the exposure to a 2 h scotophase (i.e. phase of darkness in the dark/night cycle) per day (Figure 2 B1 and Figure 3). This behaviour suggests that the scotophase period was too short to trigger a response to the photophase/ scotophase cycle and that the swimming behaviour peak in activity was trigger by an endogenous cue, like under constant illumination. It has been suggested that a minimum of 4 h of darkness is necessary to trigger synchronised DVM behaviour in zooplankton (Buchanan and Haney, 1980) explaining the unsynchronised swimming behaviour we observed in August (i.e., during Midnight sun period). *Calanus finmarchicus* circadian clocks do not express endogenous swimming rhythms over long periods of time without an exogenous cue (Häfker et al., 2017) suggesting that prior to the experiment, *in-situ* the studied individuals were exposed to some sort of exogenous cue maintaining the circadian clock despite mid-nigh sun. We assume that the diel variation in solar elevation above the horizon during mid-night sun could entertain *Calanus* spp. endogenous clock as shown in the circadian clock gene expression of *C. finmarchicus*. (Hüppe et al., 2020). The differences in endogenous cue between the laboratory and the natural environment explain the slight discrepancy between the unsynchronised *in-vitro* behaviour and the synchronised DVM *in-situ* in August.

In early September (beginning of Phase 3) a peak in activity centered around mid-night was detected in response to the scotophase (Figure 3). This change in the timing of the peak of activity during the day, compare to Phase 1, suggests a possible change in photosensitivity at this time which remains unexplained. We postulate a working hypothesis that during the period when the scotophases are short it would be adaptive to be more active at these times than during the daytime. However, the presence of visual predators when it is light nearly all the time means that it is beneficial to restrict most activity during the safety of the night. Moving from summer into autumn, the scotophases becomes longer and there is more time to forage, reflected in the increasing activity during the scotophase between ….

Between late September and December (Phase 3), *Calanus* spp. reverted to a negative phototactic response with a peak in activity centered around noon (Figure 2 B1, Figure 3). However, in contrast to the observation in spring (phase 1), the overall activity intensity in the autumn was relatively low, and a ρ value equal or lower than in spring. At this time of year *Calanu*s spp. are entering the overwintering phase and a diapause, although it has been suggested that in fjord and shelves this is not represented by complete activity quiescence (Hirche, 1996, Barth-Jensen et al., in prep) unlike in shelf seas or ocean basins (Hirche, 1996, Grigor et al., 2022). Between late September and December with decreasing daylengths, rhythm strength decreased equally (Figure 2 A1 &C1), confirming a tight coupling between the swimming activity and the photoperiod.

3.2. Seasonal changes in *in-situ* behaviour

Different DVM patterns were identified over the study period from the volume backscatter. In April (Phase 1), the backscatter value at depth (102 m) was higher at nighttime than during daytime (Figure 2 B2) suggesting a classical highly synchronised DVM behaviour. However, the backscatter signal was relatively low over the entire water column (Figure 4 A), suggesting that few zooplankton were present during this season. The AZFP only ensonified the water column between 102 m to 50 m and we hypothesize that the organisms were probably located <50 m during nighttime and hence not tractable. This hypothesis is supported by the ascent and descent of the backscatter signal to 50 m at dusk and dawn, respectively (Figure 4 A), and by the presence of a phytoplankton bloom which took place in April (Coguiec et al., 2021) which would have acted as a positive migratory cue for the zooplankton (Huntley and Brooks, 1982, Fiksen and Carlotti, 1998, Dale and Kaartvedt, 2000, Baumgartner et al., 2011).

From mid-April to early August (phase 2) during the Midnight sun period, the backscatter signal was overall low and there was no clear signal of DVM (Figure 2 B2, Figure 4 B). The absence of DVM during this time is expected (late May to late July, Figure 2 A2) as has been reported for several other zooplankton species (Bogorov, 1946, Blachowiak-Samolyk et al., 2006 and references within) although other studies showed DVM despite of midnight sun (Dale and Kaartvedt, 2000, Fortier et al., 2001). The variation of rhythm strength in the acoustic data decreasing from April to June (Figure 2 C2), suggests a loss of synchronicity leading up to the midnight sun period and is supported elsewhere (Cottier et al., 2006, Wallace et al., 2010).

During phase 2, the acoustic backscatter signal of zooplankton migration was not homogenous. Bands of higher backscatter were observed between in May and between the 24th of June and the 4th of July and were near contiguous across the Midnight sun period. Those bands can be explained by the isolume theory (Cohen and Forward, 2009, Hobbs et al., 2021) which describes zooplankton migrate following an optimal light level, i.e., isolume. According to this theory, periods of low backscatter result in the zooplankton community taking refuge in waters deeper than the AZFP, while during periods of higher backscatter the zooplankton inhabit shallower waters and were consequently detected by the AZFP. The position of the elevated backscatter bands in the water column depends on the light conditions and the depth of the optimal light level (Hobbs et al., 2021). Changes in cloud cover or in water clarity can impact the light penetration in the water and consequently the depth of the isolume (Dupont and Aksnes, 2011, Brierley, 2014). However, no relation between these bands and the cloud cover or moon phase were found during our study. More environmental data, particularly on the optical properties of the water and their seasonal changes are necessary to confirm our hypothesis.

Between late August and December (phase 3) two DVM patterns occurring simultaneously were identified. A classical DVM pattern with a strong ascent and descent signal (Figure 2 B2, Figure 4 C) and a small scale DVM at depth represented by a relatively high backscatter at depth which slightly shoaled during night time (Figure 4 C). The occurrence of different DVM patterns occurring simultaneously in different fjords has been previously demonstrated by Berge et al. (2014). Our observations suggest that while some species remain at fixed depth, such as *Calanus* spp. which overwinter during this period (Hirche, 1996, Coguiec et al., 2021), others continue to perform DVM throughout the entire water column through autumn and winter. Species continuing DVM year round include *Metridia longa* (Falkenhaug et al., 1997b,

Daase et al., 2008, Grenvald et al., 2016) and *Meganyctiphanes norvegica* (Tarling, 2003), both of which have been observed in Ramfjord (Coguiec et al., 2021),

Both DVM behaviours show a tight coupling between DVM and light. From late September to November, we observed a clear coincidence between the photoperiod length and the time between descent and ascent. From late November, with extreme short photoperiods we see 'merging' of the ascent and descent signal (Figure 2 B2). Furthermore, between August and late November, the organisms were migrating deeper than between late November and December. We suggest that the species performing classical DVM were only passing through the area detected by the AZFP with daytime positions of animals below the instrument and out of detection, while in November and December during daytime, peak backscatter was up to 80 m. Species performing small scale DVM were found up to 80 m between August and late November and up to 60 m in late November and December.

3.3. The relation between swimming behaviour and DVM

Studies on swimming behaviour in copepods often relate the swimming behaviour to DVM (Båtnes et al., 2015). However, in such setting, the proximate cues triggering DVM are absent (i.e., phytoplankton availability (Huntley and Brooks, 1982, Bandara et al., 2018), dark deep refugia (Hays, 2003) and predators (Bandara et al., 2018)) which limits the extent of the conclusions. Furthermore, those studies relate the swimming behaviour they observe to DVM observed in other studies. However, DVM behaviour is highly flexible and varies seasonally, geographically (Hobbs et al., 2018, Wiebe et al., 2022) and as a function of the community composition (Wallace et al., 2010, Berge et al., 2014). In this study, we paired *Calanus* spp. swimming behaviour in the laboratory with DVM patterns in the zooplankton population in the wild. *Calanus* spp. was chosen to investigate the swimming behaviour as it is one of the dominant species in Arctic and sub-Arctic seas (Hirche, 1991, Mumm et al., 1998, Falk-Petersen et al., 2002, Arnkvaern et al., 2005) and is regarded to be one of the major contributors of the acoustic signal within our chosen frequency range (Berge et al., 2014, Hobbs et al., 2020). In Ramfjord, the mesozooplankton population is largely composed of smaller copepods (i.e., *Oithona similis*, *Microcalanus pusillus*, *Microsetella norvegica* and *Pseudocalanus* spp.) (Coguiec et al., 2021), however *Calanus* spp. is the dominant larger copepod and considered to be one of the main contributors to the acoustic backscatter and particularly the DVM signal. Using 455 kHz frequency, older stage of *O. similis* and *M. pusillus* can be detected according to equations describing the approximative equivalent spherical diameter by Fielding et al. (2004) and Forest et al., (2012). However, those species of small copepod do generally not

perform large scale DVM but rather small-scale DVM in surface waters (Eiane and Ohman, 2004, Madsen et al., 2008, Zamora-Terol et al., 2014). Consequently, we suggest that the acoustic data reflect *Calanus* spp. DVM behaviour and is therefore directly comparable with *Calanus* spp. swimming behaviour in the laboratory. Seasonal change in both laboratory and wild swimming behaviours showed a tight coupling with the seasonality in photoperiod (Figure 2). Three swimming behaviours were categorized into phases: Phase 1 in April when *Calanus* spp. performed a classical DVM behaviour and was highly reactive to light; Phase 2 from mid-April to early August when midnight sun occurred leading to unsynchronous behaviour *in-situ* and *in-vitro*; Phase 3 between early August and December, when *Calanus* spp. were overwintering in a winter resting stage (Barth-Jensen et al., in prep) and performed small scale DVM. We suggest that the three phases demonstrate that *Calanus* spp. swimming behaviour in the laboratory can therefore be used as a proxy of *Calanus* spp. DVM behaviour *in-situ*.

4. Conclusion

Calanus spp. swimming behaviour in the laboratory correspond well with the DVM behaviour observed in-situ despite the difference that there is a large dominance of small copepod in the wild mesozooplankton community. This highlights the role of light as a proximate cue irrespective of animals being in the lab or the wild. Both environments result in swimming behaviour avoiding light and under very long or very short days unsynchronised behaviours are equally revealed. *Calanus* spp. swimming behaviour should not be directly interpreted as a DVM behaviour but can be used as a proxy of DVM for *Calanus* spp. as long as the light condition in the laboratory reproduce the natural light conditions. It is now necessary to extent the study to include other member of the zooplankton community, i.e., krill to determine species specific responses to seasonal changes in photoperiod potentially with the use of broadband acoustic data which is able to resolve individual migration *in-situ*.

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6. Figures

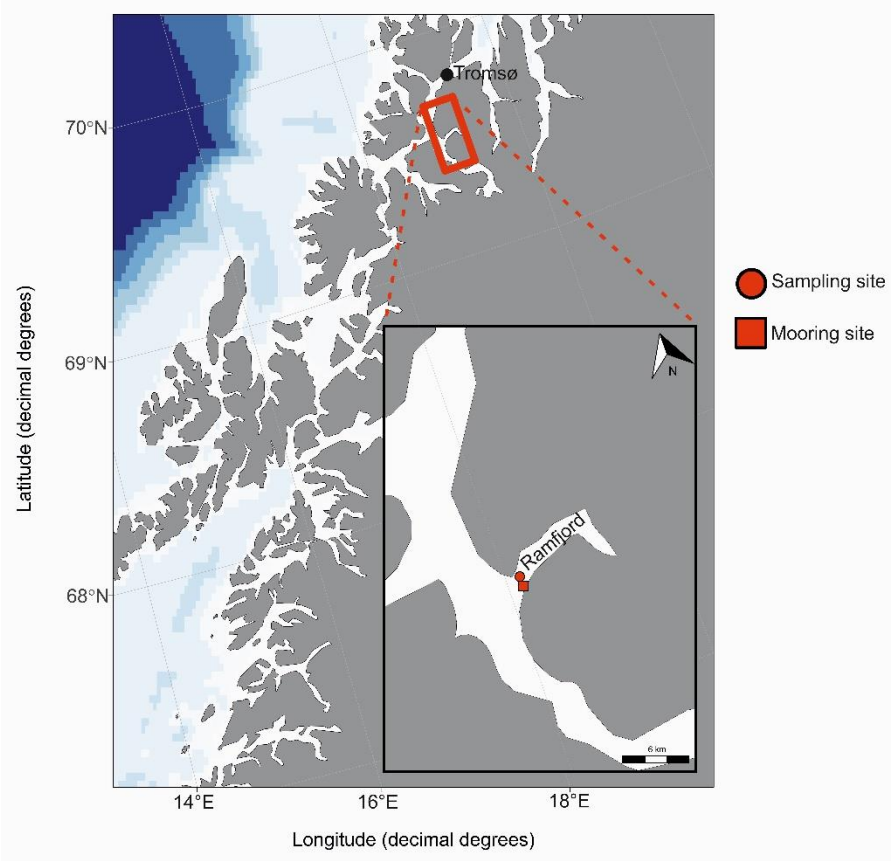

Figure 1: Map of northern Norway where Ramfjord is located. The bottom map of Ramfjord show where the sampling station and the observatory site is.

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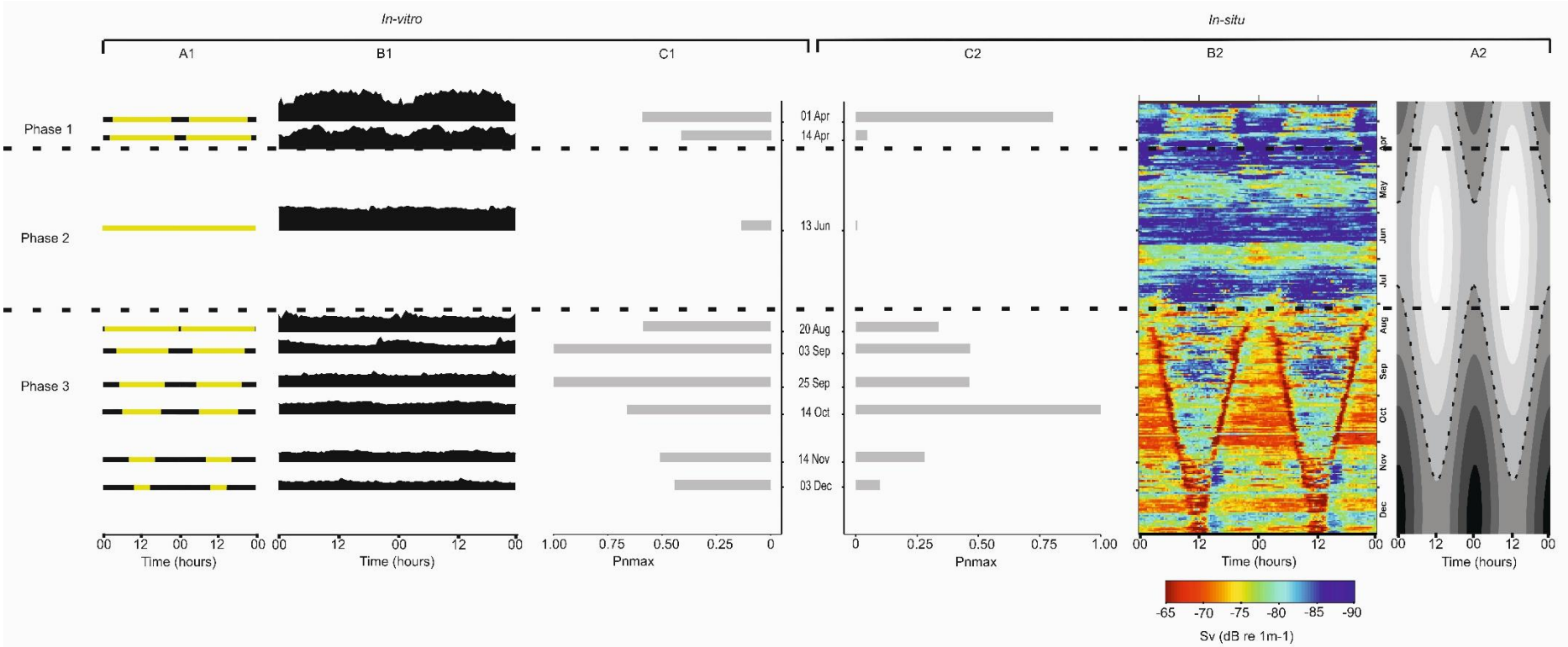

Figure 2: A1 shows the light cycle used during each experiment, where the yellow segment represents the period when the light is on and the dark segment the period when the light is off. B1 is a double plotted actogram of the daily in vitro (number of breaks per 30 min). C1 shows the average rhythm strength (PNmax). Right panels mirror the information for the in-vitro data. A2 shows seasonal changes in solar elevation, in Ramfjord (69°N). The darker grey indicates a lower solar elevation and the dotted line mark a solar elevation of 0°. B2 is a double-plotted actogram of the mean volume backscatter (Sv) over the study period (April 2019- December 2019). C2 shows the normalized strength peak (PNmax) of a Lomb-Scargle analysis for 10 days around each sampling event).

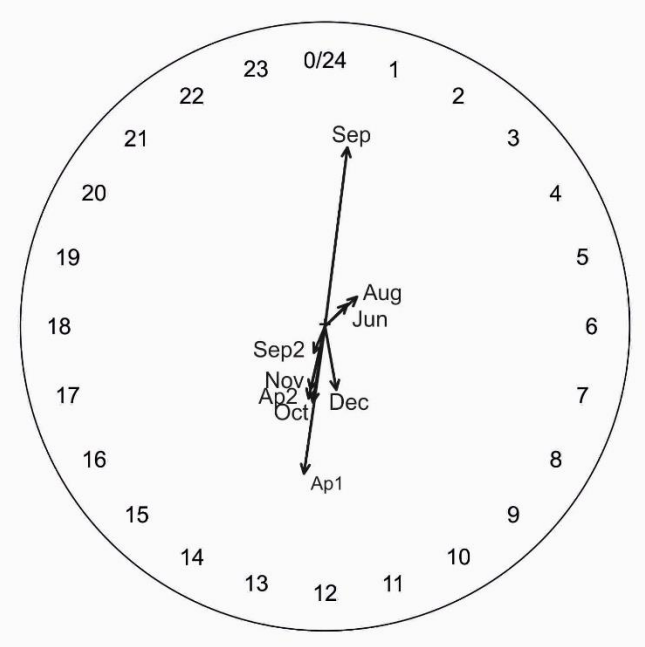

Figure 3:the mean angle (μ) and mean resultant length (ρ) of the daily activity over the 3 first days of the experiment obtained by combining all the individual sampled during each month.

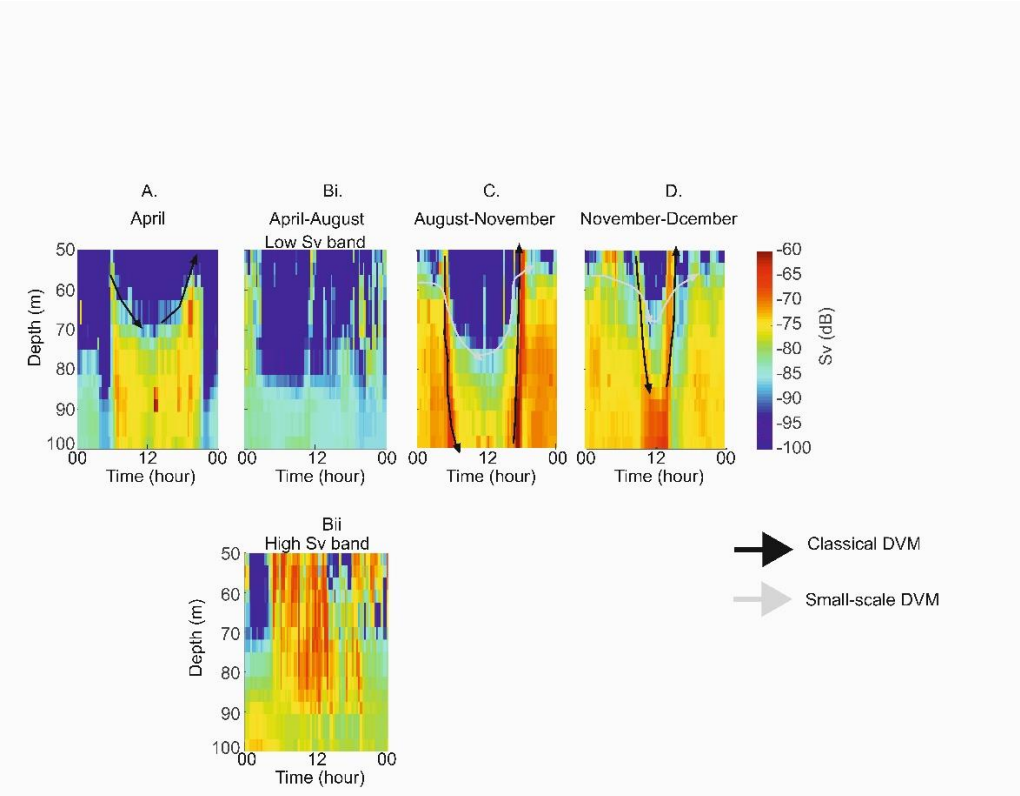

Figure 4: Echograms of hourly averaged data for a 7 d period (each data point represents the average backscatter at that time across all 7 d) in April (1st to 8th) (A), between April and August (B) with the top panel representing a week of low Sv band (01st to 08th of June) and the bottom panel a week of a high Sv band (1st to 8th of July), between early August and November (1st to 8th of October) and between November and December (3rd to 8th of December) . The black arrows are highlighting the ascent and descent signal of classical DVM while the grey arrows are highlighting the ascent and descent signal of small-scale DVM.

Table 1 Summary of the sampling dates, duration of the swimming experiment

Date	experiment duration
01/04/2019	6d
14/04/2019	5d
13/06/2019	6d
20/08/2019	7d
03/09/2019	7d
25/09/2019	7d
14/10/2019	6d
14/11/2019	5d
03/12/2019	6d

Table 2:Number of copepodite stage I to V (CI-CV) and adult stage female (AF) and male (AM), used during each experiment,

	CI	CII	CIII	CIV	CV	AF	AM	Total
early April	0	0	0	0	4	23	2	29
mid-April	0	0	7	5	2	20	1	35
June	0	0	0	0	51	3	2	56
August	0	0	0	0	48	0	0	48
early September	0	0	0	3	55	0	0	58
late September	0	0	0	2	74	4	0	80
October	0	0	0	0	113	3	0	116
November	0	0	0	1	108	0	0	109
December	0	0	0	8	95	1	0	104