UiT The Arctic University of Norway

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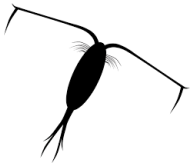
Sine-Sara Astad

Master's thesis in Biology, BIO-3950, November 2022



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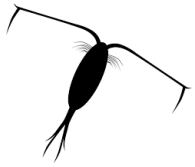



the Nansen LEGACY

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# Acknowledgement

Two years ago, I started sampling for this project with very limited knowledge about copepod ecology, molecular methods, and statistics with the use of R. The learning curve has been steep, and I have encountered many challenges along the way. I am ever so grateful for the opportunities I have been provided with through UNIS and especially grateful that Janne Søreide was my main supervisor. She has given me opportunities to go on research cruises, trusted me to handle challenges on my own, but always been around if I needed to talk.

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Sine-Sara Astad,
Longyearbyen, November 2022

# Abstract

Many species of small copepods are morphologically similar and thus often grouped together at genus level rather than studied as individual species. However, species within the same genus may differ quite a lot in life history strategies and traits. In this study, I used a species-specific polymerase chain reaction to explore the abundance, distribution and size range of *Pseudocalanus* sibling species in the Svalbard – Barents Sea region. The main aim was to investigate if they have distinct environmental preferences and the potential to be used as valid environmental indicator species. Temporal patterns in species composition were investigated monthly year-round in a high Arctic fjord (Billefjorden, Svalbard) over an entire year, while spatial patterns in species composition were investigated during a three-week cruise in the Barents Sea across the Polar Front from 73º S to 78º N.

*P. acuspes* was the most abundant species in Billefjorden and it dominated year-round except in June when the overall *Pseudocalanus* abundance was low and *P. minutus* were dominating. *P. minutus* was also present year-round in Billefjorden comprising 20 - 42 % of the total *Pseudocalanus* abundance from May to August 2021, while in the other months they had a lower total abundance between 4 and 15 %. *P. moultoni* was only present in 7 out of 12 months in Billefjorden, with the highest abundance in June and July (16-20 % of the *Pseudocalanus* community).

*P. minutus* had the overall largest average CIV and CVs, and while *P. acuspes* had the overall largest adults, *P. moultoni* had the overall largest single individuals. The gap and inconsistency between body size in CV and adult females, could be explained by differences in feeding strategy and timing of reproduction. *P. minutus* overwintered as CIV and CV in Billefjorden and started to reproduce in February and peaked in April when most CV had moulded into adults and hence were using their lipids reserves on maturation and reproduction rather than growing larger during the spring showing a capital breeding strategy. *P. acuspes* on the other hand overwintered as CIII and CIV, and adults had a late peak in July after CV were grazing during the spring and becoming adults during the summer displaying a income breeding strategy.

In the Barents Sea, *P. acuspes* was the most abundant species at the northernmost (T1, P2) and at the Polar Front stations (T3, T4, and T5). *P. moultoni* on the other hand, was the most abundant species at the southernmost stations (P1, C and the reference station), but barely present at the stations furthest North (1.3 – 3.2 % of the total *Pseudocalanus* abundance).

My study demonstrates the importance of sampling the entire populations (CI-adults) and not only parts of it when investigating species distributions, and preferable throughout the entire year to avoid biases due to seasonality in population structures and advective events (i.e., Billefjorden).

**Keywords;** Zooplankton, copepods, sibling species, life cycles, polar regions, species-specific polymerase chain reaction, annual study

# 1 Introduction

Zooplankton, heterotrophic single- and multicelled organisms in the size range 0.2 - 2000 $\mu m$ (Daase et al., 2021), are important components of the marine ecosystem. They function as a link between primary producers and higher trophic levels. Zooplankton actively graze on microalgae and other protists accumulating essential fatty acids important for the healthy development and growth of themselves as well as their predators (Saiz et al., 2013, Daase et al., 2021, Urban et al., 2022). Due to their high energy content, zooplankton are important prey items for many types of fish, birds, crustaceans and larger marine mammals (Turner, 2004, Arnkværn et al., 2005, Peters et al., 2006, Wassmann et al., 2006).

The ocean can be divided into different environmental regions based on differences in salinity, temperature and density which again shape zooplankton distribution. When currents move water masses from one location into another, zooplankton drift along passively as they can only move vertically (by regulating their buoyancy), and hence cannot withstand the ocean currents. This results in a mix of species that overlap in distribution, but differ in environmental preferences (Daase and Eiane, 2007). Some zooplankton species have previously been described as good environmental indicators due to their specific habitat and water mass preferences. Categorizing species as typically Atlantic or typically Arctic, based on where they thrive the most, allows different organisms to act as biotic indices for environmental changes. Hence, changes abundance, diversity and distribution of Atlantic species can be used to describe the current state of Atlantic water inflow into the Arctic Ocean (Mańko et al., 2020), popularly called Atlantification (Wassmann et al., 2006).

Areas like in the Arctic, where northward going currents, with warmer more saline water, meets southward going currents, with colder and less saline water, are optimal places to study these mixing biotic communities. Here, species of the same genus, i.e. sibling species, have previously been linked to slightly different habitat preferences when it comes to salinity, temperature, and food environment (Daase and Eiane, 2007, Falk-Petersen et al., 2007). In the Arctic, the Svalbard-Barents Sea region is an area where the largest contribution of warmer more saline water enters the Arctic Ocean (Basedow et al., 2018). North of Norway, the North Atlantic Current splits in two main branches, one flowing into the Barents Sea while the other flows northwards as the West Spitsbergen Current. On the north and east coast of Svalbard a

cold current of less saline Arctic water arrives from the Arctic Ocean entering the Barents Sea from both sides of Franz Josef Land (Grøsvik et al., 2018). This transition area where the different water masses meet is commonly referred to as the Polar Front (Loeng, 1991).

Differences in zooplankton environmental conditions affect the body size, life history strategy, and ecosystem function of the different zooplankton species. Life history adaptations to different environmental conditions across their different distributional range have been extensively studied for three copepod species of the *Calanus* genus. *C. glacialis*, *C. hyperboreus*, and *C. finmarchicus.* For instance, *C. hyperboreus*, the Artic and more oceanic species has adapted to uncertainties in food supply, due to long periods of ice cover, by developing a large plasticity in its life history strategies. This is a consequence of a higher need to rapidly store high energy wax esters, making the overall size much larger than its two other sibling species (Falk-Petersen et al., 2009). *C. finmarchicus* and *C. glacialis* have been used as environmental indicators as *C. finmarchicus* has its main area of distribution in the North Atlantic and *C. glacialis* has its main area of distribution in the Arctic seasonal ice-covered shelf seas. More recent studies however have shown that correct identification is more problematic than assumed (Gabrielsen et al., 2012), and that the overlapping distributional range (due to warming) makes correct identification even more difficult.

Copepods of the genus *Pseudocalanus* have similar traits as *Calanus* (i.e. herbivores, seasonal migration). Still, very little is known about how they differ in size, life history adaptations and distribution range, and if they potentially could become environmental indicators.

The inflow of warmer Atlantic water by the West-Spitsbergen current is increasing and has already led to warmer temperatures in the Svalbard-Barents Sea region over the last century (Pavlov et al., 2013, Onarheim et al., 2014, Bloshkina et al., 2021). This inflow influences both the physical and biological properties of the Arctic Ocean (Piechura and Walczowski, 2009), and a northward expansion of the distributional range of boreal species has been observed most likely related to the increased influx of Atlantic water (Fossheim et al., 2015). A change in environmental factors could potentially alter copepod community composition and population size structure (Aarbakke et al., 2014), as change in temperature and salinity

would strongly influence species distribution with continued warming trends (Questel et al., 2016).

Small copepods of the genus *Pseudocalanus* are common in all Arctic and sub-Arctic seas and can constitute up to 5-25 % of the total biomass (Ershova et al., 2017, Ershova et al., 2021b). They are primarily herbivores, but are found to be opportunistic feeders, with a diet of diatoms in the spring, flagellates in the summer, and an omnivorous/carnivorous diet in the autumn and winter (Lischka et al., 2007). Reproduction is closely linked to the main phytoplankton production period, while gonad maturation for CVs and females are fuelled by a combination of energy stored in small lipid sacs, the spring bloom and a later intensive feeding period (Lischka and Hagen, 2005). Species of the *Pseudocalanus* genus seem to have slightly different timing in reproduction, and their ability to complete a one-year life cycle seem to change with species, and potentially with latitude. Differences in distribution and life history traits may make them a potentially good species to use as an indicator species, but with limited morphological differences and challenges identifying the correct species, there are still some gaps in our knowledge about their exact distribution and life history traits.

Seven neritic copepod species of *Pseudocalanus* have been described in the Northern Hemisphere, species with both Arctic and Atlantic origin (Frost, 1989, Questel et al., 2016), but only six of these species have been accepted according to Worms - World Register of Marine Species (2022). In the Svalbard – Barents Sea region, Frost (1989) described the presences of two, possible three, species of *Pseudocalanus*: *Pseudocalanus acuspes* (Giesbrecht, 1881), *Pseudocalanus minutus* (Krøyer, 1845) and *Pseudocalanus major* SARS G.O., 1900. The latter has never been confirmed by molecular methods and is today accepted as *P. minutus* (Worms - World Register of Marine Species, 2022). In recent years, another sibling species, *P. moultoni*, was confirmed to be present in Svalbard waters with the help of molecular tools (Aarbakke et al., 2011). This species has since been identified several times in both Kongsfjorden (Aarbakke et al., 2017) and Isfjorden (Ershova et al., 2021b), and in Rijpfjorden (Aarbakke et al., 2011)

Studies conducted so far indicate different distribution patterns among the different *Pseudocalanus* species (Lischka et al., 2007, Aarbakke et al., 2017), where *P. minutus* is

considered to prefer areas of mixing Atlantic and Arctic water masses whereas *P. acuspes* is a more fjord species where an inflow of Atlantic water is less likely (Lischka and Hagen, 2005). Still, several species of the *Pseudocalanus* genus are frequently observed to co-occur in the same area (Ershova et al., 2021b). It has also been suggested that the common arctic species *Pseudocalanus sp.* can reach very high concentrations just under fast ice as it will graze on ice algae if available (Bedo et al., 1990).

The boreal *Pseudocalanus elongatus* is most abundant in the North Sea and along the west coast of Norway. In the past, this species has been morphologically identified in Raunefjorden (Frost, 1989) and Balsfjorden (Hopkins et al., 1989, Artsdatabanken, 2022), both fjords located close to Tromsø in Northern Norway. Recently, *P. elongatus* has also been confirmed to be present year-round in Ramfjord using metabarcoding (Coguiec et al., 2021). In the Svalbard-Barents Sea region, *P. elongatus* has been morphologically identified (Ellertsen et al., 1982, Węsławski et al., 1988, Timofeev, 2002), but never confirmed present by molecular methods. Overall, *P. elongatus* is rarely reported in the Arctic and it has been suggested that observations of *P. elongatus* are based on misidentifications (Dvoretsky and Dvoretsky, 2010) due to confusion around an earlier morphological description of this species (Corkett and McLaren, 1979). Sub-Arctic species can be transported into the high-Arctic with currents (Ershova et al., 2017), hence *P. elongatus* could potentially be transported into the Svalbard-Barents Sea region. Being described as the most abundant *Pseudocalanus* species in the regions of the North Atlantic, the presence of *P. elongatus* is positively correlated with warmer temperatures which is regarded as a significant explanatory environmental variable for the distribution of *P. elongatus* (Corkett and McLaren, 1979, Aarbakke et al., 2017, Ershova et al., 2021b). In the North Sea, *P. elongatus* peaks in abundance in May/June (Renz et al., 2008), but so far there is a lack of observations from the Svalbard-Barents Sea region and it is rarely observed in the high Arctic.

*Pseudocalanus acuspes* is commonly found in neritic and coastal waters. In Svalbard, it has been observed in low numbers in Kongsfjorden in a study from 1998 – 1999 with a peak in abundance in November (Lischka and Hagen, 2005). While in Billefjorden, *P. ascupes* was found with a peak in abundance in July and continuously high abundance in August and November the same year (Ershova et al., 2021b).

*Pseudocalanus minutus* is regarded more oceanic than its sibling species (Norrbin, 1991) and seems to prefer a mix of Arctic and Atlantic water prevailing in Arctic Shelf seas (Lischka and Hagen, 2005). A metabarcoding study showed that *P. minutus* was the least common of the sibling species in Billefjorden and Balsfjorden, but the most common in the northern Barents Sea (Ershova et al., 2021a). In Kongsfjorden, abundance of *P. minutus* peaked in November, and was lowest in June (Lischka and Hagen, 2005), while in Billefjorden *P. minutus* had less than 20 individuals per $m^3$ during all sampling months (May, July, August and November) (Ershova et al., 2021b).

*Pseudocalanus moultoni* has only recently been identified in the Svalbard region based on molecular tools (Aarbakke et al., 2011). It was previously considered to prefer temperate coastal waters (Frost, 1989). Aarbakke et al. (2014) suggest that *P. moultoni* has a large distributional range and could possibly be an oceanic cosmopolitan species. In Svalbard, it has been identified in several fjords including Rijpfjorden, Van Mijenfjorden, Billefjorden and Adventfjorden (Aarbakke et al., 2011, Ershova et al., 2021b). In Adventfjorden, *P. moultoni* has been found to be present throughout the year and to be the dominant among the *Pseudocalanus* species during winter, with a peak in late winter (March) and in autumn (September, November). In Billefjorden on the other hand it was present in much lower numbers with a minimum abundance in May, but still present most of the year (Aarbakke et al., 2011, Ershova et al., 2021b).

Previous studies have also reported a variety of different life history strategies between sibling species of *Pseudocalanus* such as the number of generations (Aarbakke et al., 2014). *P. acuspes* has been reported to complete only a one-year life cycle in the Canadian Arctic, but can have up to three generations per year in Balsfjorden (a northern Norwegian fjord). In Adventfjorden, Nauplii and CI-II stages of *P. acuspes* have been found to be abundant from June to November, while in Billefjorden nauplii were only present in May and July (Ershova et al 2021b). While stages CIII-IV dominated almost the whole population in Adventfjorden from December to April, adult females of *P. acuspes* showed a high abundance in May and re-appeared in the period August to September. In Billefjorden on the other hand *P. acuspes* adult females was most abundant in August and November (Ershova et al., 2021b).

For *Pseudocalanus minutus*, a one-year life cycle has been suggested for populations in Kongsfjorden and Hornsund (Kwasniewski, 1990, Lischka and Hagen, 2005), while they have two generations per year in Balsfjorden (Ershova et al., 2021a). Ershova et al., (2021b) found *P. minutus* to barely be present in Adventfjorden, while it was common in Billefjorden with nauplii appearing in May and the copepod stage CI-II appearing in July (Ershova et al., 2021b), indicating a one-year life cycle there.

Nauplii of *Pseudocalanus moultoni* were not detected in Billefjorden during any of the sampling months (May, July, August, and November), potentially due to the use of a 200 $\mu$m mesh size net (Ershova et al., 2021b). However, CIII-CV were present in all months, with a peak in adults in August and November. Similar observation has been made in the North Sea, where most development stages of *P. elongatus* were present throughout the study period, and it was not possible to distinguish any obvious cohorts. This suggests that *P. elongatus* can spawn over a long period and recruitment does not occur at a clearly defined time interval (Renz et al., 2008), potentially being a more temperate species life strategy.

Unfortunately, most research has been conducted at the genus level due to a lack of morphological species characteristics (Weydmann et al., 2014, Søreide et al., 2022) thus ignoring different preferences for water masses, feeding strategy and life cycle. A few studies have looked at morphological differences (Lischka and Hagen, 2005) or assumed the prevalence of a certain species due to previous morphological studies in the same area without confirming the assumption with molecular methods (Koski et al., 2010). One morphological trait commonly used to distinguish between species is body size, but it has more recently been shown that size alone cannot be used as a characteristic as it is highly variable with latitude and temperature (Corkett and McLaren, 1979, Bucklin et al., 1998). It has also been found that preservation in different solutions such as ethanol tends to decrease the size of individuals.

Year around ecological studies in arctic type waters with monthly resolution are rare in the Svalbard-Barents Sea region and hence there is insufficient data on population size, distribution range, and life history adaptation in this region (Ershova et al., 2021b). Only a few studies have documented seasonal changes in the community and population structure of

small copepods with high temporal resolution. Most of these studies has focused on the *Calanus* genus (Arnkværn et al., 2005, Søreide et al., 2010). There are only a few studies that have looked at seasonal changes in the population structure and abundance of different *Pseudocalanus* species in the Svalbard – Barents Sea region with the use of molecular identification.

The few studies that have utilized both morphological and molecular identification, with some exceptions (Ershova et al., 2017, Ershova et al., 2021b), have almost all been limited to only studying adult females as they are more easily identified (Aarbakke et al., 2017). The disadvantage of this is that females may often comprise <5% of the population (Ershova et al., 2021b), and hence provide a skewed picture of changes in abundance and species composition. To determine differences in the distribution and life history strategies among boreal and Arctic *Pseudocalanus* species, studies using molecular identification of all copepodite stages are needed.

The main aim of this study is to explore the occurrence of the abundance, distribution, population structure and size range of the different *Pseudocalanus* species in the Svalbard – Barents Sea region and how they differ with seasonal and hydrographic conditions, in order to assess if Atlantification could lead to a shift in the *Pseudocalanus* species distribution. To reach this goal, the following questions will be answered:

Which species of *Pseudocalanus* are present in the Svalbard-Barents Sea region?
How does the *Pseudocalanus* species composition and population structure change through time and space?

Do the different species differ in body size?

Do the species have different environmental preferences? Can individuals of the *Pseudocalanus* genus act as an environmental indicator? Why? Why not?

# 2 Methodology

## 2.0 The Svalbard – Barents Sea region

Two different sampling strategies were used in the Svalbard – Barents Sea region (Figure 1). A transect in the Barents Sea was sampled to map spatial changes in distribution of the *Pseudocalanus* community across the Polar Front, while an annual study with monthly resolution in Billefjorden was conducted to investigate temporal changes in the species composition.

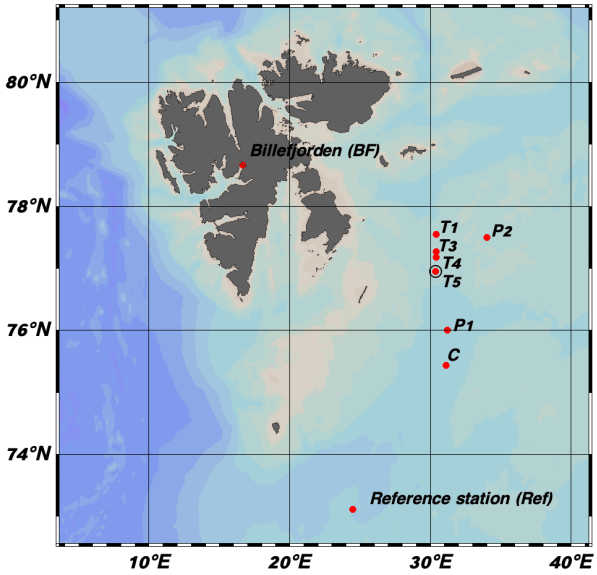


Figure 1 Map of the Svalbard – Barents Sea region including location of the different sampling sites; Billefjorden (BAB), T1, P2, T3, T4, T5, P1, C, and the reference station taken closer to Tromsø.

The first sampling area, the Barents Sea, is a shallow shelf sea located between $70^0$ and $80^0$N. The water masses here are influenced by both warm and saline Atlantic water from the North Atlantic Current and cold, less saline Arctic waters from the Arctic Ocean. In addition, the Norwegian Coastal Current transports relatively fresh waters along the northern Norwegian and Russian coast (Grøsvik et al., 2018). In the Barents Sea eight different stations were sampled in October 2020 during the Nansen Legacy SS-MSC2 Process/mooring service cruise (T1, P2, T3, T4, T5, P1, C, in addition to a southern reference station (Ref)). These stations were sampled on a depth range from 170 – 359 meters and a latitude span of $73^0$ to $78^0$ north (Table 1). The northernmost stations (T1 and P2) were located north of the Polar Front in cold and less saline Arctic Polar Water. The influence of Atlantic water increased towards the southern stations. The reference station was meant to represent a fully Atlantic influenced station. The T-stations were sampled a few hours apart, while the other locations were sampled within a few days and weeks (Table 1).

The second sampling area was Billefjorden ($78^0$39.50`N, $16^0$40.50`E), a seasonally ice-covered threshold fjord branching out of the larger Isfjorden system. The fjord consists of two basins with a shallow sill (approx.60 m deep) limiting warm Atlantic water from entering.

The sampling station in Billefjorden (BAB) is located in the inner basin Adolfbukta (approx. 191 m deep). The fjord is a typical Arctic fjord where freshwater run off from glaciers and other land sources influences the water masses. In Billefjorden samples were taken in monthly intervals at 11 occasions between November 2020 and October 2021, except for March 2021 when it was not possible to reach the station due to very thin ice cover. In addition, an extra sample was taken in July 2021 to catch any rapid changes in the copepod community and environmental factors during the summer (Table 1).

## 2.1 Field work and sampling information

Zooplankton was sampled with vertical hauls from close to the sea floor to the surface using either a 0.255 $m^2$ mouth opening WP2 mesozooplankton net or a 0.2825 $m^2$ mouth opening Bongo net with a 64 µm mesh size (HydroBios, Kiel, Germany) (Table 1). The net was lowered to approximately 10 meters above the seafloor and towed vertically with a speed of 0.3 m $s^{-1}$. Upon retrieval it was rinsed with either a hose or several buckets of sea water, to gather all the zooplankton in the cod end. The sample was split in two using a zooplankton splitter (Motoda, 1985). One half of the sample was preserved in a 250 ml plastic container with 80-96 % ethanol, while the other was archived in formalin. The ethanol was changed within 48 hours, to maintain the correct ethanol concentration, and the samples were then stored at -20°C.

## 2.2 Environmental parameters

At each sampling event, conductivity, temperature and depth were measured using a handheld SAIV SD204 CTD equipped with a Seapoint fluorescence sensor in Billefjorden and a SBE911 plus CTD in the Barents Sea. Furthermore, water samples were collected using a 12 l Niskin bottle in Billefjorden at 0, 15 and 150 m, and as part of the CTD rosette during the Barents Sea cruise at 5, 10, 20, 30, 40, 50, 60, 90, 120, 150/200, bottom to measure chlorophyll *a* (Chl *a*) concentration.

The Chl *a* samples were partially processed on board by filtering 150 - 500 ml of water sample onto 25 mm GF/F filters (Whatman), which were subsequently stored in liquid nitrogen or in a -80$^0$C freezer. Chl *a* was extracted by incubating the filters in 10 ml methanol overnight at 4 - 10$^0$C in the dark (Holm-Hansen and Riemann, 1978). Fluorescence was

measured both before and after adding 2 drops of 5 % hydrochloric acids (1.2 M) on a Turner 10-AU-005-CE fluorometer calibrated with a Sigma standard. The raw values were converted to acid-corrected Chlorophyll *a* (µg/l) using the following equation:

$$C = Fs\left(\frac{r}{r-1}\right)(Rb - Ra)\left(\frac{Vex}{Vfil}\right)$$

Where C = Chlorophyll *a* concentration (µg/l); Fs = response factor for sensitivity setting after calibration; r = acidification ratio; Rb = fluorescence before acidification; Ra = fluorescence after acidification; Vex = extraction volume; Vfil = volume filtered sample.

## 2.3 DNA extraction

Each 250 ml ethanol sample was poured into a 60 $\mu$m sieve. The container was subsequently rinsed three times with 250 ml of filtered sea water to ensure all the individuals were transferred to the sieve. The sieve with the sample was soaked in a small tray with filtered sea water for at least 30 min, to remove all traces of ethanol, before the sample was transferred to a measuring cup with approximately 1000 ml of filtered sea water. A quantitative subsample was extracted using a 5 ml pipette with a cut off tip. Subsamples were extracted until a minimum of 89 copepods were picked, but the maximum numbers of copepods varied as the entire subsamples were examined (Table 2).

Subsamples were analysed under a Leica 7.1-115x stereomicroscope. For each hand-picked *Pseudocalanus spp.* individual urosome segments, prosome length and gender was noted down, utilizing the existing morphological framework (Corkett and McLaren, 1979, Frost, 1989). Prosome length was measured using the calibrated scale in the stereomicroscope. Only copepodite and adult stages were recorded, and the counted individuals were grouped by copepodite stage CI-III, CIV, CV, and adults. Gender was registered from CIV, and the size was calculated using the ruler to measure the prosome length in the microscope (calibrated just before use). While ethanol might have decreased the size of the individuals it should be consistent for all samples. Specimens were left in individual 1.5 ml Eppendorf tubes containing milliQ water for another 30 min to remove traces of ethanol. This was conducted under the stereomicroscope in order not to lose the individual in the process.

Table 1 An overview of all sampling dates, locations, depths, equipment used between, sampling conditions and means of transportation during sampling from October 2020 to October 2021.

| Date | Station name | Location | Sampling depth | Equipment | Sampling conditions | Boat/ Transport |
|---|---|---|---|---|---|---|
| 10.10.20 | P2 | 77°29.94`N 34°0.59`E | 176 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 12.10.20 | P1 | 76°0.43`N 31°13.02`E | 300 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 14.10.20 | T1 | 77°33.03`N 30°23.87`E | 200 m | Bongo net 64 $\mu$m | Calm, cloudy, daylight, | G.O. SARS |
| 14.10.20 | T3 | 77°16.37`N 30°24.24`E | 170 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 14.10.20 | T4 | 77°10.89`N 30°23.83`E | 190 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 14.10.20 | T5 | 76°57.07`N 30°23.19`E | 220 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 22.10.20 | C | 75°25.92`N 31°6.73`E | 350 m | Bongo net 64 $\mu$m | Calm, dark | G.O. SARS |
| 25.10.20 | Ref | 73°7.08`N 24°29,58`E | 359 m | Bongo net 64 $\mu$m | Wavey, dark | G.O. SARS |
| 19.11.20 | BAB | 78°39.50`N 16°40.50`E | 185-0 m | WP2 net 64 $\mu$m | Calm, snow, no sea ice, dark | Polarsyssel |
| 11.12.20 | BAB | 78°39.50`N 16°40.50`E | 185-0 m | WP2 net 64 $\mu$m | Calm, snow, no sea ice, dark, brownish water | Polarsyssel |
| 28.01.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 m | WP2 net 64 $\mu$m | Ice formation, but drift ice, windy | Polarsyssel |
| 11.02.21 | BAB | 78°39.50`N 16°40.50`E | 180-0 m | WP2 net 64 $\mu$m | Calm, icey | Polarsyssel |
| 09.04.21 | BAB | 78°38.79`N 16°40.50`E | 135-0 m | WP2 net 64 $\mu$m | Did not get to the deepest part due to ice, | Polarsyssel |
| 09.05.21 | BAB | 78°39.50`N 16°40.61`E | 180-0 m | WP2 net 60 $\mu$m | No data, | Helmer Hansen |
| 16.06.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 m | WP2 net 60 $\mu$m | Calm, | UNIS Sila |
| 05.07.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 m | WP2 net 60 $\mu$m | Calm, sunny | UNIS Sila |
| 15.07.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 | WP2 net 60 $\mu$m | Windy and wavey, | UNIS Sila |
| 17.08.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 | WP2 net 60 $\mu$m | Calm, cloudy | UNIS Sila |
| 17.09.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 | WP2 net 60 $\mu$m | Calm, cloudy | UNIS Sila |
| 05.10.21 | BAB | 78°39.50`N 16°40.50`E | 185-0 | WP2 net 60 $\mu$m | Small storm, high waves and very windy | Polarsyssel |

The total genomic DNA was extracted utilizing the Hotshot method according to Truett et. al (2000) as it is rapid and inexpensive. In the protocol, 25 $\mu$L of lysis buffer are first added to

the copepod in an eppendorf tube, then the copepod is heated at 55° C for 30 minutes, before the liquid is cooled down and 25 $\mu$L of neutralisation

buffer was added. The samples were then stored in 4-10℃. The remains of the subsample from which individuals had been removed was transferred to a separate Falcon tube and morphologically checked by Professor Janne Søreide (Table 2).

Table 2 Overview of samples, including date, location, depth, information on whether the sample was split or not, how much the sample was diluted, the total volume of the subsample, how many zooplankton were picked per sample and % correctly picked *Pseudocalanus*. The % of *Pseudocalanus* identified molecularly was calculated by counting the molecularly identified *Pseudocalanus* individuals per subsamples and dividing it by the total number of copepods picked per subsample.

| **Date** | **Location** | **Depth** | **Split** | **Diluted (ml)** | **Subsamples (ml)** | **Number of copepods picked** | **% of *Pseudocalanus* identified molecularly** |
|---|---|---|---|---|---|---|---|
| 10.10.20 | P2 | 176-0 m | 1/2 | 1000 | 3 | 98 | 95 % |
| 12.10.20 | P1 | 300-0 m | 1/2 | 1050 | 77 | 90 | 90 % |
| 14.10.20 | T1 | 200-0 m | 1/2 | 1000 | 17 | 94 | 78 % |
| 14.10.20 | T3 | 170-0 m | 1/2 | 1050 | 36 | 94 | 94 % |
| 14.10.20 | T4 | 190-0 m | 1/2 | 1000 | 17 | 93 | 83 % |
| 14.10.20 | T5 | 220-0 m | 1/2 | 1000 | 36 | 89 | 90 % |
| 22.10.20 | C | 350-0 m | 1/2 | 1000 | 95 | 92 | 96 % |
| 25.10.20 | Ref | 359-0 m | 1/2 | 1000 | 249 | 88 | 84 % |
| 19.11.20 | BAB | 185-0 m | 1/2 | 1000 | 10 | 98 | 92 % |
| 11.12.20 | BAB | 185-0 m | 1/2 | 1000 | 7 | 94 | 87 % |
| 28.01.21 | BAB | 185-0 m | 1/2 | 1000 | 4 | 95 | 87 % |
| 11.02.21 | BAB | 180-0 m | 1/2 | 1000 | 5 | 117 | 86 % |
| 09.04.21 | BAB | 135-0 m | 1/2 | 1000 | 11 | 90 | 92 % |
| 09.05.21 | BAB | 180-0 m | 1/2 | 1000 | 11 | 117 | 35 % |
| 16.06.21 | BAB | 185-0 m | 1/2 | 1000 | 16 | 93 | 77 % |
| 05.07.21 | BAB | 185-0 m | 1/2 | 1000 | 33 | 93 | 72 % |
| 15.07.21 | BAB | 185-0 m | 1/2 | 1000 | 53 | 110 | 92 % |
| 17.08.21 | BAB | 185-0 m | 1/2 | 1000 | 14 | 106 | 77 % |
| 17.09.21 | BAB | 185-0 m | 1/2 | 1000 | 14 | 88 | 83 % |
| 05.10.21 | BAB | 185-0 m | 1/2 | 1000 | 9 | 108 | 80 % |

## 2.4 Primer testing

The original plan was to use the primer set up established by Ershova et al. (2021b) with a single primer cocktail that could separate *Pseudocalanus acuspes, P. minutus,* and *P.*

*moultoni* based on sizes of their respective PCR products. In their protocol species-specific reverse primers had been designed to attach to different locations of the cytochrome oxidase I (COI) gene (Table 3). The protocol was tested using the original PCR conditions except that the Quantabio ToughMix was replaced with Thermofisher Dreamtaq buffer. Surprisingly, no products were obtained using *P. minutus* or *P. moultoni* DNA, which at the time was the only controls available to us.

To determine whether the lack of amplification was due to problems with the primer cocktail, the primers were tested in individual pairs for each *Pseudocalanus* species (Table 3). Multiplex PCR reactions are more likely to create primer-dimer-reactions that compete with target amplification. The PCR reactions were again unsuccessful for both positive controls. Because this did not work, it was necessary to use some time to develop a new PCR assay that could at least separate the three species earlier described from the region. Changes in the original PCR set up was therefore carried out step by step (Table 4). To be certain of having good positive controls new individuals, including assumed *P. acuspes* copepods, were hand-picked, amplified using LCO1490 primer (10 µM) and HCO2198 primers (10 µ) (Folmer et al., 1994) and sent for Sanger sequencing (details in 2.6).

Table 3 List of primers that were tested with information about DNA sequence and reference.

| Primer name | DNA sequence | Reference |
|---|---|---|
| PseudoF | 5′- TTCGAATAGAGYTAGGHMVAGY-3′ | Questel et al. 2016 |
| PseudoF-mod | 5′-TTCGAATASARYTRGGHMVRGY-3′ | Ershova et al. 2021b |
| acuspesF | 5`- TCGAATAGAGTTAGGTCAAGCA-3` | A. Vader |
| acuspes280R | 5′-AGAGGAGGGTATACAGTTCACC-3′ | Ershova et al. 2021b |
| acuspes238R | 5′- AGAGGAGGGTATACAGTTCACC-3′ | Ershova et al. 2017 |
| acuspesR | 5`- GCTAACACTGGTAATGACAGC-3` | A. Vader |
| minutusF | 5′- GAATAGAGCTAGGCCAAGCA-3′ | A. Vader |
| minutusF2 | 5′- GGCCAAGCAGGGTCACTAAT-3′ | A. Vader |
| minutus398R | 5′- CGCAAACARAGGTATTTGGTCT-3′ | Ershova et al. 2016 |
| minutus480R | 5′-CGCAAACARAGGTATTTGGTCT-3′ | Ershova et al. 2021b |
| moultoni307F | 5′-GCATGCAGGAGGTTCTGTTG-3′ | Ershova et al. 2021b |
| moultoni520R | 5′-ACAATATTGTAATTGCMCCAGC-3′ | Ershova et al. 2021b |
| PtoniF | 5`- AAGTCAACAGAACCTCCTGC- 3` | S. Thompson |
| PtoniR | 5`- TAATGCCAGCCTTGATCATGTT- 3` | S. Thompson |

As the success of PCR depends on several factors, the concentration of reaction components such as the enzymes and primers can be adjusted to either improve PCR yields if there are

inhibitors or to reduce amplification of nonspecific targets or primer-dimer formation. The concentration of enzyme, DNA and primers where therefore adjusted. Still, no product was obtained for either of the available *Pseudocalanus minutus* or *P. moultoni* primer sets. Additional primer sets were then designed and tested (Table 3), now also including the new *P. acuspes* positive control.

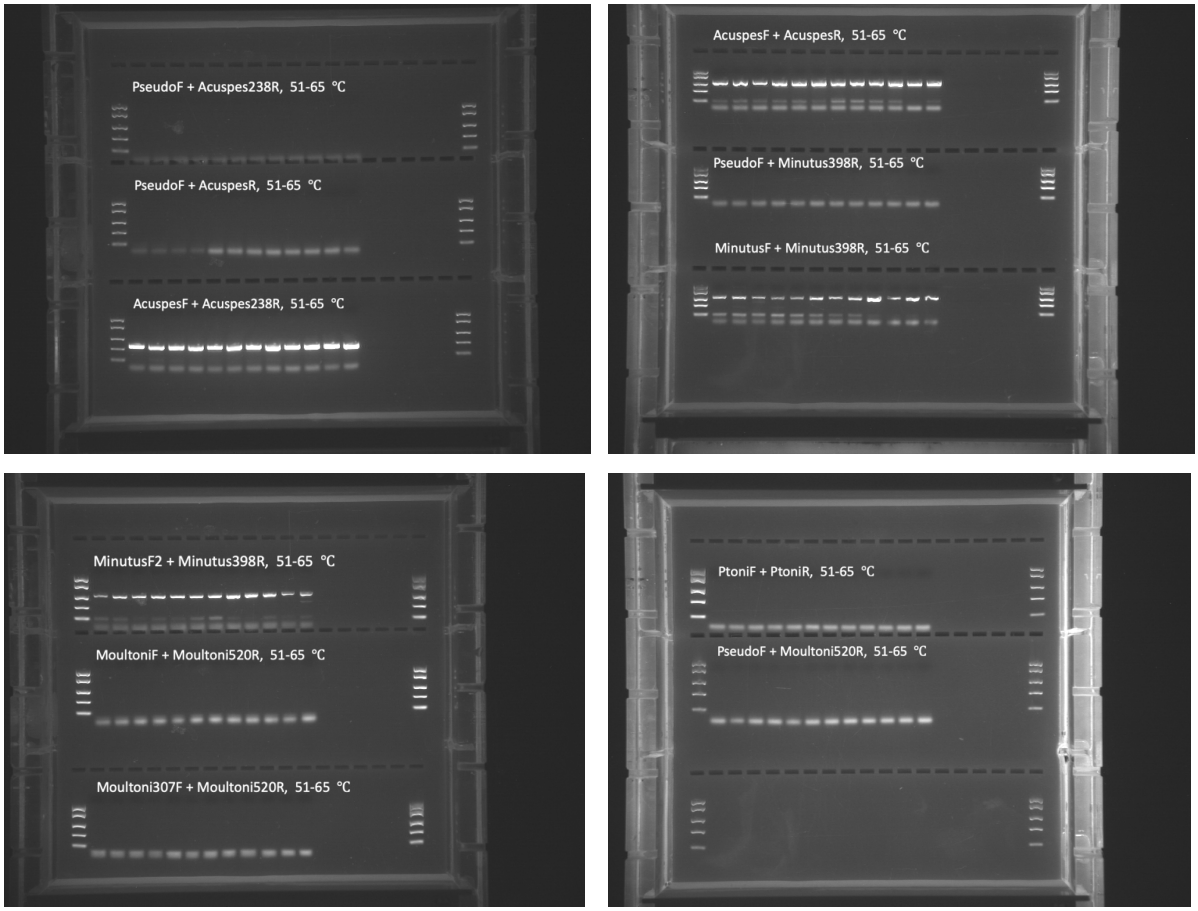


*Figure 2 An overview of the results of the 11 different primer sets tested on a temperature scale from 51 - 65 ºC.*

Several of the new primer pairs were successful (Table 5), providing PCR products for *Pseudocalanus acuspes* and *P. minutus*, but not for any of the *P. moultoni* primer sets. To optimize the PCR conditions, especially the annealing temperature which to a large degree determines the specificity of the PCR reaction, a temperature gradient PCR was performed (Figure 2). Also, primer pairs that had failed previously were included to exclude the possibility that lack of amplification was due to the annealing temperature having been set too high. The results showed that the three combinations of acuspesF and acuspes238R,

minutusF2 and minutus 398, and PtoniF and PtoniR provided the most stable and reliable results in addition to providing the best separation in base pair length.

Table 4 List containing reagents and how they were adjusted for the primer cocktail and PCR protocol from the first protocol (A) until the last protocol (E) showing the development of the assay over time. The red color indicate change adjusted per new protocol.

| **Reagents** | **Protocol A** | **Protocol B** | **Protocol C** | **Protocol D** | **Protocol E** |
|---|---|---|---|---|---|
| $H_2O$ | 4.0 | 5.5 | 6.7 | 6.7 | 6.5 |
| Dreamtaq buffer (10X) (Thermofisher) | 1.0 | 1.0 | 1.0 | 1.0 | |
| Dreamtaq Green buffer (10X) (Thermofisher) | | | | | 1.2 |
| dNTPs (2.5 mM each) (Thermofisher) | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Primers (10uM each) (Integrated DNA Technologies) | 0.5 (each) | 0.5 | 0.2 | 0.2 | 0.2 |
| Dreamtaq polymerase (enzyme) (5 U/µL) (Thermofisher) | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |
| DNA | 3.0 | 1.5 | 1.0 | 1.0 | 1.0 |
| Annealing temperature | 62° C | 62° C | 57° C | 54° C | 54° C |
| Cycles | 35 | 35 | 30 | 30 | 30 |

Because using several different PCR assays on the same sample is time consuming, another attempt at doing multiplex PCR was done. The master mix was again adjusted to include two primer sets, and the annealing temperature was set to 54°. A mix of only the *Pseudocalanus acuspes* and *P. minutus* primer sets was successful.

Table 5 Successful combinations of primers shown by the letter S, while failed primer combinations showed by the letter F. Combinations shown in bold were used in the final assays.

| | **acuspes -280R** | **acuspes -238R** | **acuspes R** | **minutus398 R** | **minutus480 R** | **moultoni520 R** | **Ptoni R** |
|---|---|---|---|---|---|---|---|
| **PseudoF** | F | F | F | F | F | F | |
| **PseudoF-mod** | F | F | F | F | F | F | |
| **acuspesF** | S | **S** | S | | | | |
| **minutusF** | | | | S | S | | |
| **minutusF2** | | | | **S** | S | | |
| **moultoni307F** | | | | | | F | |
| **PtoniF** | | | | | | | **S** |

A primer mix including *P. acuspes, P. minutus* and *P. moultoni* primers produced a very clear primer dimers and products of several lengths. It was therefore decided to use a dual assay approach with one multiplex primer mix that detected *P.* acuspes and *P.* minutus and a separate assay for *P. moultoni*.

To further reduce the total workload, the Dreamtaq buffer was replaced by Dreamtaq Green buffer (Thermofischer), which makes it possible to run the PCR product on a gel without adding loading dye.

## 2.5 PCR assay and gel electrophoresis

The optimized species-specific PCR assays (Table 6 and 7) were then used to identify *Pseudocalanus acuspes, P. minutus* and *P. moultoni.* The PCR products from the primer cocktail have a size difference of 160 base pairs which makes it possible to separate products from *P. acuspes* and *P. minutus* on a 1.5% agarose gel stained with Gel Red (Biotium). The *P. moultoni* PCR products were run on a 2.0% agarose gel to obtain a better separation between the small PCR products and potential primer dimers.

Table 6 List of primers used in the final PCR assay.

| **Name** |
|---|
| acuspesF/ acuspes238R/ minutusF/ minutus398R |
| PtoniF / PtoniR |

Table 7 The final ingredients of the 10 µL PCR reactions used to identify *Pseudocalanus acuspes, P. minutus* and *P. moultoni.*

| **Reagents** | ***P. acuspes* + *P. minutus*** | ***P. moultoni*** |
|---|---|---|
| MilliQ | 6.1 $\mu L$ | 6.5 $\mu L$ |
| Dream Taq green buffer | 1.2 $\mu L$ | 1.2 $\mu L$ |
| dNTPs | 0.8 $\mu L$ | 0.8 $\mu L$ |
| Forward primer | 0.2 $\mu L$* 2 | 0.2 $\mu L$ |
| Reverse primer | 0.2 $\mu L$* 2 | 0.2 $\mu L$ |
| Enzyme | 0.1 $\mu L$ | 0.1 $\mu L$ |
| DNA | 1.0 $\mu L$ | 1.0 $\mu L$ |

## 2.6 Sanger sequencing

After successfully identifying *Pseudocalanus acuspes, P. minutus* and *P. moultoni*, all unidentified samples (185 samples from Billefjorden and 92 samples from the Barents Sea) were amplified using general eukaryotic COI primers (Folmer et al., 1994), and the products were sent for Sanger sequencing to determine whether they contained another species of *Pseudocalanus* (e.g. *P. elongatus*). The following primer mix and protocol were used; A 25 µL master mix containing 16.8 µL sterile water, 2.5 µL Dreamtaq buffer (10x), 2.0 µL dNTP mix (2.5 mM each), 0.5 µL of LCO1490 primer (10 µM) and HCO2198 primer (10 µM), 0.5 µL bovine serum albumin (BSA, 10mg/ml), 0.2 µL Dreamtaq polymerase, and 2.0 µL extracted DNA, with a PCR protocol that consisted of 3 min at $94^0$C, 35 cycles of 30 sec $94^0$C, 60 sec $50^0$C, 60 sec $72^0$C, and then 10 min at $72^0$C. The PCR products were checked on a 1% agarose gel to ensure that a product of the expected size had been formed.

The PCR products were cleaned using SPRI beads according to a protocol made by S. Thompson, consisting of 0.1% (5% SeraMag Speed) beads, 18% Polyethylene glycol (PEG), 1M NaCl, 10 mM Trish-HCl (pH=8.0), and 1 mM EDTA (pH=8.0), and eluted in 10 µl milliQ. A mix of 7.5 µL cleaned product and 2.5 µL LCO1490 primer (10 µM) was sent to Eurofins Genomics in Germany for LightRun sequencing. The results were downloaded from the Eurofinsgenomics.eu page and compared using BLASTn to the nucleotide database at NCBI (National Center for Biotechnology information) in addition to the online identification engine for Species Level Barcode Records at Boldsystems.org during June to September 2022.

## 2.7 Data processing and visualization

Conductivity measured in Billefjorden with the SAIV handheld CTD was converted to salinity using the SAIV-MiniSoft SD200W software. The CTD data containing temperature, salinity and fluorescence from each depth was quality controlled and interpolated at 1m intervals. The software Ocean Data View (version 5.6.2 – 64 bit (Max OS X)) was used to draw a map and visualize the CTD data, while the TS plot was made in R studio (version 1.4.1717) using the OCE package (version 1.7 – 10 – developed by D. Kelley and C. Richards). Water masses in Billefjorden were categorized according to Skogseth et al. (2020).

Water masses in the Barents Sea were defined according to the Nansen Legacy water mass definitions for the central and northern Barents Sea (Sundfjord et al., 2020).

The total abundance of copepods was calculated as individuals per sample (c) divided by subsample volume (s) multiplied with total volume (v) and how many times the sample was split (p).

$$Total\ abundance\ (x) = \sum_{n=3} \left( \frac{cvp}{s} \right)$$

To get the abundance per $m^{-3}$, the total abundance was divided by the opening area of the net (a) and sampling depth (d) as illustrated below.

$$Total\ individual\ per\ m^{-3}\ (x) = \sum_{n=3} \left( \frac{cvp}{sad} \right)$$

To be able to compare the data between samples, a frequency table was made where the different life-stages and gender categories were up-scaled to fraction per $m^{-3}$. This was done by first creating an "up-scale factor" per species and sample, where the number of individuals per $m^{-3}$ was divided by the total species count for the sample. This factor was then multiplied by each life-stages and gender category to obtain average individuals per $m^{-3}$.

*Nmonth/species / Tmonth/species = Fmonth/species*

Nmonth/species; Number of individuals per $m^{-3}$ (for a specific month and species)
Tmonth/species; Total species count (for the same specific month and species)
Fmonth/species; Factor for that month and species.

Changes in abundance and species and stage composition were displayed as stacked bar plots and density ridges made with the ggplot2 package (version 3.3.6 – developed by Wickham H 2016) in R Studio.

## 2.8 Statistics

Descriptive statistics (i.e., mean, standard deviation, standard error, median, minimum, and maximum values) were summarized for body size (prosome length).

Prior to choosing a statistical method to test difference in prosome length between the different *Pseudocalanus* species, the Shapiro-Wilk normality test and QQplot was analysed. Normality was checked by using the Shapiro-Wilk normality test complemented by QQplot and boxplots with the use of stats (version 4.1.3 developed by the R Core Team), qqplotr (0.0.5), tidyverse (1.3.2 developed by the R Core Team) and dplyr (version 1.0.10 maintained by Wickham). The boxplots were added to visualize potential outliers, and to be able to assess if the outliers provided a skewed distribution.

The Shapiro-Wilk test showed that normality was rejected for most samples except *Pseudocalanus moultoni* CIV, adults, and *P. minutus CIV* in Billefjorden, and *P. acuspes* CV from the Barents Sea (Supplementary 1). QQplot – Shows that two out of three *Pseudocalanus* species deviate from the confidence interval and hence support the use of the Kruskal – Wallis test (Supplemenatry 2). Since the data was not normally distributed, the non-parametric Kruskal-Wallis test was used to test differences in prosome length across *Pseudocalanus* species, as it can analyze for differences between three or more variables. The test requires independent samples whereby the null hypothesis in this case is that the population size distribution is equal for all species. The test provides a p-value that corresponds to the two-sided test based on the chi-square distribution. An alpha of $<0.05$ was set in order to reject the null hypothesis and conclude that there are significant differences in the size distribution of the different *Pseudocalanus* species.

As the Kruskal-Wallis normality test does not provide an indication of which species had significant different body size, the Dunn`s multiple comparison procedure was used to identify which *Pseudocalanus* species of CIV and CV differed. For this the FSA (version 0.9.3) package was used in R-Studio.

In addition to general statistics both clustering and nonmetric multidimensional scaling (NMDS) was conducted on the abundance (individuals per $m^{-3}$) and environmental dataset,

the latter containing temperature, salinity, in-situ fluorescence of Chl *a* (µg/l) and in-vitro Chl *a* (µg/l). A dendrogram was drawn based on Euclidean distance to group similar samples based on abundance individuals per $m^{-3}$ of the three *Pseudocalanus* and to look for patterns. For this ggplot2 (version 3.4.0 developed by Wickham et al.) and dendextend (version 1.16.0) were used.

Hellinger transformed NMDS with Bray-Curtis dissimilarity was used to compare the *Pseudocalanus* species complex structure between samples at different locations and different month of the year based on the counts of different species between samples. The stress value provides information on the difference between the distance in the reduced dimension compared to the complete multidimensional space, and should be as optimized as possible according to the Goodness-of-fit scale (Kruskal, 1964). For the NMDS, the vegan package (version 2.5 – 7 developed by Oksanen et al.) and the FactoMineR (version 1.34 developed by Husson et al.) was used in R-Studio, and ggplot2, and factoextra (version 1.0.7 developed by Kassambara) was used to improve the visualization of the plot.

In the redundancy analysis (RDA) dominant water temperature, dominant salinity, Chl a, and in-situ fluorescence of Chl a was compared to life stage per species for each stations in the Barents Sea. An RDA provides the opportunity to find connections between species and environmental factors as it explores the relationships between two matrices. If the data is not normally distributed the RDA should rely on a permutation test. This test would check for global RDA significance, axis significance and explanatory variables significance. The RDA analysis was conducted in R-Studio with the ggord packages (version 1.0.0). The Hellinger-transformation was used with the decostand function. Pairwise correlations were removed and the adjusted $R^2$ explained the variance.

# 3 Results

## 3.0 Physical environment

In Billefjorden, no Atlantic Water (AW) or Transformed Atlantic Water (TAW) was detected during the sampling period, but all stations had some Arctic Water (ArW), Local Water (LW) and Winter Cooled Water (WCW) (Figure 3). In November 2020 also Intermediate Water (IW) was detected, while in the period June until October 2021 Surface Water (SW) and IW was also detected for all months.

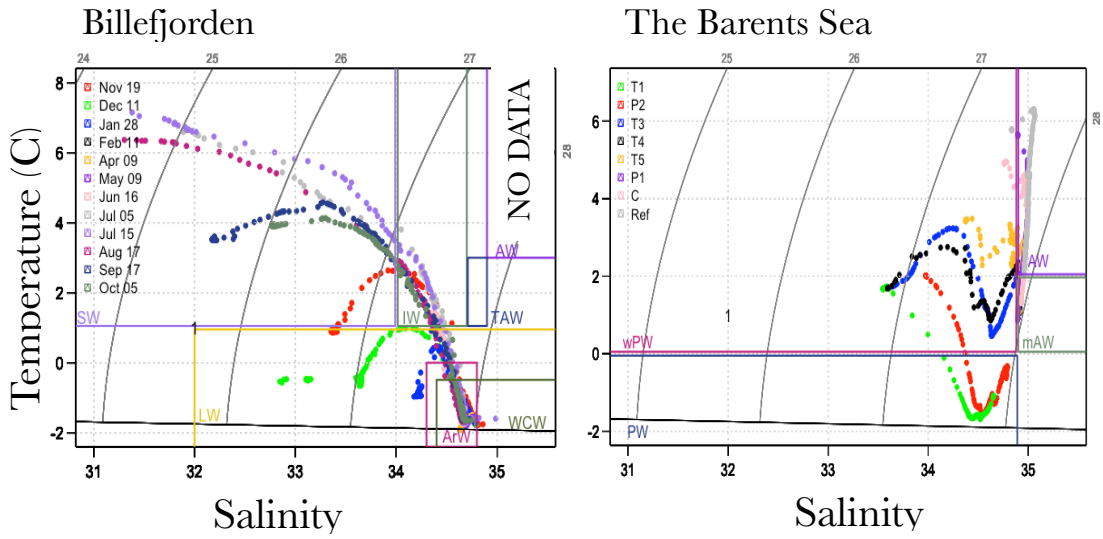


Figure 3 Temperature and salinity diagram taken with A) the SAIV SD204 CTD in Billefjorden during the sampling period from November 2020 until October 2021. The different water masses are identified according to the Skogseth et al. 2020 framework for Svalbard fjords. B) the SBE911 plus CTD in the Barents Sea during the October 2020 Nansen Legacy cruise. The different water masses in this plot are identified according to the Sundfjord et al. 2020 workshop report from the Nansen Legacy Report Series. Atlantic water (AW), Transformed Atlantic water (TAW), Intermediate Water (IW), Surface Water (SW), Local Water (LW), Arctic Water (ArW), Winter cooled Water (WCW), Polar Water (PW), warm Polar Water (wPW) and modified Atlantic Water (mAW).

In 2021 there was hardly any sea ice cover in Billefjorden during January to May. Fast sea ice was only found in the inner Adulfbukta from April to mid-May. The drift ice varied from week to week but was only covering Billefjorden in March, and then only as very open drift ice. Because of the lack of permanent sea ice there were never thick enough ice conditions to support sampling by snow mobile, and the sampling in March was not conducted.

In November the water temperature in the upper 60 meters in Billefjorden was 1.67 ºC, but it dropped in December and went below 0 ºC (Figure 4). The upper water column was more or less homogenous from January until May with the maximum and minimum temperatures ranging between 0.4 ˚C and -1.9 ˚C, while the average temperature at 0-60 m, 60-bottom, and 0-bottom all was below 0. Surface waters started warming in early June 2021 (Figure 4) when the temperature in the upper 30 meters rose to 0.7 -1.4 ˚C with an average temperature of 0.62 C˚ (Supplementary 3). This rise continued in the upper 60 meters in July and lasted until the beginning of October with maximum surface temperatures reaching 7.2 ˚C in July. The average temperature between 0 - 60 meters was above 2˚ C for all months between July and October, and the surface layers still had above 4 ˚C in September and October. The deeper water masses on the other hand seem more stabled and below -1.0 ºC for all months.

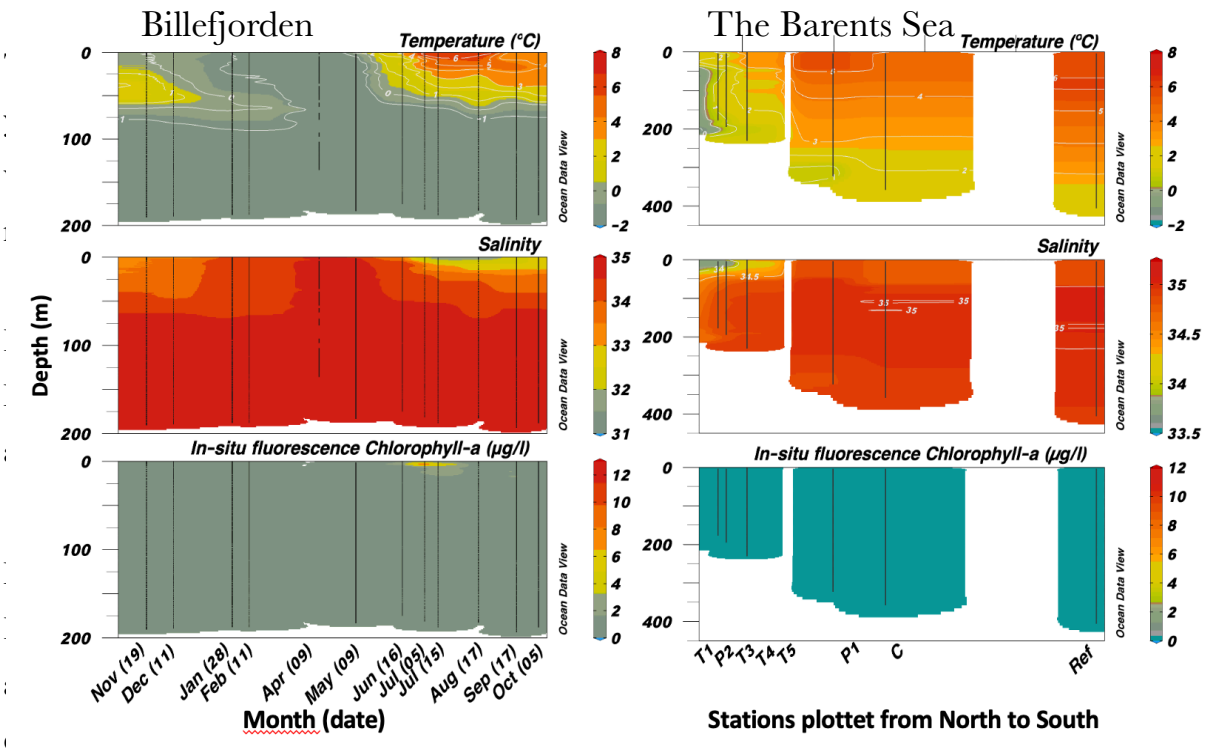


Figure 4 Seasonal changes in temperature (ºC), salinity and in-situ µg/l Chl a taken with a A) SAIV CTD 595 during the Billefjorden sampling period from November 2020 until October 2021, except for April where the in-situ florescence data is missing. B) Seabird 9E plus during the October 2020 Nansen Legacy cruise in the Barents Sea (arranged by latitude).

than the surface water at the different stations and temperature and salinity increased with decreasing latitude.

A clear gradient from colder to warmer water was observed in the Barents Sea from North to South when crossing over the Polar Front from 77 to 73º North. At the northern most stations T1 and P2, the average water temperature in the deeper layer was below 0 ºC degrees, while it gradually increased to an average of 4.10 ºC furthest South (Figure 4). A similar pattern was observed at the surface where the average temperature reached 5.48 ºC at P1 and 5.78 ºC at the Atlantic reference station, while it was much colder at T1 and P2 (Figure 4). Except for T1, most stations had average salinity above 34 at all depths, while T1 had lower salinity average at the surface. In the Barents Sea in November all stations displayed very low Chl *a* values at all stations (Supplementary 4).

## 3.1 Species-specific polymerase chain reaction (PCR) and Sanger sequencing

Of the in total 1947 samples of individual copepods, 277 samples were not properly identified with the use of polymerase chain reaction (PCR). This could be due to the absence of DNA, misidentified other copepod species as *Pseudocalanus* or the PCR not properly working. These 277 samples were sent for Sanger sequencing where two of the Billefjorden samples and 12 of the Barents Sea samples was identified as *Pseudocalanus* (Table 8 and 9), leaving an error margin of less than 0.72 % that were not identified properly by PCR.

Table 8 Among the 277 samples sent for Sanger Sequencing, only 2 individuals from the Billefjorden samples, taken between November 2020 and October 2021, were identified as *Pseudocalanus*.

| | **Nov (19)** | **Dec (11)** | **Jan (28)** | **Feb (11)** | **Apr (09)** | **May (09)** | **Jun (16)** | **Jul (05)** | **Jul (15)** | **Aug (17)** | **Sep (17)** | **Oct (05)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pseudocalanus* | 0 | 0 ( | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other species/ No DNA | 8 | 11 | 12 | 17 | 6 | 53 | 21 | 13 | 13 | 4 | 13 | 12 |
| Total | 8 | 11 | 12 | 18 | 7 | 53 | 21 | 13 | 13 | 4 | 13 | 12 |

On average, 83.5 % of the individuals picked in Billefjorden were identified as *Pseudocalanus,* and 88.75 % from the Barents Sea samples. These differences can be explained by increased preciousness in picking the right species with time, as the Billefjorden samples were processes before the Barents Sea samples. Furthermore, the May samples from Billefjorden also contained a lot of younger and smaller stages of copepods which are more difficult to identify.

Table 9 Among the 277 samples sent for Sanger Sequencing, only 12 individuals from the Barents Sea samples, collected in October 2020, were identified as *Pseudocalanus.*

| | **T1** | **P2** | **T3** | **T4** | **T5** | **P1** | **C** | **Ref** |
|---|---|---|---|---|---|---|---|---|
| *Pseudocalanus* | 6 | 0 | 0 | 0 | 1 | 0 | 0 | 5 |
| Other species/ No DNA | 13 | 6 | 6 | 16 | 8 | 8 | 12 | 11 |
| Total | 19 | 6 | 6 | 16 | 9 | 8 | 12 | 16 |

## 3.2 Abundance

In Billefjorden, the overall *Pseudocalanus* abundance ranged between 81 and 880 individuals per $m^{-3}$, with the highest abundance in January and February 2021, and the lowest abundance in July the same year. In January and February, *P. acuspes* was the most abundant *Pseudocalanus* species and peaked with >800 individuals per $m^{-3}$, before dropping drastically in numbers during the spring. *P. acuspes* abundance increased again in August and throughout September and October.

*P. minutus* was also present in all months, but it was overall less abundant compared to *P. acuspes.* Highest abundance of *P.* minutus was observed in June with 79 individuals per $m^{-3}$, and February (70 ind. $m^{-3}$) (Table 10).

Table 10 Total abundance per $m^{-3}$ for each month and species in Billefjorden including the relative abundance in % of the species complex per month recorded in ().

| | **Nov (19)** | **Dec (11)** | **Jan (28)** | **Feb (11)** | **Apr (09)** | **May (09)** | **Jun (16)** | **Jul (05)** | **Jul (15)** | **Aug (17)** | **Sep (17)** | **Oct (05)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. acuspes* | 322 (84) | 478 (96) | 816 (93) | 810 (92) | 391 (88) | 115 (73.5) | 72 (38) | 57 (66) | 52 (64) | 200 (80) | 188 (85) | 372 (89) |
| *P. minutus* | 47 (12) | 18 (4) | 53 (6) | 70 (8) | 53 (12) | 36 (22.5) | 79 (42) | 30 (34) | 16 (20) | 48 (20) | 33 (15) | 47 (11) |
| *P. moultoni* | 13 (3) | 0 (0) | 11 (1) | 0 (0) | 0 (0) | 8 (5) | 40 (21) | 0 (0) | 13 (16) | 0 (0) | 0 (0) | 0 (0) |
| Total | 382 | 497 | 880 | 880 | 444 | 158 | 191 | 86 | 81 | 248 | 221 | 419 |

*P. moultoni* on the other hand was only present sporadically and only in low numbers. It was found in November, January, May, June and mid-July, with a peak of 40 individuals per $m^{-3}$ in June, otherwise only in very low numbers (<15 ind m-3) (Table 10).

*Pseudocalanus acuspes* dominated the Billefjorden *Pseudocalanus* community throughout the study period (80 %), except in June when *P. minutus* increased and exceeded 42 % of the abundance. *P. moultoni* only exceeded 20 % of the community once in June (Table 10).

The *Pseudocalanus* abundance was highly variable across stations in the Barents Sea ranging from 6 ind $m^{-3}$ at the Atlantic reference station to 1246 ind $m^{-3}$ at P2 furthest North (Table 11). *P. acuspes* and *P. minutus* dominated the species complex composition at the northern and Polar Front stations (T1, P2, T3, T4 and T5), while *P. minutus* comprised more than 45 % of the *Pseudocalanus* individuals at the southern stations (P1, C and the southern reference station) (Table 11).

Table 11 Total abundance per $m^{-3}$ for each station sampled in the Barents Sea October 2020 including the relative abundance in % of the species complex per month recorded in ().

| | **T1** | **P2** | **T3** | **T4** | **T5** | **P1** | **C** | **Ref** |
|---|---|---|---|---|---|---|---|---|
| *P. acuspes* | 77 (47.5) | 911 (73.1) | 56 (52.3) | 103 (61.0) | 38 (53.8) | 9 (33.3) | 6 (30.7) | 1 (11.8) |
| *P. minutus* | 83 (51.3) | 295 (23.7) | 38 (35.2) | 53 (31.2) | 29 (40) | 5 (21.0) | 4 (20.5) | 2 (39.5) |
| *P. moultoni* | 2 (1.3) | 40 (3.2) | 13 (12.5) | 13 (7.8) | 4 (6.3) | 12 (45.7) | 9 (48.9) | 3 (48.7) |
| Total | 162 | 1246 | 107 | 169 | 71 | 26 | 19 | 6 |

## 3.3 Population structure (Life cycles and stages)

In Billefjorden, CI-III and CIV of *Pseudocalanus acuspes* peaked in abundance in January 2021, but thereafter decreased and had lowest values observed in May 2021. In the same period both CIV and CV increased, and CV peaked in May when adults had only started appearing. The *P. acuspes* adults were most abundant in the beginning of July (Figure 6).

The overall abundance of *Pseudocalanus minutus*, was lower than *P. acuspes* (Figure 5 and 6). A clear peak in abundance of CI-III of *P. minutus* was seen in June of 2021, while CIV and CV increased during the fall. CIV peaked in January and February, and the first females were found in February, but were more abundant in April.

*P. moultoni* was only sporadically found, but in June and mid-July, all life stages of *P. moultoni* were present in Billefjorden. CIV and CV were the dominating stages, except for November and May when also CI-III was found (Figure 5).

In the Barents Sea in October, CI-III was the most abundant life stage of *Pseudocalanus* spp., accounting for 86 % of the *Pseudocalanus* populations at P2 of which 79 % of the CI-III were identified as *P. acuspes* (Figure 6). *P. acuspes* was well represented in the Barents Sea and had presents of CI-CV at all stations. CV was found at some of the stations, but adults were in general not present.

*P. minutus* was the most abundant species at T1, and the second most abundant species at all other northern and Polar Front stations. In the northern stations *P. minutus* CI-CIV were found in high numbers, and at T1 some CV and adults were also identified (Figure 6).

While highest relative abundance of *P. moultoni* was found in the southern reference station, highest total abundance was observed at P2. There younger life stages such as CI-III were more abundant, while at the more southern stations later life stages such as CV were present in higher abundance (Figure 6).

In Billefjorden, adults of *Pseudocalanus* were mostly represented by females. While *P. minutus* females occurred already in early February and peaked in abundance in April, females of *P. acuspes* first occurred in April and did not peak before the beginning of July. Surprisingly, females were present in 6-7 months of the year for both *P. minutus* and *P. acuspes* (Figure 7). Only a few *P. moultoni* adults were identified, and all of these were females detected in June and July. In the Barents Sea in October 2020 there were almost no adults present, hence the lack of gender data there.

## 3.4 Prosome length

There was a large overlap in the prosome length frequency distribution of all three *Pseudocalanus* species when comparing all sampling dates and sites (Figure 8). While *P. acuspes* adults had the overall largest mean body size of 1049 µm, adult *P. moultoni* had the overall largest individuals with a maximum prosome length of 1380 µm (Supplementary 5). Still, only a few adults *P. moultoni* were identified, which probably was the reason for the multimodal size distribution.

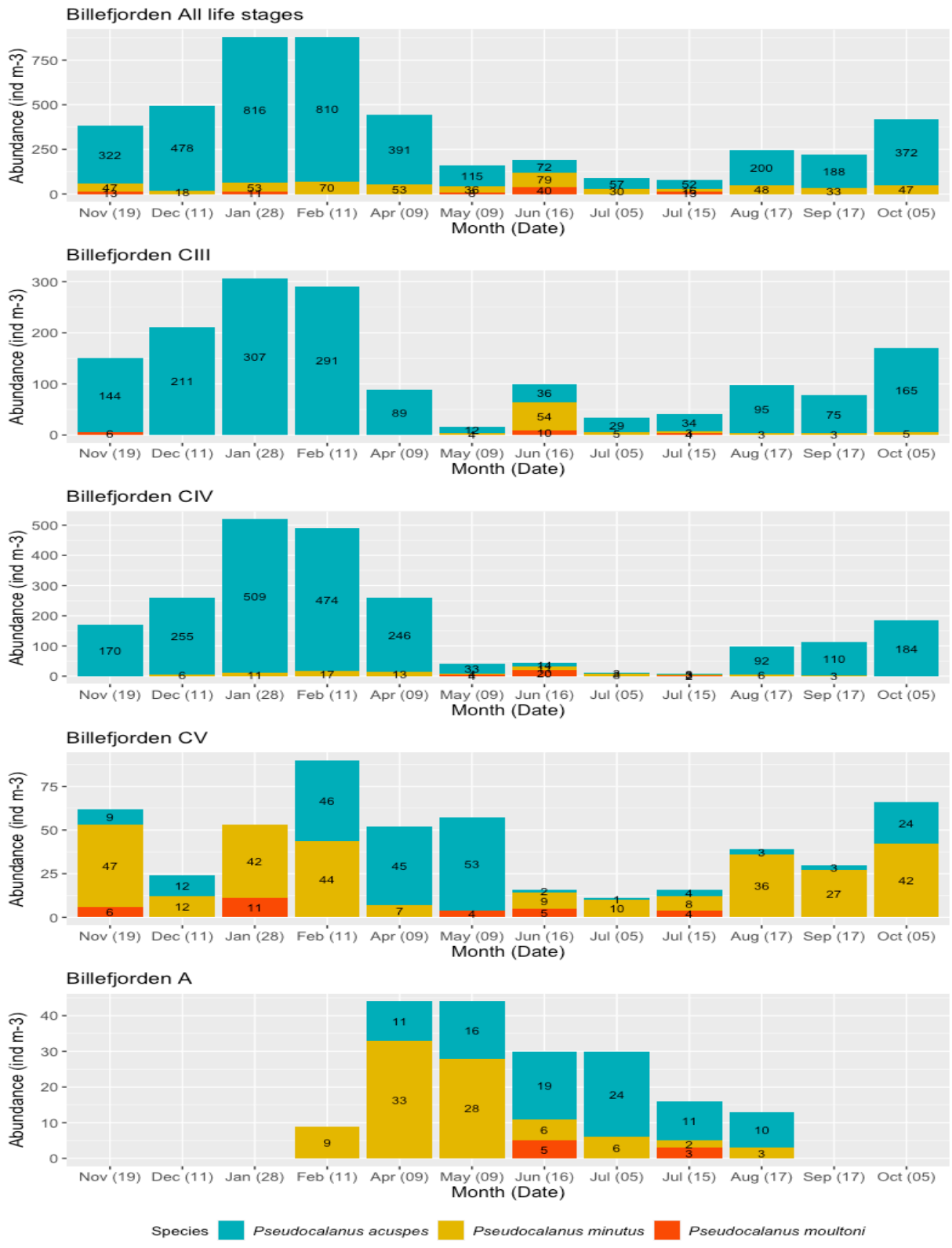


Figure 5 Seasonal changes in abundance and stages composition of *Pseudocalanus* in Billefjorden 2020-2021 A) *Pseudocalanus* all life stages B) *Pseudocalanus* CIII C) *Pseudocalanus* CIV, D) *Pseudocalanus* CV E) *Pseudocalanus* Adult (A).

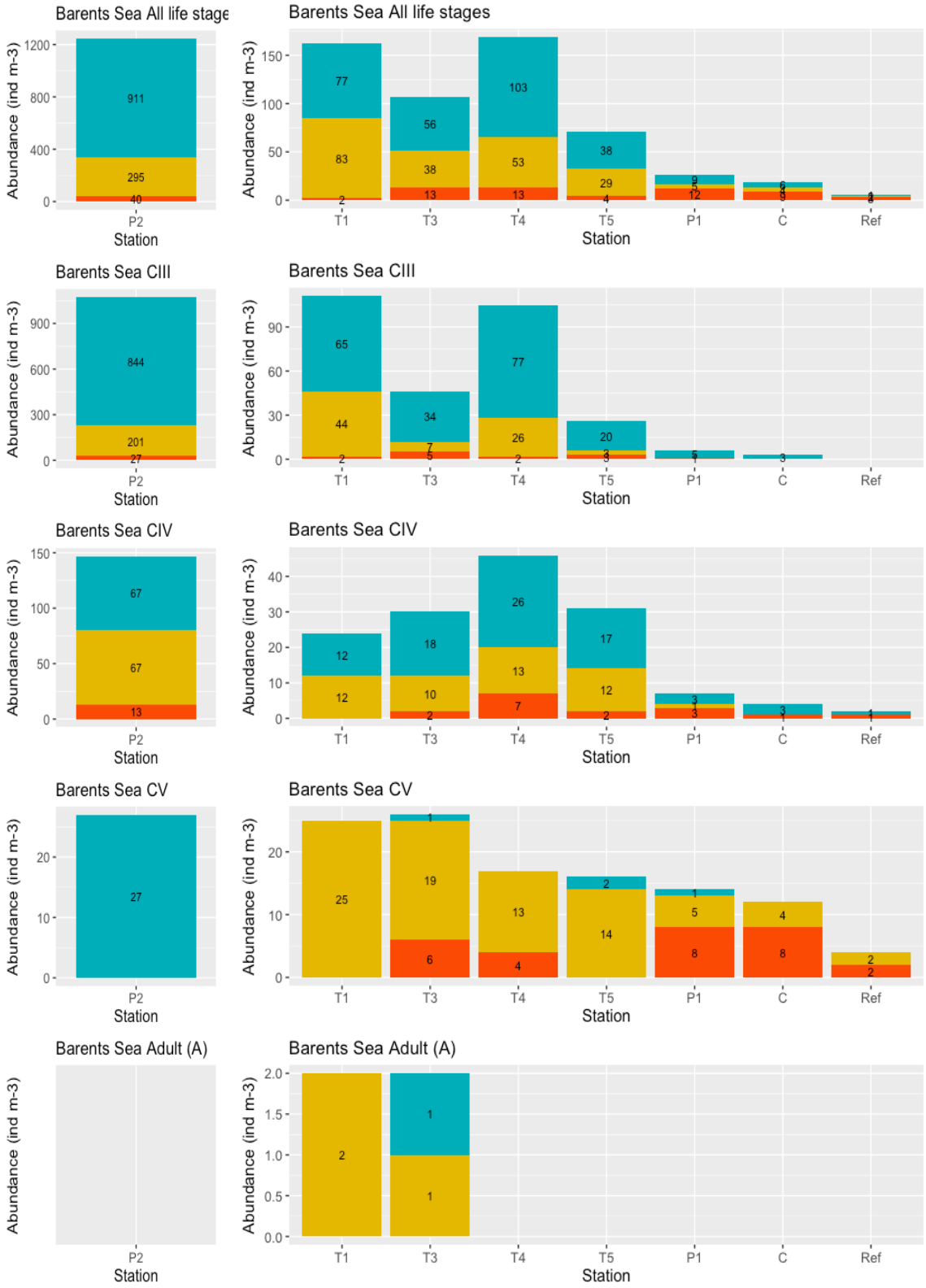


Figure 6 Abundance and species composition of different life stages of *Pseudocalanus* at the different stations in the Barents Sea for A) All life stages B) All CI-III C) CIV, D) CV E) Adults (A).

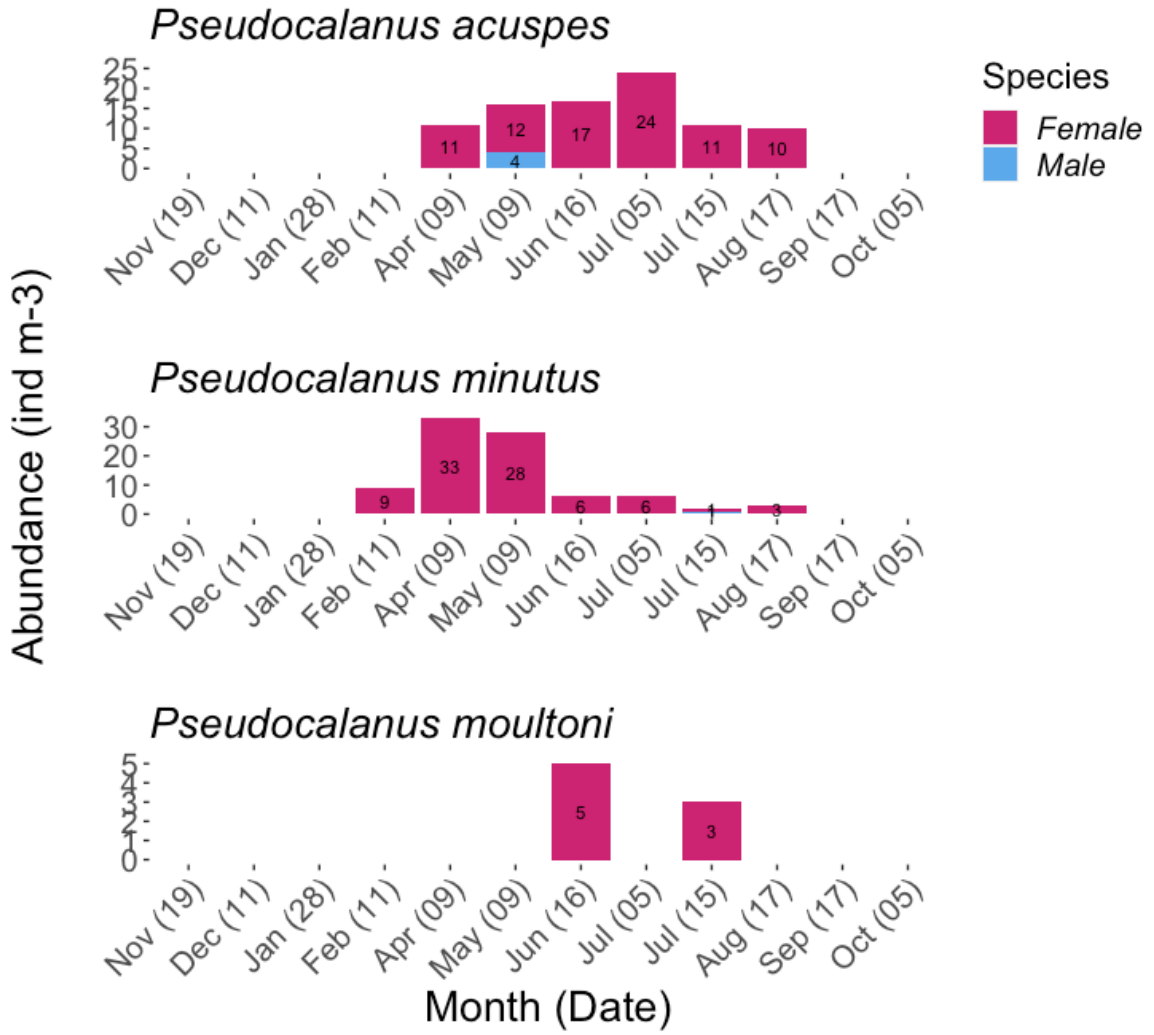


Figure 7 Seasonal changes in abundance of adult females and males of A) *Pseudocalanus acuspes* B) *P. minutus* C*) P. moultoni* in Billefjorden.

At younger life stages *Pseudocalanus minutus* (CIV and CV) had the largest individuals with a size range of 600 to 1020 µm and 660 to 1200 µm, respectively. These two life stages had quite large and overlapping ranges. Also *P. acuspes* and *P. moultoni* had overlapping size ranges between CIV and CV, and the smallest *P. acuspes* CIV was 420 µm while the smallest *P. moultoni* was 720 µm (Supplementary 5). For *P. moultoni* there were less gap between the smallest and the largest individuals, but also fewer individuals were measured.

Kruskal – Wallis test shows that there are statistically significant differences between the three *Pseudocalanus* species in each of the life stages, CIV and CV, in both the Billefjorden

and the Barents Sea habitats. On the contrary, no differences were detected between the adults across both habitats (Supplementary 6).

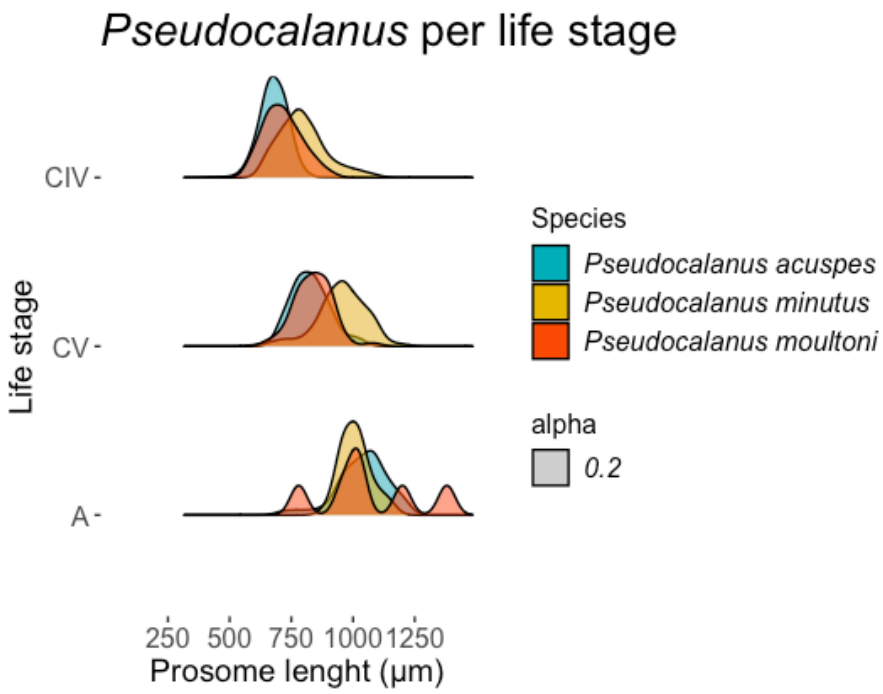


Figure 8 Combined prosome length frequency distribution for all stations and sampling months for the different life stages and species of *Pseudocalanus.*

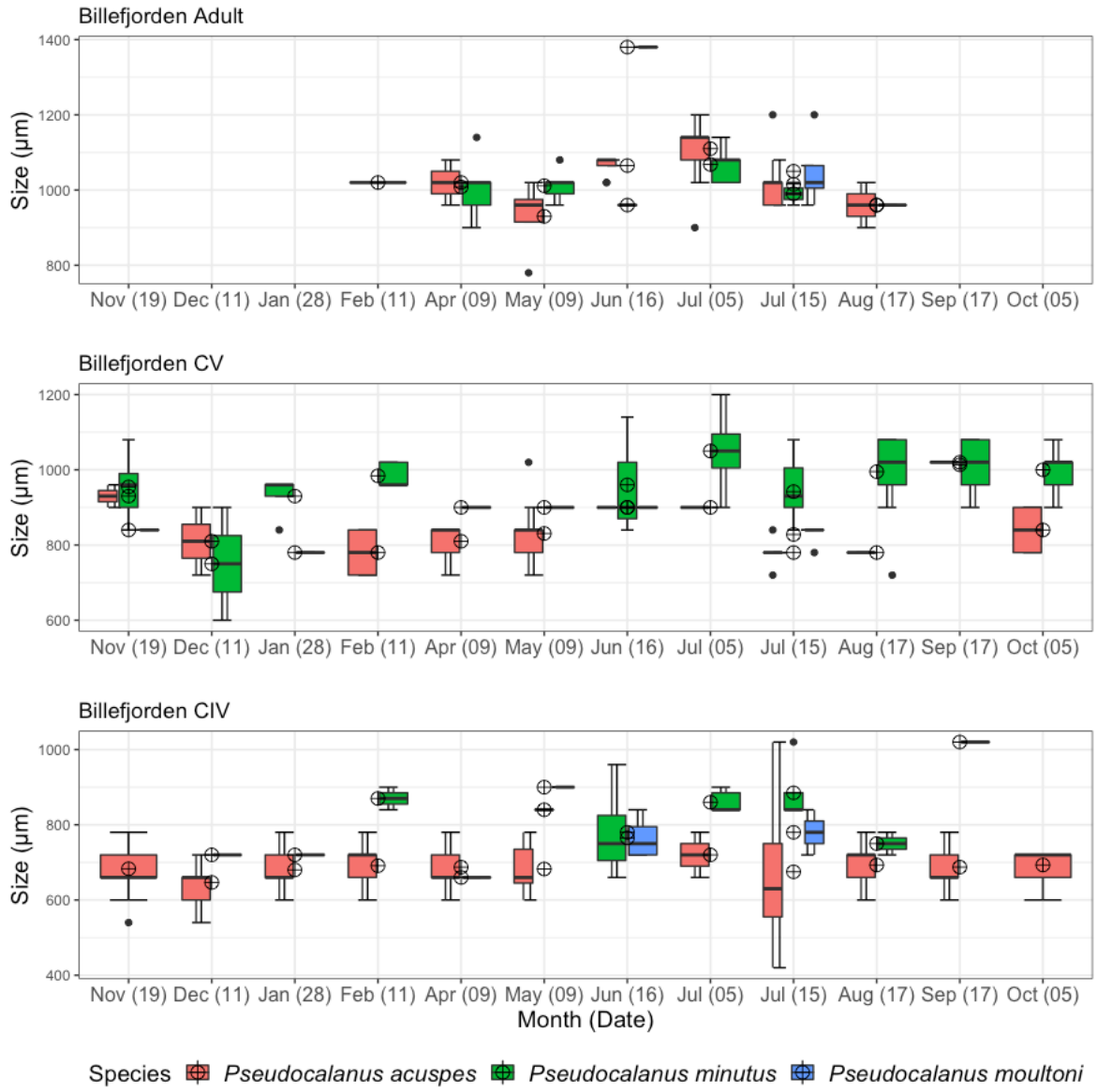


Figure 9 Boxplot of the different body sizes per life stages CIV, CIV and adults of *Pseudocalanus* in Billefjorden Nov 2020 – Oct 2021.The cryptic circles with the plus inside are boxes with too few samples to plot the boxplot for the species, while the black dots are outliers.

In Billefjorden, Dunn`s multiple comparison analysis showed that prosome length was statistical larger for *P. minutus* CV compared to *P. acuspes* and *P. moultoni*, while for CIV *P. acuspes* was also significantly smaller than both *P. minutus* and *P. moultoni* (Figure 9, Supplementary 7).

During the mid-July large variations in prosome length was seen for *P. acuspes* CIV ranging from 420 – 1020 µm. A 300 µm variation between the smallest and the largest individuals in prosome length was also seen in *P. minutus* CV in December, June, July and August (Figure 10, Supplementary 8 and 9).

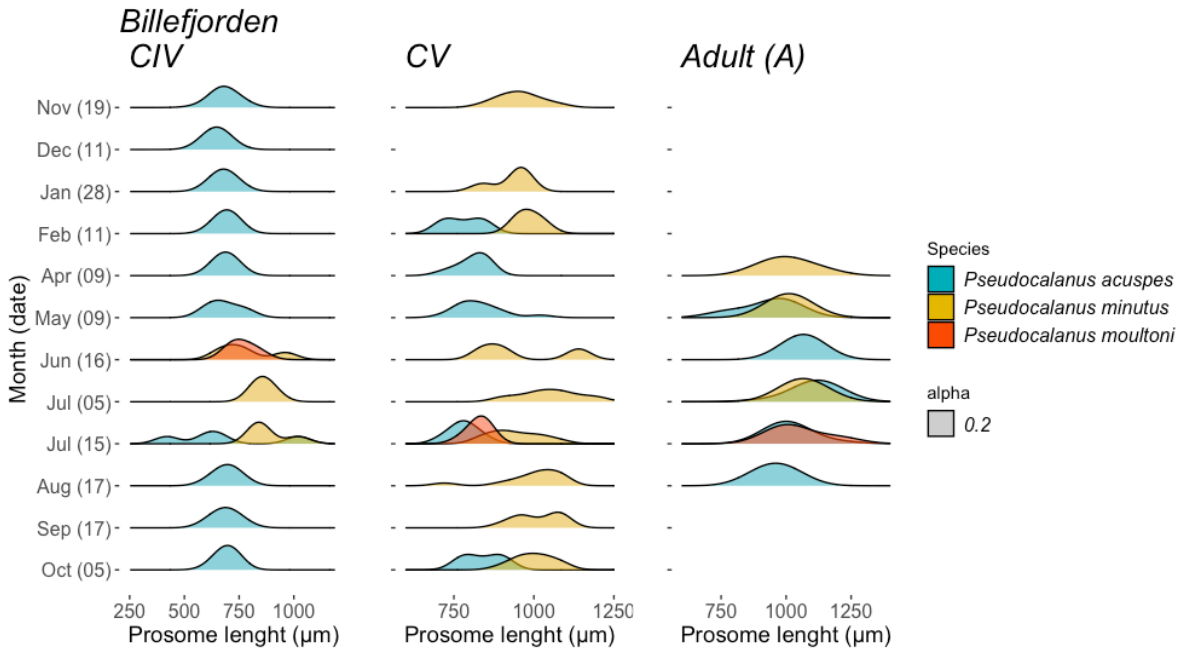


Figure 10 Prosome length frequency distribution of CIV, CV and adults of the three *Pseudocalanus* species from November 2020 to October 2021 in Billefjorden.

In the Barents Sea, Dunn`s multiple comparison analysis showed that prosome length was statistical larger for *P. minutus* CIV and CV compared to both *P. acuspes* and *P. moultoni* (Figure 11, Supplementary 10, 11 and 12).

Large variations between body sizes in and between different stations were also seen in the Barents Sea for *P. minutus* CV. At T1 and P1 the largest difference in minimum and maximum value constituted a difference of 420 µm, potentially indicating several populations present in the area (Figure 12).

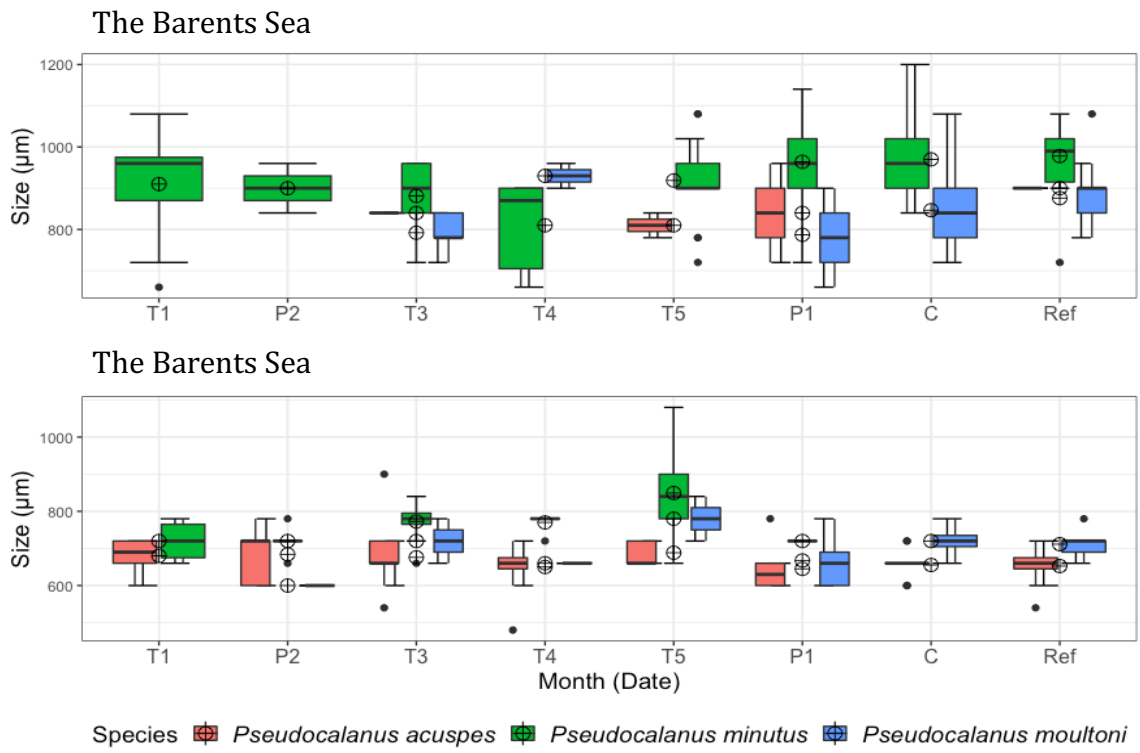


Figure 11 Boxplot of different body sizes per life stages CIV and CV of *Pseudocalanus* in eight different locations of the Barents Sea October 2020. The cryptic circles with the plus inside are boxes with too few samples to plot the boxplot for the species, while the black dots are outliers.

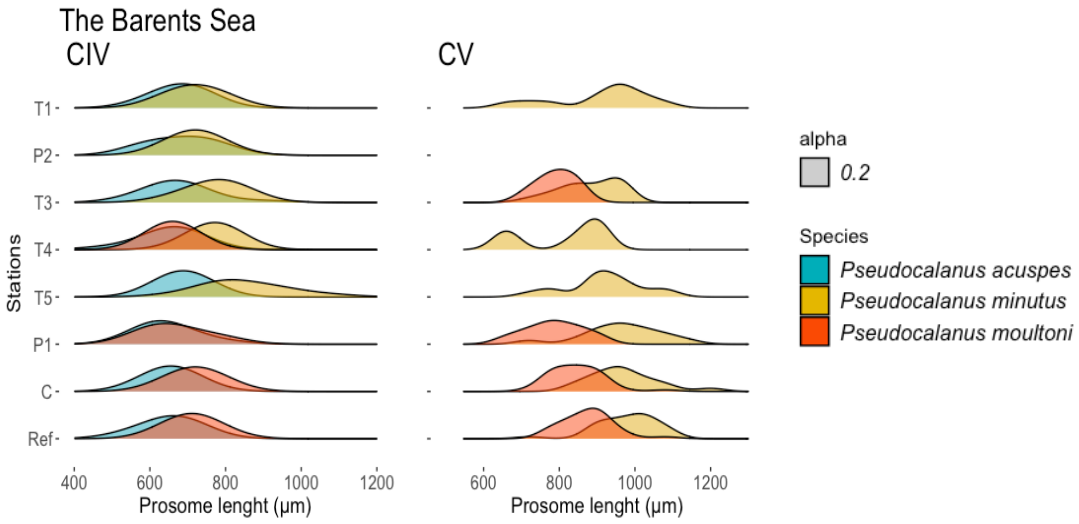


Figure 12 Prosome length frequency distribution of CIV and CV of the three *Pseudocalanus* species at the 8 stations in the Barents Sea October 2020.

## 3.5 Comparison of *Pseudocalanus* community composition

The cluster analysis compared and divided the samples into six clusters. The samples from Billefjorden clustered by season, and the samples from the Barents Sea clustered almost all together. Group 1 consisted of August and September, group 6 consisted on October to December, group 4 consisted of January to February, group 5 consisted of April, group 2 consisted of samples from May to July including all the Barents Sea samples except for P2, and group 3 consisting of P2 (Figure 13).

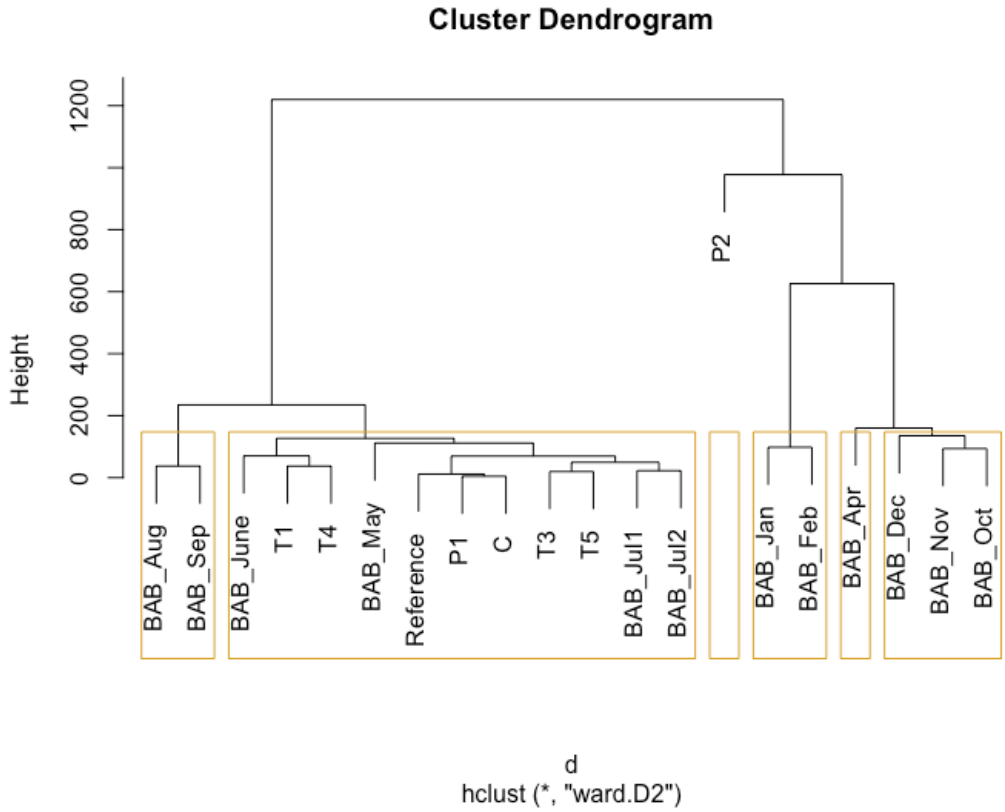


Figure 13 Dendrogram displaying the Euclidean distance between all stations and months, clustered based on total abundance individuals per $m^{-3}$ of each of the life stages (CI-III, CIV, CV, and adults) of the three different *Pseudocalanus* species.

Interestingly, P2 is closer related to the samples from October to April in Billefjorden than to the other samples in the Barents Sea. Since most of the Barents Sea samples clustered together, a separate dendrogram was displayed for these samples based on Euclidean distance. This showed the southern reference stations clustering together, while the Polar Front stations were separated into two groups, and P2 still standing alone constituting the northern stations (Figure 14).

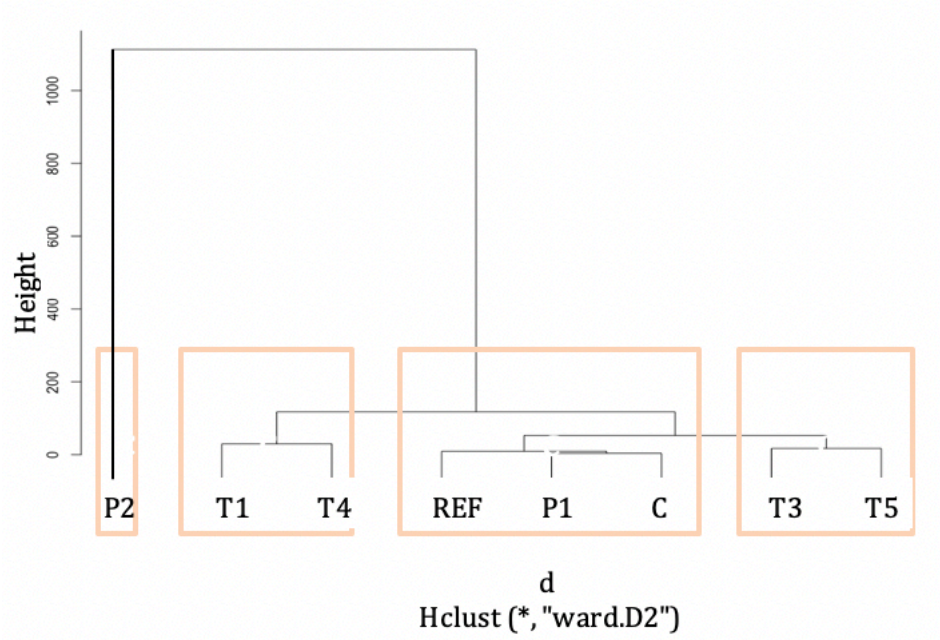


Figure 14 Dendrogram displaying the Euclidean distance between the 8 stations in the Barents Sea sampled in october 2020, clustered based on total abundance individuals per $m^{-3}$ of each of the life stages (CI-III, CIV, CV, and adults) of the three different *Pseudocalanus* species.

Overall in Billefjorden, the clustering seems to confirm two branches of samples; one related to colder and more stable water in the late fall, winter and early spring, and the other group related to warmer more mixed water masses in late spring, summer and early fall. The Barents Sea samples seem to show a gradient from north to the south.

There were seasonal differences in the hydrography in Billefjorden. The winter (January to May) was characterized by low water temperatures and deep mixing. Surface temperatures started to increase in Spring (May to June) and then layers of different water masses started to form. The summer (July to August) was characterized by warm surface water and clear thermoclines, and in autumn (September and until the end of the sampling season in October) water still had plus degrees, but showed indication of slowly starting to cool down (Figure 4). The timing of the seasons was similar to seasons earlier described in (Skogseth et al., 2020).

The NMDS analysis showed that all the Barents Sea samples were clustered together some closer than others (Figure 15). In Billefjorden a pattern of the winter and autumn samples were found relatively close together, while the spring and summer samples showed the same

trend, but the spring and summer samples were a bit more scattered (Figure 15). The 0.10 stress value, with stress type 1 and weak ties, provided a fair representation of the relationship between the stations showing that abundance individuals per $m^{-3}$ clustered together by season in Billefjorden.

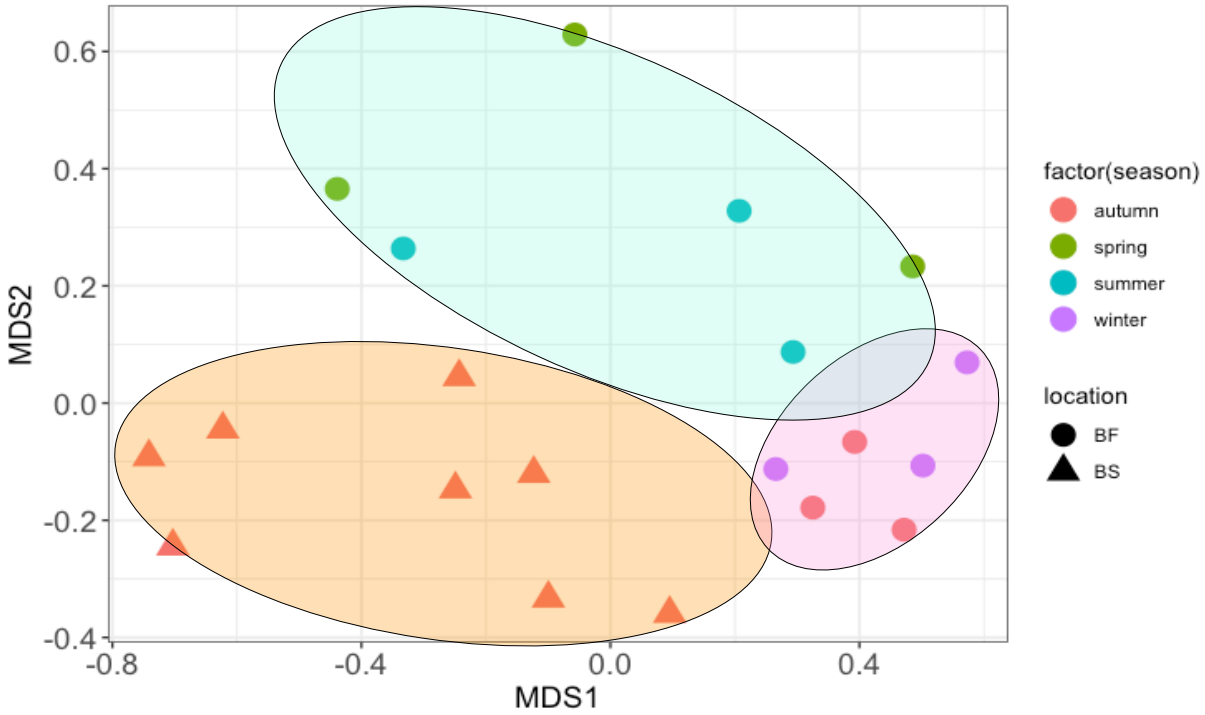


Figure 15 A nonmetric multidimensional scalling (NMDS) with the Bray-Curtis dissimilarity, where all stations and months were separated into categories season and location based on different life stages per each of the three *Pseudocalanus* species.

## 3.6 A potential indicator species?

In the Barents Sea, the P1, C and the southern more Atlantic influence reference station was positively correlated with higher salinity, temperature, deeper depths, while P2 and T1 were negatively correlated to the same variables (Figure 16). The RDA analysis explained 7.34 % and 0.75 % of the total variation, indicating that there is still a lot of the variation that is not explained.

The linear regression plotted for abundance individual per $m^{-3}$ on a temperature scale where the stations were arranged by increasing water temperature, showed that *P. minutus* would decrease in abundance when water temperature rose ($R^2$ value of 0.56), while the regression

coefficient was 0.37 for *P. acuspes* and 0.17 for *P. moultoni* (Figure 17). When the linear regression was plotted as relative abundance however, the $R^2$ value for *P. minutus* and *P. moultoni* rose above 0.5, and the model showed a decrease in relative abundance with higher water temperature for *P. acuspes,* and an increase in relative abundance for *P. moultoni* with higher water temperature (Figure 18).

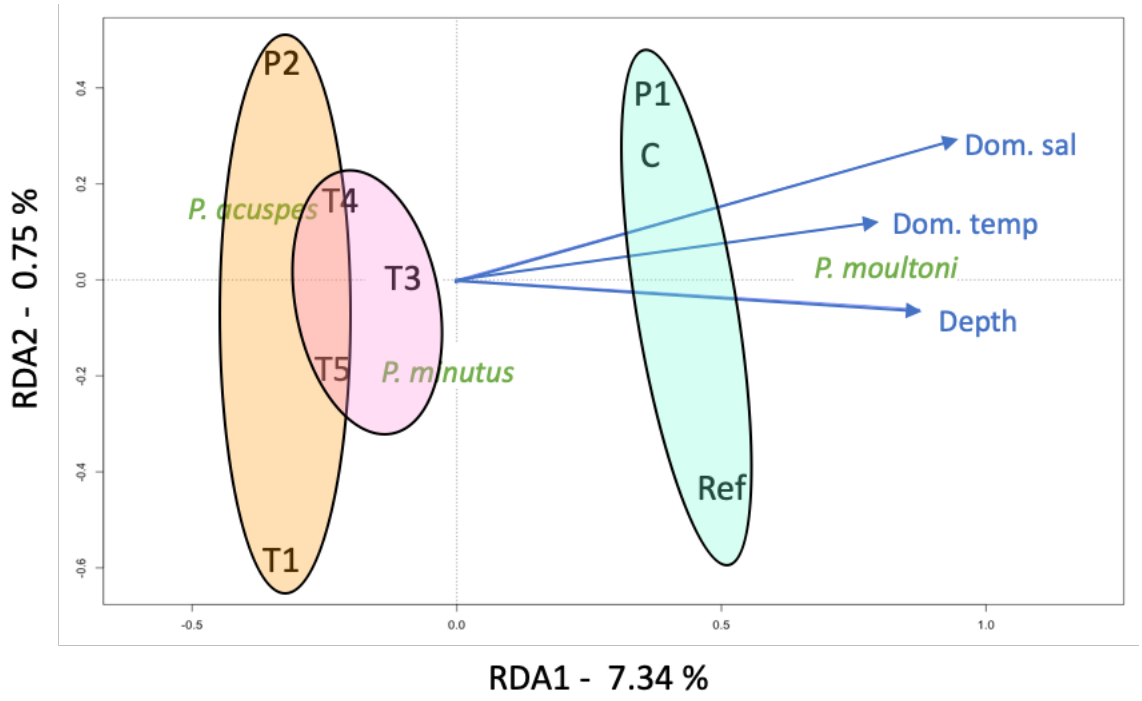


Figure 16 The redundancy analysis (RDA) compared the total abundance in each of the three *Pseudocalanus* species to depth, dominant salinity and dominant temperature at each of the 8 stations in the Barents Sea in October 2020. The dominant salinity and temperature were calculated exclusively by the average of the most dominant water mass at each station.

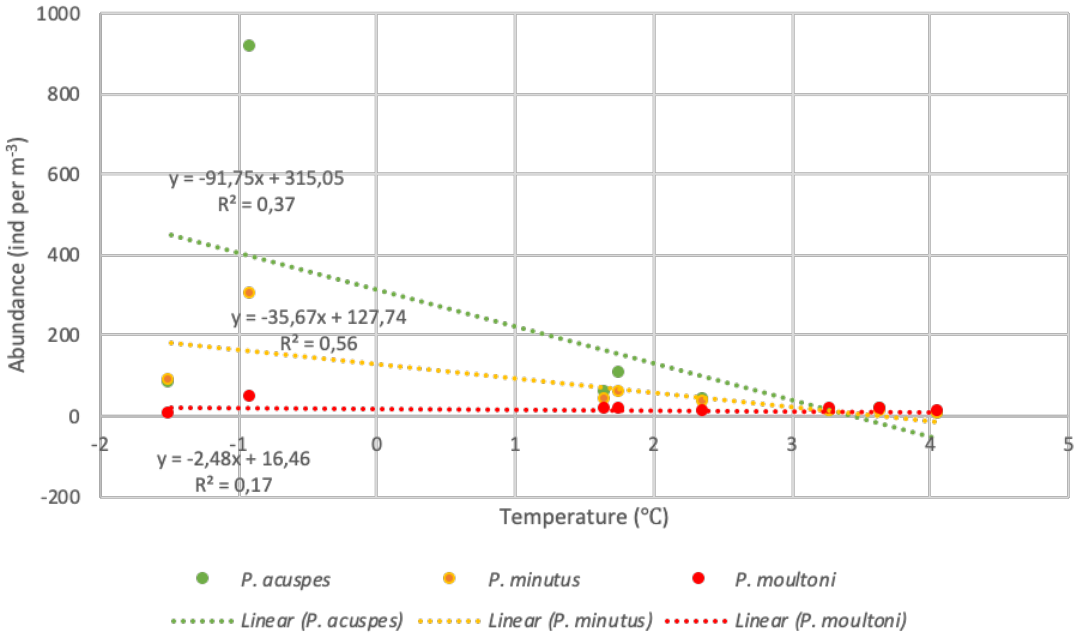


Figure 17 A linear regression showing abundance (individual per $m^{-3}$) for each of the *Pseudocalanus* species arranged based on average dominant water temperature at each station in the Barents Sea October 2020.

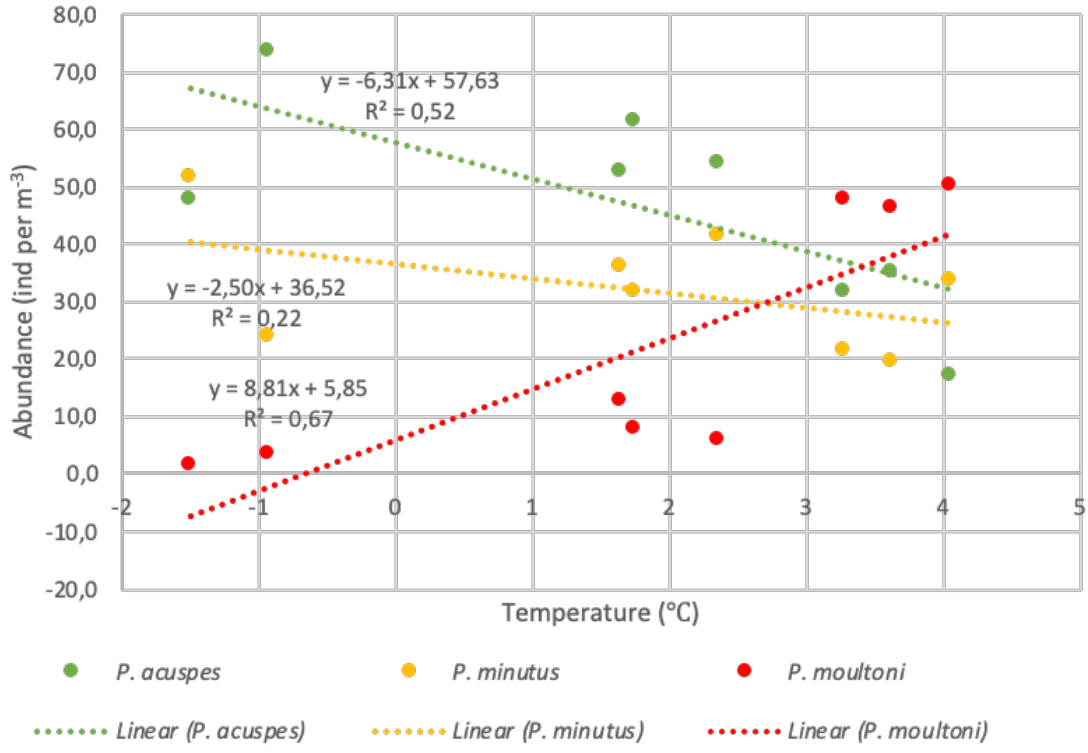


Figure 18 A linear regression showing relative abundance of each of the *Pseudocalanus* species arranged based on average dominant water temperature at each station in the Barents Sea October 2020.

# 4 Discussion

## 4.0 *Pseudocalanus* abundance and species composition

The three species earlier identified in the Svalbard – Barents Sea region (Aarbakke et al., 2011), *Pseudocalanus acuspes, P. minutus* and *P. moultoni,* were all present in both Billefjorden and the Barents Sea, while the boreal *P. elongatus* was never detected. The lack of *P. elongatus* is not surprising as it has only been detected morphologically in the Svalbard - Barents Sea region previously (Ellertsen et al., 1982, Węsławski et al., 1988, Timofeev, 2002), and their presence have been questioned as a potential misidentification (Dvoretsky and Dvoretsky, 2010). Also, the lack of Atlantic and Transformed Atlantic water in Billefjorden during the year, and the dominance of Polar- and warm Polar Water in most of the Barents Sea stations in October (Figure 3) suggest no strong influence of Atlantic Water and thus intrusion of *P. elongatus* as seen in Ramfjorden, Northern Norway, where these four species co-occur (Coguiec et al., 2021).

Overall, Billefjorden had higher *Pseudocalanus* average abundance in October (~ 419 individuals per $m^{-3}$) compared to the average between all stations in the oceanic Barents Sea (~ 223 individuals per $m^{-3}$) (Figure 5 and 6). A reason for this could be that Billefjorden, is a sill fjord, and more protected from large water mass exchanges and thus represent a more stable environment. There was also a gradient of high to low abundance of individuals per $m^{-3}$ between the northern Barents Sea P2 station (~ 1250 individuals per $m^{-3}$) and the southern Barents Sea reference station (~ 6 individuals per $m^{-3}$). The northern stations and most of the Polar Front stations were situated on the shallower banks compared to the southern stations which were in the deeper trenches and potentially are more affected by changing Atlantic Water inflow (Table 1 and Figure 1).

High abundance of *P. acuspes* (~ 800 individuals per $m^{-3}$) in Billefjorden prevailed in winter with colder water masses, while abundance of *P. moultoni* increases during summer with warmer water masses (Figure 5) most likely due to more exchange of water between Billefjorden and the outside Isfjorden. In July the total abundance was low (~ 80 individuals per $m^{-3}$) compared to abundances found in Hornsund (~ 144 - 668 individuals per $m^{-3}$) and Isfjorden (~ 332 - 759 individuals per $m^{-3}$) (Gluchowska et al., 2016).

Interestingly, the maximum abundance of *Pseudocalanus spp.* (~ 800 individuals per $m^{-3}$) were found during January and February in Billefjorden (Figure 5), months that have rarely been sampled before. This indicated an increase in total population numbers either by birth or immigration or that the WP2 net somehow were able to catch some form of accumulation. In January, it was the *Pseudocalanus* spp. CIV that was found in particularly high numbers with no reproducing adults observed. Therefore, it is likely that the increase in abundance are due to inflow of water masses rich in *Pseudocalanus* copepods rather than birth of new individuals. No change in water masses was detected in Billefjorden during January and February (Figure 3), indicating a stable environment, but the CTD data only look for shifts in water masses based on change in salinity and temperature. On the other hand, currents and wind conditions were not measured in Billefjorden during this study, but the wind driven ice conditions were varying from week to week between very open drift ice and open water with a bit of permanent sea ice in the inner part of Adolfbukta (Supplementary 13). A possible theory could therefore be that, the sea ice was pushed back and forward by wind-driven currents bringing copepods along, but as *Pseudocalanus* is situated in deeper water during the winter they should not be subjected for wind driven advection (Weydmann et al., 2013, Søreide et al., 2022). While one could speculate if there could have been formation of copepod patchiness during winter months, it is unlikely that this happened since the high abundances were caught in consecutive months (Figure 5). Hence, as some wind-driven currents were observed this could also have led to a vertical trawling rather than sampling the deepest part, which could also explain accumulation of *Pseudocalanus* as they are distributed higher up in the water column during winter and spring compared to other species doing seasonal vertical migration (Søreide et al., 2022).

During winter-spring transition, the *Pseudocalanus* abundance dropped as previously seen in Billefjorden (Ershova et al., 2021b, Søreide et al., 2022) and elsewhere in the Arctic (Lischka and Hagen, 2005). In July, young CI-III stages started to increase (Figure 4) similar to other studies in the same fjord (Ershova et al., 2021b, Søreide et al., 2022). *P. acuspes* adults were first abundant in June and early July, thus probably reproduce 1-2 months later than *P. minutus* for which the adults peaked in numbers in May. These differences in timing of reproduction reflect their breeding strategy (Varpe et al., 2009). *P. minutes*, with a capital breeding strategy, prioritize to reproduce early using internal energy stores accumulated from

the previous year and in addition to early ice algae bloom (Runge and Ingram, 1991) and thus do not grow much larger from overwintering stage CV to adult. The benefit is that the offspring gets a long growth season, if born early, and can utilize both the phytoplankton bloom (Varpe et al., 2009) and the later post bloom consisting of smaller algae cells and microplankton (Hegseth et al., 2019). *P. acuspes* on the other hand, has more of an income breeding strategy, where it chooses to feed on the spring bloom to grow larger before molting to adult, and then maturing and reproducing (Varpe et al., 2009).

The high abundance of *P. acuspes* CI-III in October and only a few CVs detected in November and December (and absent in January), indicates that only CIII and CIV of *P. acuspes* overwinter in Billefjorden. It might seem like CIII and CIV develop to CV during the spring before becoming adult the same year, thus supporting a one-year life cycle as also seen in the Canadian Arctic (Frost, 1989).

In this study it was proven that *P. minutus* can complete its whole life cycle in Billefjorden, as it has earlier been suggested, but not yet conclude since only parts of the year was sampled (Ershova et al., 2021b). They were present year-round with CI-III in summer and early fall, then CIV and CV during the fall and winter, and adults during the winter, spring, and early summer. The overwintering of CV and the early occurrence of adults could potentially be indications of a 1-2 year life cycles, even though *P. minutus* has been reported to complete a one year life cycle in other Svalbard fjords (Lischka and Hagen, 2005). In Billefjorden, *P. minutus* were also present with a slightly similar abundances (~ 40 average individuals per $m^{-3}$) to previous years (~ 20 individuals per $m^{-3}$) under similar environmental conditions (Ershova et al., 2021b). *P. minutus* did not show any clear patterns of high abundance peaks during any months in Billefjorden nor in the eight stations in Barents Sea in October, this could imply that temperature alone does not explain the abundance of *P. minutus.*

Another interesting result was the presences of *P. moultoni* in January, since it was not expected to be able to complete a life cycle in Billefjorden. The lack of *P. moultoni* in other months during the winter could potentially be due to *P. moultoni* being advected from Isfjorden where it is more abundant (Ershova et al., 2021b) or that *P. acuspes* was (too) dominant during certain months. In some of the months when *P. acuspes* was very abundant,

less than 1 % of the subsample was used to pick the minimum of 89 copepods, while in four of the five months when it was identified, 1 - 5.5 % of the subsample were examined. This indicates that *P. moultoni* could be detected for several months if a larger percentage of the total sample had been checked but does not exclude that it was advected from Isfjorden, which also could explain the high numbers of *P. acuspes* in January. More sample analyses are therefore needed before a conclusion can be drawn on *P. moultoni* is able to complete its life cycle or not in Billefjorden.

## 4.1 Prosome length

Overall, it seems to be impossible to morphologically distinguish between the three co-occuring *Pseudocalanus* species based on body size. The *Pseudocalanus* species differ in size based on individual life stages, but the species size ranges are overlapping, and with outliers that prevent body size from being a good morphological trait to species determine within the genus. This was also the case in the eastern North Pacific and the Pacific Arctic Region where both overlapping size ranges and temperature-dependent size shift were found (Questel et al., 2016).

An interesting finding was that there were significant differences in prosome length of CIVs and CVs between the *Pseudocalanus* species in both Billefjorden and the Barents Sea, with *P. minutus* being larger than its sibling species, while at the same time no significant difference was found in the adult life stages in neither Billefjorden nor the Barents Sea. A potential theory could be that *P. minutus* had early occurrences of adult life stages due to them moulding to adult females on internal resources, as seen in species of the *Calanus* genus to speed up maturation and be able to reproduce early so that the offspring gets a long growing season (Falk-Petersen et al., 2009). For *P. acuspes,* the late peak in abundance of adults could potentially explain why the average body size were larger than *P. minutus,* as they can feed on the spring bloom and thus grow much larger before they molt to adults and mature, i.e., an income breeding strategy (Varpe et al., 2009).

The few findings of the largest adult *P. moultoni* in June is a bit of a puzzle. Especially since the adults in July seemed smaller. It could be that either the larger *P. moultoni* were advected in during June from nearby areas where it had already utilized an early bloom, or that smaller

adults were advected in during July. Earlier studies has found that adult summer specimens were smaller than spring specimens due to the spring specimens having sufficient fat reserves that was absent during the summer (Ershova et al., 2016), and that small versus large body size can be due to multiple generations per year. This could explain why female adults of *P. moultoni* reduced in size if adult females spend their energy on egg production rather than maintaining growth.

The explanation for the lack of significant difference between adult body size is most likely due to the few individuals found of some of the species (i.e., *P. moultoni* (n = 5)). Still, one should keep in mind that among the few *P. moultoni* adults identified, one individual was 300 µm larger than the mean adult *P. acuspes* (1049 µm) (Supplementary 5). On the other hand, earlier studies in Billefjorden have found larger single individuals of *P. acuspes* (~ 1300 µm) and smaller single adult individuals of *P. moultoni* (~ 800 µm). Differences in populations can occur due to food availability as it was shown that northern populations of adult *P. minutus* were larger than populations in the southern, central and eastern regions of the Barents Sea (Dvoretsky and Dvoretsky, 2010).

At the present point, there is no indication that a potential shift in environmental conditions with warmer more saline water might decrease the average body size of *Pseudocalanus* individuals as *P. moultoni* has the largest individuals and are equally in size or larger than *P. acuspes.* However, *P. moultoni`s* life cycle should be better understood since it is unclear if they can complete a life cycle in Billefjorden, and what type of breeding/feeding strategy they primarily use. To be able to draw these conclusions, the mechanisms behind why some individuals are larger than others (for instance feeding and reproductive strategies) for species of *Pseudocalanus* must be better understood. In its southernmost distribution area it is suggested that *P. moultoni* reproduces continuously throughout the year (McLaren et al., 1989), while *P. minutus* can produce two generations and *P. acuspes* can produce three generations (Norrbin, 1987).

## 4.2 Can individuals of the *Pseudocalanus* genus act as environmental indicators?

The seasonality in Billefjorden and the gradient from North to South clustered nicely together as expected in both the dendrogram and the NMDS analysis (Figure 13, 14 and 15). The scattered spring and summer samples compared to the more clustered autumn and winter samples shown in the NMDS could potentially reflect the changes during the summer and spring compared to the stable autumn and winter period. During the summer the copepods are primarily found in the surface and different water masses that flow into Billefjorden (Ershova et al., 2021b, Søreide et al., 2022). Still, the lack of Atlantic Water and Transformed Atlantic Water, and the fact that the Intermediate Water was most likely a mix of Surface water and Local water (Figure 3), strongly indicates that either little or diluted Atlantic water entered Billefjorden during the sampling year confirming that advective processes are minor in Billefjorden and that the local in-fjord processes are the main ones (Søreide et al., 2022). Large amount of run off from glacier and rivers could potentially dilute and mix Atlantic water entering Billefjorden. Based on these conditions I would expect less *P. minutus* and less *P. moultoni* compared to years with higher inflow of Atlantic water masses, as the first prefer a mix of Arctic and Atlantic water (Norrbin, 1991), while the latter is considered a temperate coastal species (Frost, 1989, Aarbakke et al., 2011).

In the Barents Sea, there was a nice and clear temperature and salinity gradient from the north to the south (Figure 16). Since, *P. minutus* is regarded more oceanic than its sibling species, and also found to be more abundant in the northern Barents Sea, I was hoping that this study would reveal a preferred habitat or some preferred environmental conditions for *P. minutus* among the 8 stations sampled in the Barents Sea. For the Barents Sea samples, my impression based on the RDA (Figure 16) and linear regression (Figure 17 and 18) is that there is a missing explanatory variable regarding *P. minutus* abundance, as neither temperature, depth and salinity (in the temperature and depth ranges I have measured) captured the drivers of high abundances of *P. minutus.* In the Pacific Arctic region, the dominance varies between *P. minutus* and *P. acuspes* over time. In 2004, 2009 and 2012, *P. acuspes* was the most dominant species in the same area (~ 630, 1998 and 1784 individuals per $m^{-3}$ respectively), while abundance of *P. minutus* was lower (~ 232, 372 and 171 individuals per $m^{-3}$ respectively) (Ershova et al., 2017). In 2013 however, *P. minutus* was reported to be dominant in the

Chukchi Sea and Beaufort Gyre in 2013, when *P. acuspes* had extremely low abundances in the same area, but number of individuals per $m^{-3}$ was not reported (Questel et al., 2016). So far, the lack of a solo dominance of *P. minutus* and their overall high proportion of the species complex at all stations and months supported a theory that *P. minutus* has less environmental preferences and could be considered more of an all-around present species in the present study area. Further studies in the Barents Sea region could for instance investigate if the abundance of *P. minutus* would increase with shallower depths as the average depth in Chukchi Sea and Beaufort Gyre is 80 and 124 m respectively, even though *P. minutus* has been reported to be more oceanic than the other *Pseudocalanus* species (Norrbin, 1991).

*P. acuspes* has earlier been described as Arctic, neritic and coastal in both the Pacific Arctic and the Atlantic Arctic (Frost, 1989, Questel et al., 2016, Ershova et al., 2021b). The dominance of *P. acuspes* during most of the year in Billefjorden except in June, and in most of the Northern and Polar Front stations where colder less saline Polar water was present confirms earlier results and also indicates that *P. acuspes* is the most Arctic of the three species.

My observations further confirm that *P. minutus* seems to thrive in several water masses and could be considered a widespread species with no special environmental preference in the Svalbard-Barents Sea region (Figure 16 and 17), while *P. acuspes* and *P. moultoni* has habitat preferences (Figure 16 and 18) that can be compared as relative composition between the Arctic and the boreal species respectively.

## 4.3 Species-specific primers and sequencing

Body size is not a good species trait for morphological identification as there is a large overlap in size between the *Pseudocalanus* species. It is only in recent years that *Pseudocalanus* has been identified molecularly to species level with the use of species-specific primers (Ershova et al., 2017).

Unfortunately, the initial primer mix made by Ershova et al. (2021b) did not work as expected, and a new protocol had to be tested and developed prior to analysing the DNA samples. The disadvantage of the primer cocktail not working was that the samples had to be

analysed first for a primer cocktail and then for a single primer reaction, doubling the workload. After that, all unidentified samples had to be run again with universal primers to prepare them for Sanger sequencing which further extended the workload.

Sending 277 samples out of 1947 for Sanger Sequencing might seem like a large number, and the high number is mostly due to picking other species resembling *Pseudocalanus* individuals and which did not give a signal on the gel electrophoresis since not being *Pseudocalanus* in the first place. Improvement in the assay could be done to reduce this numbers by more practice before picking individuals as it can be both challenging to pick the right species and to identify young life stages. Still, the error margin of 0,72 % with the use of species-specific primers was considered very low compared to the costs and use of time spent on Sanger sequencing with the set up that was made available. This indicates that the gained value of using Sanger Sequencing for this purpose was small, especially since none of the samples identified other *Pseudocalanus* species as hoped (i.e., *P. elongatus*). For future studies on the same topic, I would exclude the use of Sanger sequencing, and rather assess a combination of species-specific PCR and metabarcoding, as conducted in more recent studies on *Pseudocalanus* in the area (Ershova et al., 2021a).

## 4.4 Methods – reliable?

In Billefjorden, one assumption made was that the zooplankton is homogenously distributed throughout the basins. During April and May, the samples were collected on slightly different coordinates compared to the rest of the year. This was due to sea ice preventing entry to the deepest part of the fjord. Also, in the summer the boats drifted with waves, as no automatic positioning system was available. A closer look at the vertical distribution of *Pseudocalanus* spp. in Billefjorden show that *Pseudoacalanus* has a shallower weighted mean depth than for instance *Calanus* during the winter-spring period so most likely did not the slight change in position to a shallower location have a big impact since it still was rather deep (~150 m) (Søreide et al. 2022).

One issue that came up during the winter (i.e., November, December and January) in freezing temperatures was that the depth counter was running a bit slow, and the net tended to hit the bottom even though the protocol states that the sampling should be conducted 10 meter above

the seabed. As *Pseudocalanus* conducts seasonal vertical migration to deeper depths during winter, it would be interesting to know how much the individuals utilize the lower 10 meters, and if some life stages have different depth preferences compared to others during the seasonal migration.

In the lab there are two issues that potentially could have altered the outcome. The first is the number of individuals picked per sample, and if this study should have aimed for more than a minimum of 89 individuals per sample as the total of four different life stages and three different species were to be identified. In this case, the minimum limit of 89 individuals was chosen mostly because the lab work was already time consuming and a large part of this thesis, but also because when aiming for 89 individuals they would most likely fit on a 96 well plate, but also as 89 individuals provided the information needed to answer the research questions in this thesis. Increasing the number of individuals picked would increase the need for extra consumables and create a bottle neck of using the available machines and instruments in the lab. If there was a way to morphologically identify the *Pseudocalanus* samples, then the leftover samples should have been investigated to establish if certain life stages or species (i.e., *P. moultoni*) as for instance adults where present in the remaining parts of the year, thought in low numbers.

# 5 Conclusion

This study gained valuable information of the *Pseudocalanus* species specific composition in the Svalbard-Barents Sea region. One interesting finding were the unexpected peak in abundance in January and February, and the fact that *P. minutus* can complete a life cycle in Billefjorden.

I found that *Pseudocalanus acuspes* prefers Arctic and Polar water, while *P. minutus* could be considered a more evenly distributed species in the region studied. These findings along with *P. moultoni* being confirmed as a temperate species suggests that relative composition of *P. acuspes* and *P. moultoni* can be used as a valid environmental indicator for the dominant "climate" in the sea (i.e., Atlantic versus Arctic), and could be a valuable indice to detect environmental changes in specie composition in the Svalbard-Barents Sea region by the use of molecular tools.

Further research on *Pseudocalanus* should investigate if *P. moultoni* can complete a one-year life cycle in Arctic environments (e.g. Billefjorden), and which variables that possible could explain the increase in *P. minutus.* Further, more insights on the vertical distribution of the different species throughout the year would be beneficial, which again may explain the pulses of *P. moultoni* observed in Billefjorden since here mainly the upper 60 m (sill depth 50 m) is exchanged with Isfjorden outside. As seen in this research a shift in environmental conditions promoting *P. moultoni* could lead to lower total *Pseudocalanus* abundance, lower winter abundance, but potentially higher summer abundance. Still, the average *Pseudocalanus* body size will likely not decrease with warmer ocean climate since *P. moultoni* had the largest individual adults in this study. Body size of *Pseudocalanus* cannot be used to identify the individuals to species level. Further improvements of the PCR assay should therefore be done to make the identification more efficient.

Also, as we do not know the full extent of how and why these individual species change their abundance / composition from one year to another, as salinity, temperature and depth can only explain a small part of the variation in abundance. I would, therefore, recommend further studies to gain more insight on these species distinct life histories and their response (phenology) to the rapidly changing Arctic environment.

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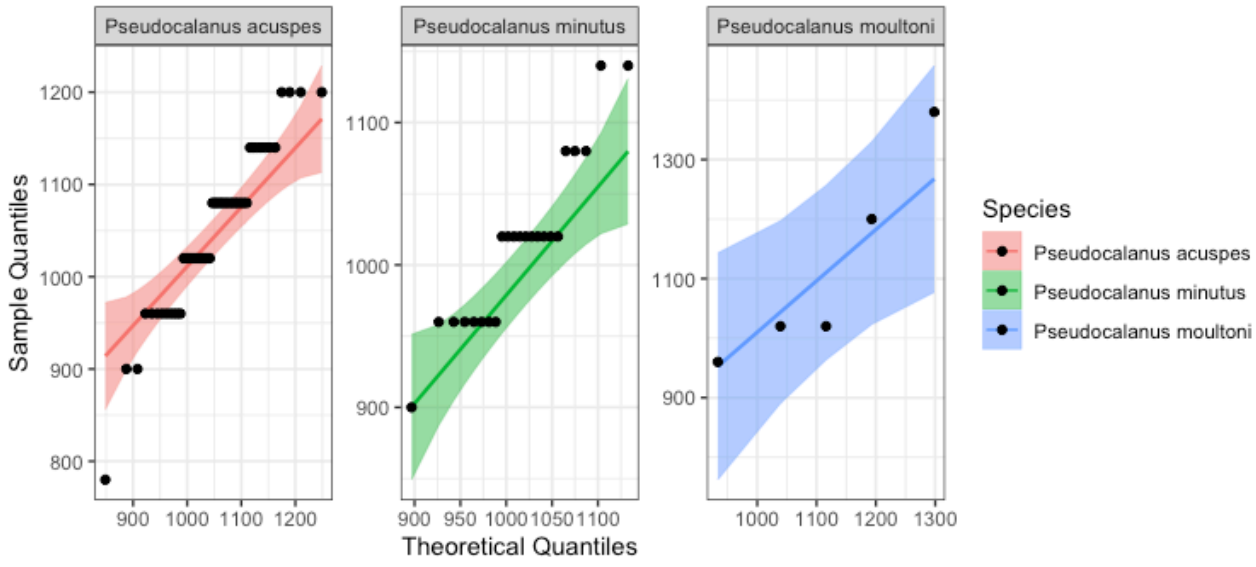
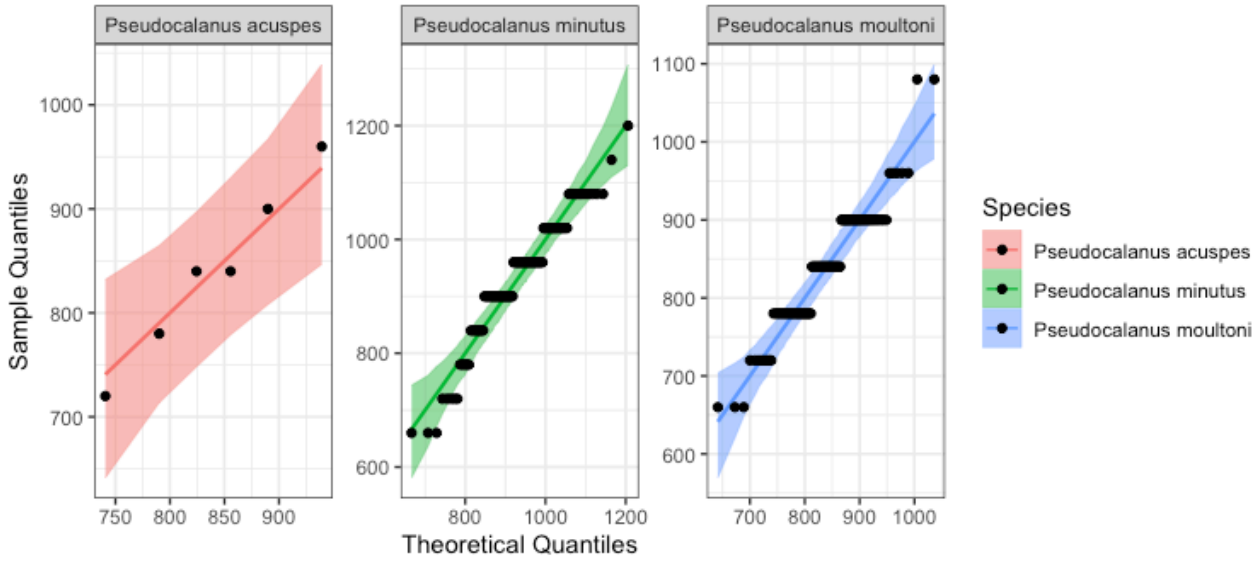
# 7 Supplementary

*Supplementary 1 Shapiro-Wilk normality test for CIV – Adult in Billefjorden (November 2020 until October 2021) and the Barents Sea (October 2020).*

| Habitat | Life Stage | Species | W Stat | p value | Result |
|---|---|---|---|---|---|
| Billefjorden | Adult | *Pseudocalanus acuspes* | 0.94 | 0.02 | Rejected |
| Billefjorden | Adult | *Pseudocalanus minutus* | 0.89 | 0.02 | Rejected |
| Billefjorden | Adult | *Pseudocalanus moultoni* | 0.87 | 0.27 | |
| Billefjorden | CV | *Pseudocalanus acuspes* | 0.91 | 0.00 | Rejected |
| Billefjorden | CV | *Pseudocalanus minutus* | 0.92 | 0.00 | Rejected |
| Billefjorden | CV | *Pseudocalanus moultoni* | 0.83 | 0.05 | Rejected |
| Billefjorden | CIV | *Pseudocalanus acuspes* | 0.83 | 0.00 | Rejected |
| Billefjorden | CIV | *Pseudocalanus minutus* | 0.93 | 0.09 | |
| Billefjorden | CIV | *Pseudocalanus moultoni* | 0.86 | 0.15 | |
| Barents Sea | CV | *Pseudocalanus acuspes* | 0.98 | 0.96 | |
| Barents Sea | CV | *Pseudocalanus minutus* | 0.94 | 0.00 | Rejected |
| Barents Sea | CV | *Pseudocalanus moultoni* | 0.93 | 0.00 | Rejected |
| Barents Sea | CIV | *Pseudocalanus acuspes* | 0.87 | 0.00 | Rejected |
| Barents Sea | CIV | *Pseudocalanus minutus* | 0.86 | 0.00 | Rejected |
| Barents Sea | CIV | *Pseudocalanus moultoni* | 0.91 | 0.02 | Rejected |

*Supplementary 2 QQplot of all life stages per species in Billefjorden (November 2020 until October 2021) (top) and the Barents Sea (October 2020) (below).*






*Supplementary 3 The average values for water temperature [ºC], salinity and fluorescence [in-situ Chl a μg/l] in the upper (0-60m), lower (60 to deepest depth) and whole water column (0- deepest depth) for each month in Billefjorden from November 2020 until October 2021.*

| | **Nov (19)** | **Dec (11)** | **Jan (28)** | **Feb (11)** | **Apr (09)** | **May (09)** | **Jun (16)** | **Jul (05)** | **Jul (15)** | **Aug (17)** | **Sep (17)** | **Oct (05)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average temperature, 0 - 60 m | 1.67 | -0.02 | -0.61 | -1,00 | -1.89 | -1.56 | 0.62 | 2.88 | 3.44 | 3.12 | 2.93 | 3.06 |
| Average temperature, 60 - Bottom | -1.48 | -1.42 | -1.26 | -1.23 | -1.89 | -1.7 | -1.66 | -1.59 | -1.6 | -1.41 | -1.46 | -1.43 |
| Average temperature, 0 - Bottom | -0.59 | -0.93 | -1.05 | -1.15 | -1.89 | -1.66 | -0.86 | -0.02 | 0.09 | 0.15 | 0,00 | 0.06 |
| Average salinity, 0-60 m | 33.81 | 33.77 | 34.26 | 34.36 | 34.64 | 34.69 | 34.47 | 33.86 | 33.73 | 33.65 | 33.6 | 33.66 |
| Average salinity, 60 - Bottom | 34.76 | 34.73 | 34.7 | 34.59 | 34.66 | 34.73 | 34.68 | 34.68 | 34.69 | 34.63 | 34.66 | 34.66 |
| Average salinity, 0 - Bottom | 34.49 | 34.39 | 34.56 | 34.59 | 34.65 | 34.71 | 34.61 | 34.39 | 34.37 | 34.3 | 34.31 | 34.33 |
| Average fluorescence, 0-60 m | 0.02 | 0.02 | 0.02 | 0.02 | No data | 0.13 | 0.13 | 1.28 | 0.79 | 0.32 | 0.61 | 0.22 |
| Average fluorescence, 60 - Bottom | 0.01 | 0.02 | 0.01 | 0.01 | No data | 0.03 | 0.11 | 0.03 | 0.08 | 0.07 | 0.07 | 0.07 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average fluorescence, 0 - Bottom | 0.01 | 0.02 | 0.01 | 0.01 | No data | 0.06 | 0.12 | 0.47 | 0.32 | 0.15 | 0.25 | 0.12 |

*Supplementary 4 The average values for water temperature [ºC], salinity and fluorescence, in-situ [µg Chl a µg/l], in the upper (0-60m), lower (60 to deepest depth) and whole water column (0 to deepest depth) at different stations arranged by latitude in the Barents Sea (October 2020).*

| | T1 | P2 | T3 | T4 | T5 | P1 | C | Ref |
|---|---|---|---|---|---|---|---|---|
| Average temperature, 0 - 60 m | 0.37 | 0.64 | 1.93 | 1.75 | 3.28 | 5.48 | 4.92 | 5.78 |
| Average temperature, 60 - Bottom | -1.52 | -0.89 | 1.53 | 1.76 | 2.05 | 3.09 | 3.04 | 4.10 |
| Average temperature, 0 - Bottom | -0.99 | -0.40 | 1.66 | 1.76 | 2.37 | 3.51 | 3.34 | 4.33 |
| Average salinity, 0 - 60 m | 33.92 | 34.21 | 34.19 | 34.29 | 34.47 | 34.92 | 34.76 | 34.83 |
| Average salinity, 60 - Bottom | 34.53 | 34.69 | 34.88 | 34.91 | 34.91 | 34.97 | 34.97 | 35 |
| Average salinity, 0 - Bottom | 34.36 | 34.54 | 34.65 | 34.72 | 34.8 | 34.96 | 34.94 | 34.97 |
| Average fluorescence, 0 - 60 m | 0.1 | 0.1 | 0.13 | 0.09 | 0.17 | 0.25 | 0.2 | 0.16 |
| Average fluorescence, 60 - Bottom | 0.02 | 0.02 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.02 |
| Average fluorescence, 0 - Bottom | 0.04 | 0.05 | 0.06 | 0.05 | 0.07 | 0.07 | 0.06 | 0.04 |

*Supplementary 5 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of the different Pseudocalanus species in all sampling stations and months separated by life stages (CIV, CV and adults).*

| Species name | Life stage | Number of individuals | Mean | Standard deviation | Standard error | Median | Minimum values | Maximum values |
|---|---|---|---|---|---|---|---|---|
| *Pseudocalanus acuspes* | A | 48 | 1049 | 88 | 13 | 1080 | 780 | 1200 |
| *Pseudocalanus minutus* | A | 23 | 1015 | 60 | 13 | 1020 | 900 | 1140 |
| *Pseudocalanus moultoni* | A | 5 | 1116 | 173 | 77 | 1020 | 960 | 1380 |
| *Pseudocalanus acuspes* | CV | 44 | 826 | 74 | 11 | 840 | 720 | 1020 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Pseudocalanus minutus* | CV | 74 | 977 | 96 | 11 | 960 | 600 | 1200 |
| *Pseudocalanus moultoni* | CV | 9 | 840 | 42 | 14 | 840 | 780 | 900 |
| *Pseudocalanus acuspes* | CIV | 340 | 683 | 52 | 3 | 660 | 420 | 1020 |
| *Pseudocalanus minutus* | CIV | 24 | 820 | 103 | 21 | 840 | 660 | 1020 |
| *Pseudocalanus moultoni* | CIV | 7 | 789 | 73 | 28 | 780 | 720 | 900 |

*Supplementary 6 Kruskal-Wallis test showing the overall chi-squared value, degrees of freedom and p-value for the different life stages in Billefjorden from November 2020 until October 2021 and in the Barents Sea October 2020.*

| **Billefjorden** | | | | |
|---|---|---|---|---|
| **Life stage** | **Kruskal-Wallis chi-squared** | **df** | **p - value** | **Results** |
| A | 4.10 | 2 | 0.13 | |
| CV | 60.81 | 2 | 0.00 | There is statistically significant difference between the prosome length of CV life stages in Billefjorden |
| CIV | 54.62 | 2 | 0.00 | There is statistically significant difference between the prosome length of CIV life stages in Billefjorden |
| **The Barents Sea** | | | | |
| **Life stage** | **Kruskal-Wallis chi-squared** | **df** | **p - value** | **Results** |
| A | 3.16 | 2 | 0.21 | |
| CV | 57.22 | 2 | 0.00 | There is statistically significant difference between the prosome length of CV life stages in the Barents Sea |
| CIV | 50.88 | 2 | 0.00 | There is statistically significant difference between the prosome length of CIV life stages in the Barents Sea |

*Supplementary 7 The Dunn`s multiple comparison showing the adjusted p-value (holm) for each combination of the three Pseudocalanus species per life stage in Billefjorden from November 2020 until October 2021 and in the Barents Sea October 2020.*

| **Billefjorden** | **Species 1** | **Species 2** | **Adj. p-value (holm)** | **Results** |
|---|---|---|---|---|
| A | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.18 | |
| A | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 0.67 | |
| A | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.34 | |
| CV | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.00 | Statistically significant difference |
| CV | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 0.82 | |
| CV | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.00 | Statistically significant difference |
| CIV | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.00 | Statistically significant difference |

| | | | | |
|---|---|---|---|---|
| CIV | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 0.00 | Statistically significant difference |
| CIV | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.95 | |
| **The Barents Sea** | **Species 1** | **Species 2** | **Adj. p-value (holm)** | **Results** |
| A | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.31 | |
| A | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 1 | |
| A | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.37 | |
| CV | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.03 | Statistically significant difference |
| CV | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 0.94 | |
| CV | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.00 | Statistically significant difference |
| CIV | *Pseudocalanus acuspes* | *Pseudocalanus minutus* | 0.00 | Statistically significant difference |
| CIV | *Pseudocalanus acuspes* | *Pseudocalanus moultoni* | 0.08 | |
| CIV | *Pseudocalanus minutus* | *Pseudocalanus moultoni* | 0.00 | Statistically significant difference |

*Supplementary 8 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of Pseudocalanus acuspes in Billefjorden from November 2020 to October 2021 separated by life stages CIV, CV, and adults.*

| ***Pseudocalanus acuspes*, adult, Billefjorden** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Months** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| April | 2 | 1020 | 84.9 | 60 | 1020 | 960 | 1080 |
| May | 4 | 930 | 104 | 52 | 960 | 780 | 1020 |
| June | 8 | 1065 | 27.80 | 9.82 | 1080 | 1020 | 1080 |
| July | 31 | 1070 | 83.6 | 15 | 1080 | 900 | 1200 |
| August | 3 | 960 | 60 | 34.6 | 960 | 900 | 1020 |
| ***Pseudocalanus acuspes*, CV, Billefjorden** | | | | | | | |
| **Months** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| November | 2 | 930 | 42.4 | 30.00 | 930 | 900 | 960 |
| December | 2 | 810 | 127 | 90 | 810 | 720 | 900 |
| February | 5 | 780 | 60 | 26.80 | 780 | 720 | 840 |
| April | 8 | 810 | 45.4 | 16 | 840 | 720 | 840 |
| May | 13 | 831 | 76.9 | 21.30 | 840 | 720 | 1020 |
| June | 1 | 900 | | | 900 | 900 | 900 |
| July | 6 | 800 | 62 | 25.30 | 780 | 720 | 900 |
| August | 1 | 780 | | | 780 | 780 | 780 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| September | 1 | 1020 | | | 1020 | 1020 | 1020 |
| October | 5 | 840 | 60 | 26.80 | 840 | 780 | 900 |

***Pseudocalanus acuspes,* CIV, Billefjorden**

| Months | Number of individuals | Mean | Standard deviation | Standard error | Median | Minimum values | Maximum values |
|---|---|---|---|---|---|---|---|
| November | 39.00 | 683.00 | 52.60 | 8.43 | 660.00 | 540 | 780 |
| December | 41.00 | 647.00 | 43.50 | 6.79 | 660.00 | 540 | 720 |
| January | 48.00 | 680.00 | 43.50 | 6.27 | 660.00 | 600 | 780 |
| February | 52.00 | 691.00 | 36.60 | 5.08 | 720.00 | 600 | 780 |
| April | 44.00 | 687.00 | 37.60 | 5.67 | 660.00 | 600 | 780 |
| May | 8.00 | 682.00 | 71.30 | 25.20 | 660.00 | 600 | 780 |
| July | 6.00 | 690.00 | 200.00 | 81.60 | 660.00 | 420 | 1020 |
| August | 29.00 | 693.00 | 49.60 | 9.22 | 720.00 | 600 | 780 |
| September | 35.00 | 687.00 | 53.20 | 8.98 | 660.00 | 600 | 780 |
| October | 38.00 | 693.00 | 33.30 | 5.40 | 720.00 | 600 | 720 |

*Supplementary 9 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of Pseudocalanus minutus in Billefjorden from November 2020 to October 2021 when (separated by life stages CIV, CV, and adults).*

***Pseudocalanus minutus*, A, Billefjorden**

| Months | Number of individuals | Mean | Standard deviation | Standard error | Median | Minimum values | Maximum values |
|---|---|---|---|---|---|---|---|
| February | 1 | 1020 | | | 1020 | 1020 | 1020 |
| April | 5 | 1008 | 89 | 39.80 | 1020 | 900 | 1140 |
| May | 7 | 1011 | 41.4 | 15.60 | 1020 | 960 | 1080 |
| June | 2 | 960 | 0 | 0.00 | 960 | 960 | 960 |
| July | 7 | 1046 | 58.6 | 22.10 | 1020 | 960 | 1140 |
| August | 1 | 960 | | | 960 | 960 | 960 |

***Pseudocalanus minutus*, CV, Billefjorden**

| Months | Number of individuals | Mean | Standard deviation | Standard error | Median | Minimum values | Maximum values |
|---|---|---|---|---|---|---|---|
| November | 11 | 955 | 68.2 | 20.60 | 960 | 840 | 1080 |
| December | 2 | 750 | 212 | 150.00 | 750 | 600 | 900 |
| January | 4 | 930 | 60 | 30.00 | 960 | 840 | 960 |
| February | 5 | 984 | 32.9 | 14.70 | 960 | 960 | 1020 |
| April | 1 | 900 | | | 900 | 900 | 900 |
| June | 3 | 960 | 159 | 91.70 | 900 | 840 | 1140 |
| July | 18 | 990 | 101 | 23.90 | 990 | 840 | 1200 |
| August | 12 | 995 | 104 | 30.00 | 1020 | 720 | 1080 |
| September | 9 | 1013 | 70 | 23.30 | 1020 | 900 | 1080 |
| October | 9 | 1000 | 60 | 20.00 | 1020 | 900 | 1080 |

| ***Pseudocalanus minutus*, CIV, Billefjorden** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Months** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| December | 1 | 720 | | | 720 | 720 | 720 |
| January | 1 | 720 | | | 720 | 720 | 720 |
| February | 2 | 870 | 42.4 | 30.00 | 870 | 840 | 900 |
| April | 2 | 660 | 0 | 0.00 | 660 | 660 | 660 |
| May | 1 | 840 | | | 840 | 840 | 840 |
| June | 4 | 780 | 130 | 64.80 | 750 | 660 | 960 |
| July | 10 | 870 | 58.3 | 18.40 | 840 | 840 | 1020 |
| August | 2 | 750 | 42.4 | 30.00 | 750 | 720 | 780 |
| September | 1 | 1020 | | | 1020 | 1020 | 1020 |

*Supplementary 10 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of Pseudocalanus acuspes for 8 stations in the Barents Sea October 2020 (separated by life stages CIV, CV, and adults).*

| ***Pseudocalanus acuspes*, adult, the Barents Sea** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| T3 | 1 | 840 | | | 840 | 840 | 840 |
| P1 | 1 | 720 | | | 720 | 720 | 720 |
| ***Pseudocalanus acuspes*, CV, the Barents Sea** | | | | | | | |
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| T3 | 1 | 840 | | | 840 | 840 | 840 |
| T5 | 2 | 810 | 42.4 | 30.00 | 810 | 780 | 840 |
| P1 | 2 | 840 | 170 | 120.00 | 840 | 720 | 960 |
| Ref | 1 | 900 | | | 900 | 900 | 900 |
| ***Pseudocalanus acuspes*, CIV, the Barents Sea** | | | | | | | |
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| T1 | 6 | 680 | 49 | 20.00 | 690 | 600 | 720 |
| P2 | 5 | 684 | 80.5 | 36.00 | 720 | 600 | 780 |
| T3 | 15 | 676 | 80.1 | 20.70 | 660 | 540 | 900 |
| T4 | 12 | 650 | 66.9 | 19.30 | 660 | 480 | 720 |
| T5 | 19 | 688 | 30.80 | 7.06 | 660 | 660 | 720 |
| P1 | 8 | 645 | 62.1 | 22.00 | 630 | 600 | 780 |
| C | 13 | 655 | 38.4 | 10.70 | 660 | 600 | 720 |
| Ref | 8 | 652 | 59.5 | 21.00 | 660 | 540 | 720 |

*Supplementary 11 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of Pseudocalanus minutus for 8 stations in the Barents Sea October 2020 (separated by life stages CIV, CV, and adults).*

| ***Pseudocalanus minutus*, adult, the Barents Sea** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| T1 | 1.00 | 960.00 | NA | NA | 960.00 | 960.00 | 960.00 |
| T3 | 1.00 | 960.00 | NA | NA | 960.00 | 960.00 | 960.00 |
| ***Pseudocalanus minutus*, CV, the Barents Sea** | | | | | | | |
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| C | 18.00 | 970.00 | 90.30 | 21.30 | 960.00 | 840.00 | 1200.00 |
| P1 | 15.00 | 964.00 | 105.00 | 27.10 | 960.00 | 720.00 | 1140.00 |
| P2 | 2.00 | 900.00 | 84.90 | 60.00 | 900.00 | 840.00 | 960.00 |
| Ref | 30.00 | 978.00 | 79.00 | 14.40 | 990.00 | 720.00 | 1080.00 |
| T1 | 12.00 | 910.00 | 127.00 | 36.80 | 960.00 | 660.00 | 1080.00 |
| T3 | 16.00 | 881.00 | 78.10 | 19.50 | 900.00 | 720.00 | 960.00 |
| T4 | 6.00 | 810.00 | 118.00 | 48.40 | 870.00 | 660.00 | 900.00 |
| T5 | 16.00 | 919.00 | 99.70 | 24.90 | 900.00 | 720.00 | 1080.00 |
| ***Pseudocalanus minutus*, CIV, the Barents Sea** | | | | | | | |
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| P1 | 2 | 720 | 0 | 0.00 | 720 | 720 | 720 |
| P2 | 5 | 720 | 42.4 | 19.00 | 720 | 660 | 780 |
| T1 | 6 | 720 | 53.7 | 21.90 | 720 | 660 | 780 |
| T3 | 8 | 772 | 59.5 | 21.00 | 780 | 660 | 840 |
| T4 | 6 | 770 | 24.50 | 10.00 | 780 | 720 | 780 |
| T5 | 13 | 849 | 109 | 30.30 | 840 | 660 | 1080 |

*Supplementary 12 Differences in prosome length, mean, median, minimum and maximum size with standard deviation and standard error of Pseudocalanus moultoni for 8 stations in the Barents Sea October 2020 (separated by life stages CIV, CV, and adults).*

| ***Pseudocalanus moultoni*, adult, the Barents Sea** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |
| C | 1.00 | 780.00 | | | 780.00 | 780.00 | 780.00 |
| ***Pseudocalanus moultoni* CV, the Barents Sea** | | | | | | | |
| **Stations** | **Number of individuals** | **Mean** | **Standard deviation** | **Standard error** | **Median** | **Minimum values** | **Maximum values** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C | 36.00 | 847.00 | 68.50 | 11.40 | 840.00 | 720.00 | 1080.00 |
| P1 | 26.00 | 787.00 | 74.60 | 14.60 | 780.00 | 660.00 | 900.00 |
| Ref | 30.00 | 876.00 | 66.10 | 12.10 | 900.00 | 780.00 | 1080.00 |
| T3 | 5.00 | 792.00 | 50.20 | 22.40 | 780.00 | 720.00 | 840.00 |
| T4 | 2.00 | 930.00 | 42.40 | 30.00 | 930.00 | 900.00 | 960.00 |

***Pseudocalanus moultoni*, CIV, the Barents Sea**

| Stations | Number of individuals | Mean | Standard deviation | Standard error | Median | Minimum values | Maximum values |
|---|---|---|---|---|---|---|---|
| C | 4.00 | 720.00 | 49.00 | 24.50 | 720.00 | 660.00 | 780.00 |
| P1 | 8.00 | 668.00 | 7.80 | 26.50 | 660.00 | 600.00 | 780.00 |
| P2 | 1.00 | 600.00 | | | 600.00 | 600.00 | 600.00 |
| Ref | 7.00 | 711.00 | 41.40 | 15.60 | 720.00 | 660.00 | 780.00 |
| T3 | 2.00 | 720.00 | 84.90 | 60.00 | 720.00 | 660.00 | 780.00 |
| T4 | 3.00 | 660.00 | | | 660.00 | 660.00 | 660.00 |
| T5 | 2.00 | 780.00 | 84.90 | 60.00 | 780.00 | 720.00 | 840.00 |

*Supplementary 13 Ice charts of the ice conditions in Billefjorden from January 2021 to May 2021.*