



UiT The Arctic University of Norway

Department of Arctic and Marine Biology

## **Meroplankton on Arctic inflow shelves**

Diversity, seasonality and origins of benthic invertebrate larvae on the Barents and Chukchi shelves

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A dissertation for the degree of Philosophiae Doctor – July 2022





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Thesis submitted in partial fulfilment of the requirements for the  
degree Philosophiae Doctor in Natural Science

Tromsø, Norway

July 2022



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## ACKNOWLEDGEMENTS

**Bodil**, you have been an incredible advisor, mentor, friend and role-model. Your dedication to your students is unparalleled. I take such inspiration from you, as a researcher and, most importantly, as a human being. One day, when I have my own students, I will strive to welcome them at the airport with a bag full of groceries 😊. To my other PhD advisors, **Kim, Paul, Randi and Jørgen**, your humanity, enthusiasm and sheer genius are remarkable! It's been an absolute joy and honour working with all of you.

To all my co-authors, thank you for your contributions and shared passion for the work. **Liza** and **Owen**, you have been more than co-authors to me, you have been mentors and friends. You have taught me so much. To **Mats**, thank you for your patience and guidance through the particle tracking process. **Finlo**, thank you for getting me onboard and into the **Arctic PRIZE** family. What a blast it has been! Thank you also to the **ArcticSIZE** group for being the catalyst for this research, giving me the rare opportunity to create a PhD project from scratch and allowing me to follow my own curiosity. This was a risky project and could have failed on multiple accounts, but it didn't, far from it, thanks to all of you!

I would like to thank the **crew and fellow scientists** onboard the RV Helmer Hanssen, RV Kronprins Haakon, and RRS James Clark Ross for their support during at-sea sampling. Thank you also to the scientists at the **Institute of Marine Research** who collected additional zooplankton samples for me as part of the SI Arctic and Ecosystem Survey cruises in fall 2017. I am grateful to **Melissa Brandner, Julie Bitz-Thorsen, Helena Michelsen, Carolin Uhlir and Kristine Cerbule** for help with lab work and to **Michael Greenacre** for advice on statistical analyses. A big thank you to **Katrine Kongshavn** and colleagues at the University Museum in Bergen and Tromsø Museum for all your help getting new Arctic benthic species sequenced and added to the Barcode of Life. To those who proofread various sections of the thesis synopsis, **Bodil Bluhm, Irina Zhulay, Maeve McGovern, Malcolm Jobling, Elizaveta Ershova and Terri Souster**, I greatly appreciate your time.

To **all my friends** in Tromsø, Christine, Daniel, Morgan, Irina, Liza, Pauke, Maeve, Bernhard, Erin, Emily, Karen, Kathy and so many others, you have seen me through the ups and downs of this PhD journey. Thank you for the laughs, the cries, the discussions (the serious ones and the silly ones) and the adventures. To **Forsker Grand Prix** and **Emerging Leaders** friends and mentors, we've been through short but intense moments together and you have made me grow as a scientist and as a person.

À **papa et maman** qui avez supporté et nourris mes rêves et ambitions depuis que j'étais toute petite, merci. **Annabelle** tu m'impressionnes constamment. J'admire ta force au quotidien. **Mamie Anita, mamie Gigi, mamie Pierrette et papi Réal** merci de votre support malgré le fait que la recherche me porte si loin de vous. Je vous aime tous très fort.

I would like to dedicate this thesis to **Flavie** and all the children of our blue planet who I hope will get to grow up to a diverse, productive and beautiful ocean.

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## FUNDING

This PhD has been jointly funded by UiT the Arctic University of Norway and the Tromsø Research Foundation under the project “Arctic Seasonal Ice Zone Ecology,” project number 01vm/h1 with additional funding from the Fram Centre Flagship “Climate Change in Fjord and Coast” grant number 272019 and the Fonds de Recherche Nature et Technologies du Québec (file number 270604). The ArcticPRIZE project (NE/P006302/1 – United Kingdom Natural Environment Research Council) and the Nansen Legacy project (Norwegian Research Council project 276730) contributed ship time for sampling. The UiT library covered the costs of open access publications.





## LIST OF PAPERS AND AUTHOR CONTRIBUTIONS

### Paper I:

Descôteaux R, Ershova E, Wangenstein OS, Præbel K, Renaud PE, Cottier F, Bluhm BA (2021) Meroplankton diversity, seasonality and life-history traits across the Barents Sea Polar Front revealed by high-throughput DNA barcoding. *Front Mar Sci* 8:677732, <https://doi.org/10.3389/fmars.2021.677732>

### Paper II:

Descôteaux R, Huserbråten M, Jørgensen LL, Renaud PE, Ingvaldsen, RB, Ershova EA, and Bluhm BA. Origin of marine invertebrate larvae on an Arctic inflow shelf. [accepted with minor revisions in *Marine Ecology Progress Series*]

### Paper III:

Ershova EA, Descôteaux R, Wangenstein OS, Iken K, Hopcroft RR, Smoot C, Grebmeier JM, Bluhm BA (2019) Diversity and distribution of meroplanktonic larvae in the Pacific Arctic and connectivity with adult benthic invertebrate communities. *Front Mar Sci* 6:490, <https://doi.org/10.3389/fmars.2019.00490>

### Author Contributions:

	<b>Paper I</b>	<b>Paper II</b>	<b>Paper III</b>
Concept and idea	RD, EE, PR, BB	RD, MH, BB	EE, RD, BB
Study design and methods	RD, EE, OW, KP, BB	RD, MH, LJ, PR, RI, BB	EE, RD, BB
Data gathering and interpretation	RD, EE, FC, OW	RD, MH, LJ, PR, EE	BB, JG, KI, EE, RH, CS, RD, OW
Manuscript preparation	RD, EE, OW, KP, PR, FC, BB	RD, MH, LJ, PR, RI, EE, BB	EE, OW, RD, BB, KI, RH, CS, JG

Abbreviations for authors (in alphabetical order): Bodil A. Bluhm (BB), Caitlin Smoot (CS), Elizaveta A. Ershova (EE), Finlo Cottier (FC), Jacqueline M. Grebmeier (JG), Katrin Iken (KI), Kim Præbel (KP), Lis L. Jørgensen (LJ), Mats Huserbråten (MH), Owen S. Wangenstein (OW), Paul E. Renaud (PR), Raphaëlle Descôteaux (RD), Randi B. Ingvaldsen (RI), Russ R. Hopcroft (RH)

## PUBLISHED DATA

### Paper I:

Descôteaux et al. (2021), Data from: Meroplankton diversity, seasonality and life-history traits across the Barents Sea Polar Front revealed by high-throughput DNA barcoding, Dryad, Dataset, <https://doi.org/10.5061/dryad.n8pk0p2vf>

### Paper II:

Descôteaux et al. (2022), "Replication Data for: Origin of marine invertebrate larvae on an Arctic inflow shelf", DataverseNO, <https://doi.org/10.18710/DVYBTY>

### Paper III:

Ershova (2019), "Diversity and distribution of meroplanktonic larvae in the Pacific Arctic", Mendeley Data, V1, <https://doi.org/10.17632/rhd9z8x86h.1>

## SUMMARY

Marine benthic invertebrates display a wide range of reproductive strategies. Many taxa release their eggs or larvae into the water column to drift with currents as meroplankton, sometimes for several months, before the developing young settle onto a substrate and grow to become adults. The planktonic early life stages play an important role in dispersal and at times represent a substantial proportion of the zooplankton. Information about the larval communities present on the Arctic inflow shelves of the Barents and Chukchi Seas is incomplete and limited because of infrequent sampling and low resolution of identification of the marine larvae to species or taxonomic group. Indeed, meroplanktonic larvae are microscopic and difficult to identify to species-level based on morphological characteristics alone.

The aims of the work described in this thesis were to characterize the meroplanktonic communities of the Barents and Chukchi Sea shelves in greater taxonomic, spatial and temporal resolution than previously achieved, and to investigate the possible origins of the identified larvae. My colleagues and I specifically optimized DNA barcoding methods for identifying meroplankton to unmask the hidden diversity in the plankton as well as detect potential newcomers to the Arctic. I hypothesized that diversity would be higher than previously realized and would be comprised of a mix of local and advected larvae. I also expected to find seasonal changes in the meroplankton communities with a peak in abundance around the spring phytoplankton bloom.

Paper I explored the seasonal cycle of the meroplankton community on the Barents Sea shelf, an Arctic inflow shelf highly influenced by advection of warm Atlantic water. Together, seasonal sampling (5 time points in one year) north and south of the Polar Front coupled with DNA barcoding of individual larvae captured a much greater diversity than previously realized with 72 taxa identified, most of them molluscs, echinoderms and polychaetes. The meroplankton community varied spatially (north and south of Polar Front, though not statistically significant) but the bulk of the variation in community composition was driven by seasonality. Contrary to expectation, larval abundances did not peak around the phytoplankton bloom but rather in later summer and fall. Winter communities, while low in abundance, were very diverse.

Paper II further characterized the Barents Sea meroplankton community with special emphasis on dispersal and origins of larvae. DNA metabarcoding facilitated a more in-depth and broader geographic coverage than was realistic with barcoding of individual larvae. Most larval taxa found over the shelf belonged to species known from the Barents Sea and adjacent coasts except for seven taxa, mostly nudibranchs, whose adults had not been recorded there before. Particle tracking analysis showed that larvae likely originated from within the Barents Sea given a 3-months drift. However, some ‘larvae’ released along the coast of Norway were able to drift to the north of Svalbard in that time. Together these results point to a largely regional-scale connectivity in the Barents Sea while recognizing the potential for some long-lived larvae to travel to the Arctic shelves from further south.

Finally, Paper III shifted the geographical focus by concentrating on the meroplankton community of the Chukchi Sea, the inflow shelf on the Pacific side of the Arctic. No seasonal samples were available on this side of the Arctic but a compilation of samples collected there in August-September over five years built a strong picture of late-summer meroplankton communities in this region. There, as in the Barents Sea, molluscs, echinoderms and polychaetes were abundant but this time cirripeds and decapods also contributed substantially to the community. Surprisingly given the strong advective nature of the system, no southern expatriate was found inside the Chukchi Sea. However, the distribution of many larvae inside the Chukchi Sea did not match that of the adults, showing once again largely regional-scale connectivity.

Findings from this thesis highlight the immense potential gained from species-level identification and shine a light on critical yet poorly-known life stages of the rich Arctic shelf benthic communities in a time of rapid climate change. Identification of larvae of boreal taxa drifting into the Arctic shelves can give an indication of which species may be able to settle into the Arctic first as the temperature and productivity cycles there continue to change. Incorporating DNA-based meroplankton analysis to ongoing zooplankton surveys could be an efficient way to detect incoming species and gain insight into what the future Arctic may look like.

## **ABBREVIATIONS**

BLAST – Basic Local Alignment Search Tool

BOLD – Barcode of Life Database

CTD – Conductivity, temperature, depth instrument

COI – Cytochrome c oxidase subunit I

DNA – Deoxyribonucleic acid

GBIF – Global Biodiversity Information Facility

MOTUs – Molecular operational taxonomic units

NCBI - National Center for Biotechnology Information

NUMTs – Nuclear mitochondrial DNA

# **1. BACKGROUND**

## **1.1 Historical perspective**

Larvae of marine invertebrates are generally too small to be visible to the naked eye, so it was with the advent of the microscope that these early life stages became known to science. In the decades following recognition of their existence, much of the research focused on anatomy and metamorphosis, but the larvae were often identified and classified incorrectly or they were not recognized as early life stages at all (Young et al. 2002). The field gradually evolved from the study of embryology and larval development to attempts to elucidate animal phylogeny, using larval forms to find evolutionary relationships between animal phyla (Young et al. 2002). In the mid-1900s, Gunnar Thorson (best known for Thorson's Rule, discussed later) studied the larval communities of Greenland and elsewhere, emphasizing their ecology (Thorson 1950). Shortly after, larval dispersal came into the spotlight and its implications for connectivity of marine populations recognized (Scheltema 1986). Until recently, however, the difficulty to identify larvae to species limited the depth of information obtained from in-situ surveys. The recent advent of molecular methods, particularly DNA barcoding, has promised to facilitate species identification. It is in this context that the present thesis is situated. The studies presented here build on previous partially-successful attempts at identifying larval invertebrates using DNA barcoding (Heimeier et al. 2010; Gallego et al. 2014; Brandner et al. 2017), optimizing methods so as to obtain a highly reliable species identification for larvae across a wide range of phyla. Such species-level identification opens up countless new windows into the biology and ecology of these early life stages. A few of these opportunities are explored here.

## **1.2 Fundamentals of larval biology**

### **1.2.1 Planktonic versus benthic development**

The collective work of the last 200 years has demonstrated, amongst other things, that marine benthic invertebrates display a wide range of reproductive strategies with varying levels of parental care (Levin and Bridges 1995). On one end of the spectrum, parents brood their young through most of their development, releasing them as juveniles into the surrounding environment. For these benthic developers, the young are released into a reliably favourable environment, one in which their parents had themselves successfully survived and reproduced. On the other end of the spectrum, early life stages (at various stages of development ranging

from gametes to embryos to larvae, depending on the species) are released into the water column and develop while drifting with water currents before settling back down to the seafloor (Figure 1, Levin and Bridges 1995). It is on these taxa with planktonic early life stages (Box 1) that the present thesis is focused.

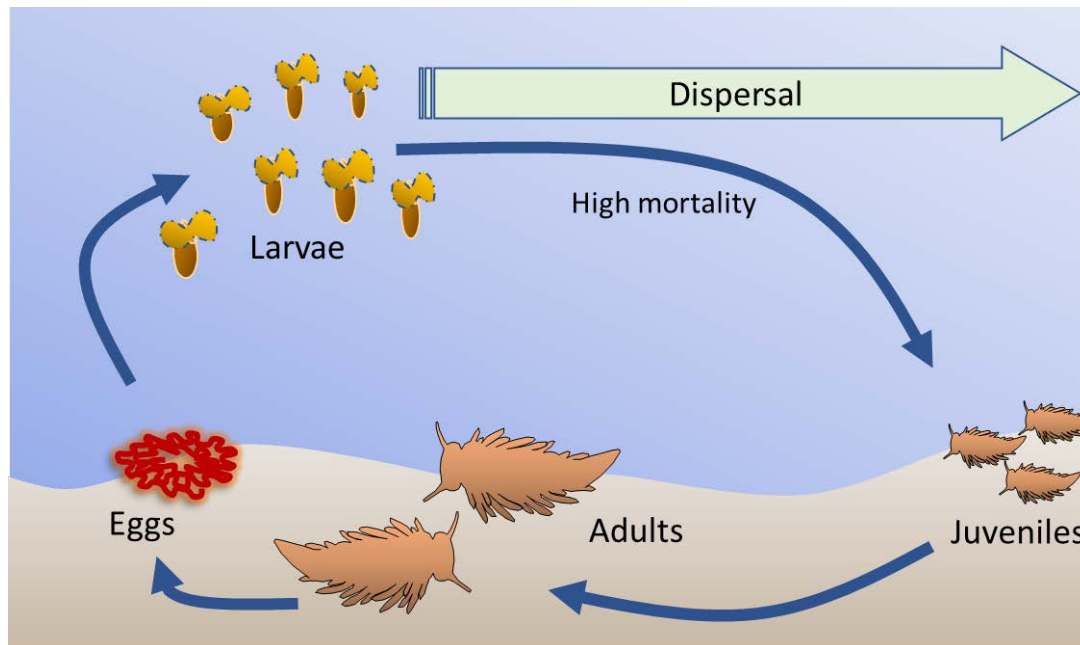


Figure 1: Example life cycle for a taxon with planktonic larval development, in this case Nudibranchia. In this example the parents lay the eggs on the substrate and larvae are planktonic after hatching. In other taxa with planktonic larval development, fertilization and embryo development may also occur in the water column.

The reproductive strategy that consists in releasing early life stages into the water column to drift generally involves less parental care resulting in very high mortality rates from predation, starvation or exposure to inhospitable physical conditions (Morgan 1995) and a much lower probability of finding a favourable seafloor habitat where to settle and grow than benthic developers (Pechenik 1999). Species with planktonic larval development generally release large numbers of propagules (e.g., Levin et al. 1987) which can help compensate for these high

## Box 1: Important terms

### **Meroplankton**

The planktonic early life stages of benthic invertebrates belong to the *meroplankton* as they are in the plankton only temporarily, for part of their life cycles. This contrasts with holoplankton such as most copepods, chaetognaths, larvaceans and others, which spend their entire life cycle in the water column. By definition, the term meroplankton includes all taxa whose presence in the plankton is only temporary, at any life stage. This includes planktonic early life stages of demersal fish as well as the planktonic adult stage of several jellyfish, harpacticoid copepods and others whose younger stages are benthic. However, the term meroplankton is most often associated with planktonic early life stages (mostly larvae, but sometimes also embryos and juveniles) of benthic invertebrates and is used as such in this thesis and associated papers.

### **Larva**

Another term that warrants discussion is '*larva*', used frequently throughout this thesis. This term can have many different definitions (Haug 2020) and is used inconsistently across authors, leading to confusion. Here, a rather broad ecological definition of larva is used where all immatures whose ecological niche differs significantly from that of the adult (eco - larva sensu lato according to Haug 2020) are included. By this definition, all post-embryonic planktonic stages are included, including some later stages which could be characterized as juveniles by some authors based on their morphological resemblance to the benthic adult (e.g. in polychaetes).

mortality rates. In the mid-1900s Thorson postulated that fewer benthic invertebrates in polar regions would release larvae into the planktonic environment compared to their lower-latitude compatriots, an idea known as Thorson's Rule (Thorson 1950; Mileikovsky 1971). Thorson speculated that inadequate feeding conditions in the plankton at high latitudes would fail to support planktonic larval development in most taxa.

### 1.2.2 Larval dispersal

While mortality rates are high and probability to find a suitable habitat low, a planktonic phase allows for dispersal over longer distances than possible for benthic developers (Levin and

Bridges 1995). Long-distance dispersal can be useful for gene flow, or horizontal connectivity between populations (Cowen and Sponaugle 2009; Hardy et al. 2011; Le Corre et al. 2020), as well as to allow taxa to quickly colonize habitats after disturbance events. In Antarctica, some of the most widespread benthic taxa have long-lived planktotrophic larvae, a strategy that may be particularly useful in shallow-water habitats where ice scouring creates frequent disturbance (Pearse et al. 1991). In such a scenario, a high capacity for dispersal could facilitate rapid recolonization. It should be acknowledged however, that the presence of a planktonic stage may also be a consequence of other evolutionary pressures which have nothing to do with dispersal itself (Burgess et al. 2016). By removing the larvae from the seafloor, for example, a planktonic phase can reduce competition for resources with adults and avoid benthic predators (Pechenik 1999).

The duration of the planktonic phase differs widely across taxa ranging from a few minutes to several months (Shanks 2009) and, in some rare cases, years (Strathmann and Strathmann 2007). The drift duration depends in part on the source of nutrition for the developing larvae. In some species, the propagules are equipped with a yolk sac to supply nutrition during dispersal (lecithotrophic larvae) while for others the propagules feed while in the plankton (planktotrophic larvae, Levin and Bridges 1995). Many taxa, however, exist somewhere along the spectrum between the two feeding modes (McEdward 1997). The maximum lifespan of a purely lecithotrophic larva is determined by the reserves in its maternally-derived yolk sac (Scheltema 1986). A lecithotrophic larva must metamorphose and settle to the seafloor prior to exhaustion of its reserves or it will die. Planktotrophic larvae can generally survive longer in the water column as long as nutrition is adequate both in terms of quality and quantity (Scheltema 1986) and can sometimes delay metamorphosis until environmental cues indicative of a suitable settling environment have been detected (Pechenik 1990). For both lecithotrophic and planktotrophic larvae, however, the duration of the planktonic phase is also strongly affected by temperature. Within the range of the organism's thermal window, outside of which the larvae would simply die, low temperatures slow down development and high temperatures speed it up (O'Connor et al. 2007).

The lifespan of a larva in the water column in turn has strong implications for its potential for dispersal. Generally, the longer the drift, the further a larva can disperse though, in many cases, the actual distance covered during larval development is much lower than predicted for passive



dispersal (Shanks 2009). Indeed, larval behaviour such as vertical migrations often reduces dispersal distance (Shanks 2009) though in some cases such as in the deep sea it can in fact enhance dispersal (Gary et al. 2020). Unfortunately, what we know of larval dispersal is largely derived from low-latitude studies (Bradbury et al. 2008) and may not be directly transferable to high-latitude environments such as the Arctic inflow shelves.

### 1.2.3 Seasonal variations

The timing of release of planktonic larvae into the water column varies across taxa and can be related to a variety of factors including physical conditions favourable to larval survival (temperature, salinity, etc.), food availability and current flow patterns (Scheltema 1986; Burgess et al. 2016). Some taxa, particularly in tropical environments or in the deep sea where the seasonal cycle is attenuated, reproduce semi-continuously throughout the year (Young 2003). Most taxa, however, reproduce at discrete periods of the year with the initiation of reproduction often triggered by an environmental cue such as daylight, temperature, food availability or other (Young 2003). Generally speaking, planktotrophic larvae tend to display distinct seasonality while lecithotrophic larvae, which are not dependent on food to survive, tend to be less constrained in their timing (Pearse et al. 1991). Seasonality may still be observed in lecithotrophic taxa, however, as other life stages, such as the reproductive adults and/or settling juveniles may themselves be constrained by food availability and therefore indirectly drive seasonality in the larval stages.

The seasonality of food availability depends on the preferred food for each taxon as well as on the location. The little that is known about larval diet originates largely from lab experiments where the larval growth and survival was assessed after being fed a range of prey items (e.g., Carboni et al. 2012). Many taxa are generally assumed to feed on phytoplankton but others target smaller prey such as flagellates or bacteria or even assimilate organic solutes (Rivkin et al. 1986; Levin and Bridges 1995). The availability of these different food items is in turn dictated by the geographical location and time of the year, especially in highly-seasonal environments like the Arctic (Marquardt et al. 2016; Dong et al. 2020).

## 1.3 Meroplankton on Arctic inflow shelves

### 1.3.1 The physical environment

The Arctic Ocean has characteristics of a mediterranean sea, being encircled by land masses around most of its circumference (Figure 2A). Despite its largely landlocked geography, however, the Arctic is deeply connected to the rest of the world's oceans through water currents. Water from the Atlantic enters through the Fram Strait and the Barents Sea opening, while water from the Pacific enters through the Bering Strait onto the Chukchi Sea shelf. Outflow from the Arctic Ocean is largely directed through the Canadian Arctic Archipelago and around Greenland southwards. The Barents (Box 2) and Chukchi (Box 3) seas are characterized as Arctic inflow shelves where water from the south is transported into the Arctic (Wassmann et al. 2020).

#### Box 2: The Barents Sea

The Barents Sea (Figure 2B) is a relatively large ( $1.5 \times 10^6 \text{ km}^2$ ) and deep shelf (average 230 m depth, Hunt et al. 2013) which is delimited in the north and west by the shelf break, in the south by the Norwegian and Russian mainland, and in the east by Franz Josef Land and Novaya Zemlya (Ozhigin et al. 2011). On the northwest corner of the Barents Sea is the island archipelago of Svalbard. The Barents Sea shelf receives large inputs of warm and salty Atlantic Water flowing east and northward. One branch of Atlantic Water follows the shelf break, flowing along the western and then northern coast of Svalbard while the other branch veers eastward into the southern Barents Sea shelf. This relatively nutrient-rich Atlantic Water is mostly restricted to the southern half of the Barents shelf while the northern half is dominated by colder, fresher and nutrient-poor Arctic Water flowing in from the north and east (Hunt et al. 2013). These two major water masses meet at the Polar Front, the location of which is relatively constant, at least west of  $32^\circ \text{E}$  where it is constrained by the bathymetry (Oziel et al. 2016). Atlantic Water cools and mixes with other water masses on its way north, becoming denser in the process (Oziel et al. 2016). This modified Atlantic Water enters the northern Barents Sea both from the north and south (Lind and Ingvaldsen 2012). The northern Barents Sea is seasonally ice-covered while the south remains ice-free year-round. There is relatively little input of freshwater to the Barents Sea resulting in rather weak stratification, except in the north where sea ice enhances stratification (Carmack and Wassmann 2006).

### Box 3: The Chukchi Sea

The Chukchi Sea (Figure 2C) is a comparatively smaller ( $0.6 \times 10^6 \text{ km}^2$ ) and shallower shelf (average 80 m depth, Hunt et al. 2013) which is bounded by the narrow Bering Strait to the south and the shelf break to the Canada Basin to the north. It extends as far west as Wrangell Island on the Russian side and as far east as Point Barrow on the Alaskan side. The Chukchi Sea receives water from the Pacific moving north through the Bering Strait. As the Bering Strait is narrow and shallow, the total flow entering into the Arctic from the Pacific is much lower than that entering from the Atlantic (Hunt et al. 2016). The Chukchi shelf is dominated by Bering Sea-Anadyr Water (also referred to as Bering Sea Water), a salty, cold and nutrient-rich water mass flowing northward. Bering Sea-Anadyr Water is created from the mixing of Anadyr Water and Bering Shelf Water as they enter Bering Strait (Hunt et al. 2013). Once into the Chukchi Sea, the Bering Sea-Anadyr Water splits into three branches following the contours of Herald and Hanna Shoals. Of the three branches, the western-most branch holds the highest nutrient concentrations and the eastern-most branch the lowest, indicative of proportionally higher contributions of Anadyr Water (nutrient-rich) in the west and Bering Shelf Water (lower in nutrients than Anadyr Water) in the east (Hunt et al. 2013). This pattern of nutrient richness is largely reflected in the primary production (Woodgate et al. 2015).

The Chukchi shelf is a highly seasonal system. The northward flow through the Bering Strait is strongest during summer, slowing down and on occasion even reversing due to a switch in wind direction in winter (Woodgate et al. 2005). In winter, a cold, deep water mass (Winter Water) forms at the bottom, created by extrusion of salt during sea ice formation (Pickart et al. 2016). The Alaska Coastal Current moving northward (warm, fresh, nutrient-poor) and, on the Russian side, the Siberian Coastal Current moving southward (cold, fresh, nutrient-poor), appear seasonally, driven largely by river discharge (Weingartner et al. 1999; Woodgate et al. 2015). The freshwater input from these coastal currents enhances summer stratification compared to the Barents Sea shelf (Carmack and Wassmann 2006) but in winter the water column is well-mixed and the entire shelf is covered in sea ice (Hunt et al. 2013). In addition to seasonal variability, the system shows high interannual variability. Under the typical northward winds, the warm and fresh Alaska Coastal Waters are confined along the Alaskan Coast. In times of anomalous strong southward winds, however, the Alaska Coastal Water ‘spills’ eastward onto the Chukchi shelf (Pisareva et al. 2015).

### 1.3.2 Primary production and the seasonal cycle

In the Barents and Chukchi Sea regions, winters are dark and do not support primary production. In spring, light returns, the water warms, ice begins to melt, and sea ice algae and phytoplankton bloom. The timing of the blooms varies spatially, depending largely on stratification of the upper water column. In the ice-free southern Barents Sea, surface warming is the main driver of stratification and generally results in a phytoplankton bloom in May. In the ice-covered northern Barents Sea, however, the timing of the bloom is more variable, occurring anytime between May and July depending on the timing of the sea ice retreat (Dong et al. 2020; Dalpadado et al. 2020).

Contrary to the Barents Sea, the entire Chukchi shelf is covered in sea ice in winter with gradual retreat northward in the spring until the shelf is mostly ice-free around September (Serreze et al. 2016). The phytoplankton bloom follows the sea ice retreat from south to north but is typically most intense in the southwestern Chukchi Sea (Wang et al. 2005). The water currents entering the Chukchi Sea are richer in nutrients than those entering the Barents Sea, leading to exceptionally high primary production there (Hunt et al. 2013).

### 1.3.3 Long-term change – climate change and borealization

Climate change is warming the Arctic rapidly and the two Arctic inflow shelves are strongly affected. The Barents Sea is expected to warm by up to 5°C compared to the long-term average and become ice-free year-round by the end of the century (Onarheim and Årthun 2017; Drinkwater et al. 2021). A strong warming and sharp sea ice decline is also predicted for the Chukchi Sea (Wang et al. 2012). In addition, increased inflow of Atlantic and Pacific water into the Arctic (individually termed ‘atlantification’ and ‘pacification’, respectively; termed ‘borealization’ collectively, Polyakov et al. 2020) will result in further warming and loss of sea ice, exacerbating the effects of climate change (Ingvaldsen et al. 2021). Together, the warming temperatures, reduced sea ice, weaker stratification and the increased flow speed of the boreal currents flowing onto the Arctic inflow shelves have important consequences on timing and extent of phytoplankton blooms (Grebmeier 2012; Palmer et al. 2014; Oziel et al. 2017) as well as species range distributions in the region (Oziel et al. 2020; Ingvaldsen et al. 2021). Combined, these changes can have important consequences on benthic invertebrate communities and their larval stages.

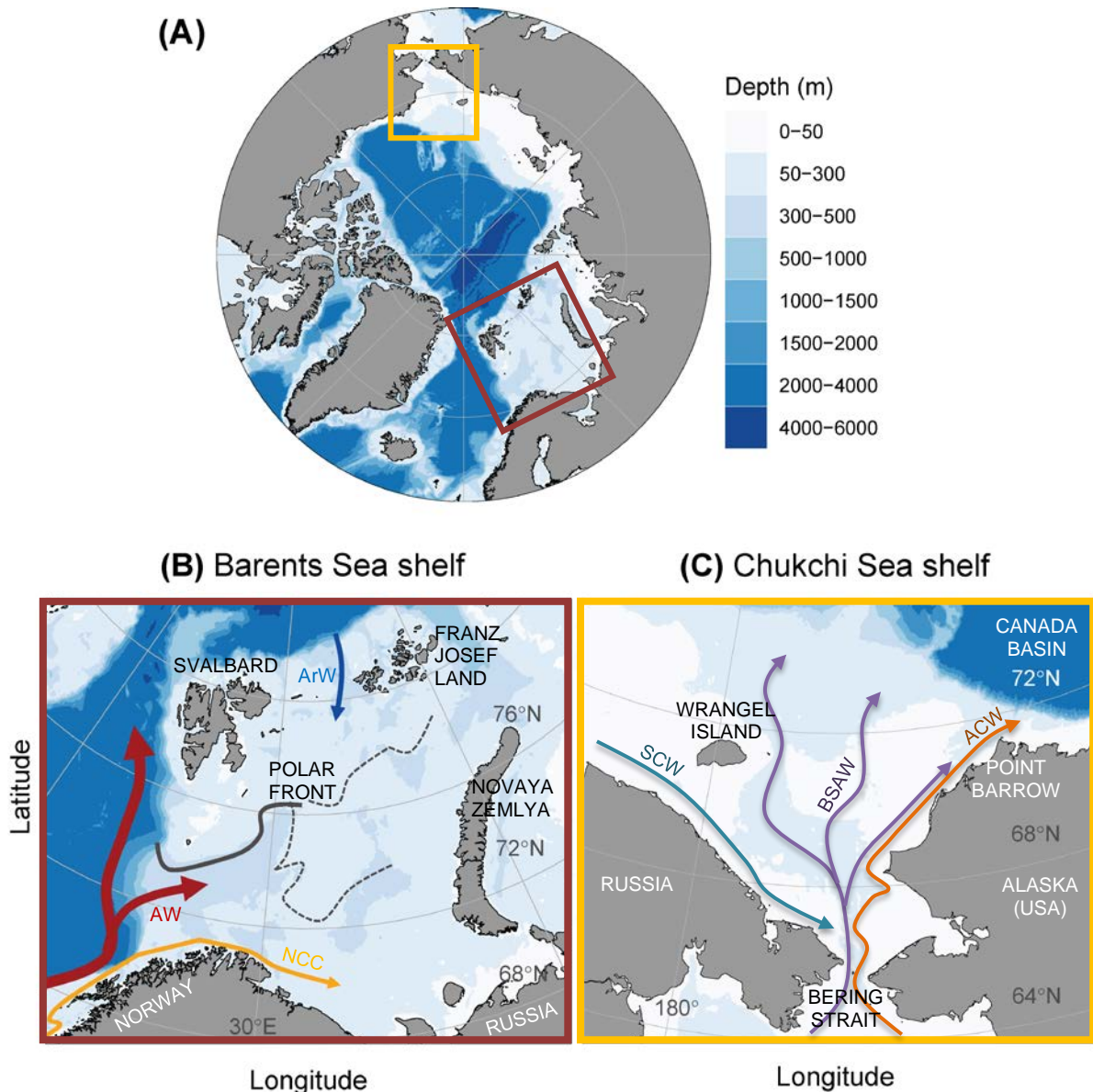


Figure 2: Maps of the study areas showing main landmarks and oceanographic features. **(A)** The two inflow shelves are highlighted in coloured boxes on the Arctic polar projection map, showing their positions in relation to other Arctic seas as well as the Atlantic and Pacific Oceans. **(B)** The Barents Sea (brown box) showing the Atlantic Water (AW, red arrows), Arctic Water (ArW, blue arrow), Norwegian Coastal Current (NCC, yellow arrow) and Polar Front (grey lines) based on (Ozhigin et al. 2011). **(C)** The Chukchi Sea (yellow box) with the Bering Sea-Anadyr Water (BSAW, purple arrows), Alaska Coastal Water (ACW, orange arrow) and Siberian Coastal Water (SCW, teal arrow) based on (Stabeno et al. 2018).

#### 1.3.4 Benthic invertebrates on Arctic shelves

Arctic shelves harbour rich benthic invertebrate communities which play an important role in carbon cycling (Souster et al. 2020), remineralization of nutrients (Renaud et al. 2007), as prey for fish (Eriksen et al. 2021), birds (e.g., Merkel et al. 2007), and mammals (Dehn et al. 2007) as well as supporting important commercial fisheries (e.g., the Northern shrimp *Pandalus*

*borealis*, Garcia 2007), and subsistence harvesting (Rapinski et al. 2018). Both inflow shelves support rich and diverse benthic communities (Grebmeier et al. 2015a; Jørgensen et al. 2015; Zakharov et al. 2020). Within each shelf, the benthic communities are spatially variable with environmental conditions driving assemblages characterized by different taxa. In the Barents Sea, sponges, arthropods and echinoderms dominate the epifaunal seafloor biomass (Zakharov et al. 2020) while polychaete worms and bivalves dominate the infauna living within the sediments (Cochrane et al. 2009). In the Chukchi Sea, echinoderms and crustaceans dominate in trawl catches (Ravelo et al. 2014) while polychaete and bivalves dominate the infauna (Grebmeier et al. 2015a). All taxonomic groups listed above, as well as most other phyla on Arctic shelves, contain members with planktonic larval stages.

#### 1.3.5 State of knowledge on Arctic meroplankton communities

Knowledge about the larval communities on Arctic shelves is fragmentary, incomplete and superficial taxonomically, spatially and temporally. On the Atlantic side, seasonality of meroplankton communities has been investigated, but all studies were conducted either in one Svalbard fjord (Kuklinski et al. 2013; Stübner et al. 2016; Brandner et al. 2017; Weydmann-Zwolicka et al. 2021) or along the northern Norwegian coast (Silberberger et al. 2016; Michelsen et al. 2017). In these nearshore studies, meroplankton abundance and taxon richness peaked in spring and early summer, shortly after the phytoplankton bloom. At the seasonal peak, meroplankton could dominate the zooplankton. Cirripedes and bivalves tend to be particularly abundant but polychaetes, echinoderms, bryozoans, nemertean, gastropods and decapods are also present (Kuklinski et al. 2013; Stübner et al. 2016). Whether these patterns seen in nearshore larval communities translate to the shelf remains to be seen as seasonal studies are missing over the shelf. Schlüter et al. (2001) studied larval stages on the Barents Sea shelf in May/June and Fetzer and Arntz (2008) studied the meroplankton in the adjacent Kara Sea in August-September. In both cases, the meroplankton communities were more diverse than those recorded in the Svalbard fjord.

Frequent summer zooplankton surveys in the Chukchi Sea have revealed the presence of an abundant meroplankton community that includes larvae of echinoderms, bivalves, cirripedes, polychaetes and decapods (Hopcroft et al. 2010; Questel et al. 2013; Ershova et al. 2015). No comparative seasonal studies exist for this region though a May-June study gives a glimpse into

the community composition early in the productive season, when only cirripedes and polychaetes were found to contribute to the meroplankton (Ashjian et al. 2021).

While a few taxa, particularly polychaetes and decapods, are identified to family, genus or even species levels in some studies (Eisner et al. 2013; Michelsen et al. 2017), most taxa are generally not resolved past phylum or class level. This makes it impossible to capture the true diversity of the system, to provide information on life-history parameters of individual species, or to discern between local taxa and those advected onto the inflow shelves from more distant seas. By using molecular tools to identify larvae to species level the work described in this thesis aims to turn the spotlight on meroplankton, and to bring the species composition of meroplankton communities of the Arctic inflow shelves to the fore.

## 2. QUESTIONS AND HYPOTHESES

The goal of this thesis was to characterize the meroplankton communities on the Chukchi and Barents Sea shelves in greater taxonomic, spatial and temporal resolution than previously possible and to investigate the likely origins of identified larvae (Figure 3).

**Question 1:** Which species characterize meroplankton communities on Arctic inflow shelves?

**Hypothesis 1.1:** The meroplankton communities on Arctic inflow shelves are more diverse than previously realized and are dominated by echinoderm, mollusc, polychaete and arthropod larvae.

**Question 2:** How do the communities differ temporally and spatially?

**Hypothesis 2.1:** Meroplanktonic larvae are present on the shelf year-round but most species peak in abundance around the spring phytoplankton bloom (Barents Sea).

**Hypothesis 2.2:** The meroplankton community differs between years (Chukchi).

**Hypothesis 2.3:** The meroplankton community differs across water masses.

**Question 3:** Where do the meroplanktonic larvae come from?

**Hypothesis 3.1:** Chukchi Sea and Barents Sea meroplankton communities comprise a mix of locally-produced larvae and advected taxa from more southern seas.

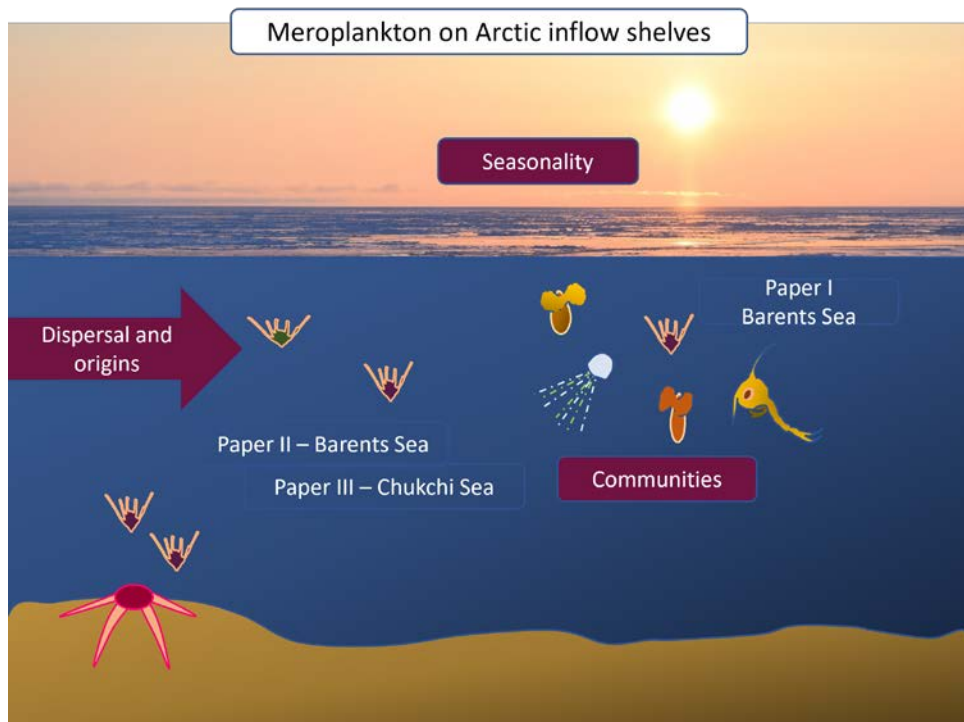


Figure 3: Conceptual figure summarizing major paper objectives.



### 3. METHODS

#### 3.1 Sample collection and processing

##### 3.1.1 Seasonal cruises – Barents Sea and Svalbard

Samples from the Barents Sea and Svalbard (**Papers I and II**) were collected in a series of seven seasonal cruises from September 2017 to August 2018 in three regions: north of Svalbard (**Paper II** only), and east of Svalbard north and south of the Polar Front (Figure 4A). At each station and sampling month, a CTD cast preceded zooplankton sampling to determine the depth distribution of water masses based on the vertical profiles of temperature, salinity and density. Zooplankton samples were collected from each water layer individually using a WP2 or Multinet (Hydro-Bios) with 64 or 180  $\mu\text{m}$  mesh, retrieving two to four samples per station, together covering the entire water column from around 10 m above the seafloor to the surface. Zooplankton samples were concentrated on a 64  $\mu\text{m}$  sieve and immediately transferred into 96% ethanol for preservation. A subset of the samples (a total of 27 samples: one cluster of seasonal samples north and one south of the Polar Front) were selected for **Paper I**. A subsample from each of the 27 samples was counted quantitatively and larvae photographed and individually-barcoded for identification. Most of these same samples, as well as additional ones (for a total of 61 samples) from the same regions and from the shelf north of Svalbard were used for bulk zooplankton metabarcoding in **Paper II**. These samples were split in half (after removal of the subsample from **Paper I**) and blended until homogenous prior to DNA extraction.

##### 3.1.2 Annual cruises – Chukchi Sea

Data on Chukchi Sea annual meroplankton distribution (**Paper III**) were obtained from zooplankton surveys, conducted during August-September of 2004, 2007, 2009, 2012, and 2015 (Figure 4B) using a 150  $\mu\text{m}$  Bongo net hauled vertically from a few meters off the bottom to the surface. A total of 31-63 stations were sampled each year, with spatial coverage of stations partially overlapping and partially differing among years. For all surveys, meroplankton was counted quantitatively from formalin-preserved samples and identified to macrotaxa level (i.e., Bivalvia, Echinodermata, Polychaeta, etc.) and individual larvae used for species identification through DNA barcoding were picked from a subset of the corresponding ethanol-preserved samples. Crabs were counted quantitatively from semi-oblique 505  $\mu\text{m}$  Bongo net which is much better at capturing larger and faster-swimming planktonic organisms.

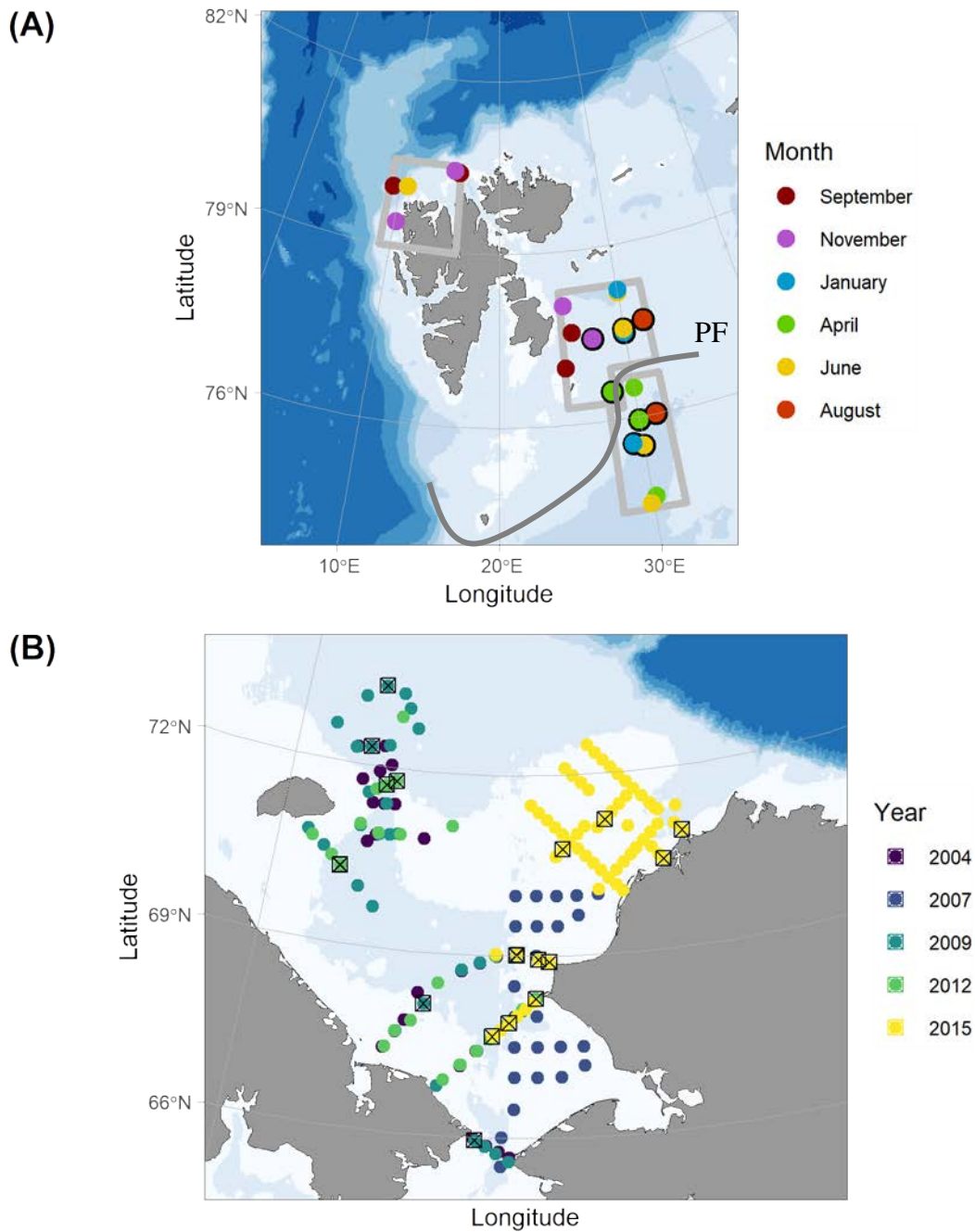


Figure 4: Maps of **(A)** the Barents Sea and Svalbard with sampling locations color-coded by month of collection for metabarcoding (all coloured dots) and individual barcoding (dots with outer black edge only), the grey polygons showing the three areas of interest and the grey line (PF) depicting the Polar Front; and of **(B)** the Chukchi Sea with sampling locations color-coded by year of collection and stations where meroplankton samples were DNA-barcoded highlighted with crossed-squares. Note that due to repeated sampling at some of the same locations over the years, some samples are hidden behind those of later years on this map. Refer to **Paper III** for full station coverage for each year.

## 3.2 Molecular work

### 3.2.1 Morphological ID versus DNA barcoding

Benthic invertebrate larvae are very small (most less than 500  $\mu\text{m}$ ) and often lack morphological features that would allow differentiation of species. Crab larvae are a notable exception to this and were identified to species visually in **Paper III** but for all other taxa collected here we relied on DNA barcoding to identify the meroplankton community. DNA barcoding, however, has its own challenges and previous attempts to identify meroplankton with molecular methods showed mixed success (e.g., Heimeier et al. 2010; Brandner et al. 2017). Here, methodologies were optimized to produce highly successful, time and cost-efficient barcoding of meroplankton (summarized in Figure 5).

### 3.2.2 Gene marker and primers

The first challenge to successful DNA barcoding of meroplankton is to extract sufficient DNA out of the microscopic larvae. Wash steps and vial transfers often found in commercial extraction kits involve some amount of DNA loss out of an already small DNA pool. The HotShot method used here for DNA barcoding of individual larvae (**Papers I and III**) circumvents this loss by removing these steps completely. The entire extraction process occurs within a single vial and requires only two pipetting steps. This method is cheap, fast and demonstrated here to be very effective at extracting DNA from meroplanktonic larvae across multiple phyla.

Extracting DNA out of a whole zooplankton sample for DNA metabarcoding on the other hand involves much larger volumes of samples and was therefore done using the PowerMax Soil kit (**Paper II**), a commercial extraction kit used in other zooplankton metabarcoding studies (e.g. Carroll et al. 2019). To date, zooplankton metabarcoding studies have used a wide range of extraction methods without reaching a consensus on one ideal method. Here, the PowerMax Soil kit successfully extracted DNA from a wide range of meroplanktonic invertebrate phyla but future studies should compare the success of different extraction methods to ensure best possible results.

Once the DNA has been extracted, the next challenge to barcoding of meroplankton is the selection of a gene marker and associated primers effective across all marine benthic invertebrate phyla. In marine metazoans, DNA barcoding studies have focused on the mitochondrial COI gene (cytochrome c oxidase subunit I) and 16S or a few nuclear markers

such as 18S and 28S (Lindeque et al. 2013; Bucklin et al. 2016). Of these, COI seems to have the best ability to discriminate between species (Andújar et al. 2018) and to date has the most complete reference database (BOLD database) so was selected for this study. While its high variability across species makes COI advantageous for species discrimination, it also makes it difficult for a single primer set to amplify across all marine phyla. The Leray-XT primer set (Wangensteen et al. 2018) overcomes that problem because it contains many degenerate (flexible) positions, allowing amplification across a wide range of phyla and was therefore selected for this study. The Leray-XT COI fragment at ~313 base pairs maintains high species discrimination but is also sufficiently short for high-throughput sequencing.

### 3.2.3 Individual barcoding versus metabarcoding

In **Papers I and III**, zooplankton were sorted quantitatively and larvae picked and barcoded individually. This method is extremely powerful in obtaining quantitative abundance counts of individual taxa, possibility to take morphometric measurements from photographs (allowing, amongst other things, to compare larval size over time as in **Paper I**) and validation of adequate taxonomic assignment to phylum or class by DNA barcoding. However, this method takes considerable time and is therefore not ideal for processing large numbers or volumes of samples.

Traditionally, barcoding of individual specimens (as in **Papers I and III**) would be done through Sanger sequencing where a single sequence is produced per sample. This method is costly, time-consuming and requires high concentrations of relatively pure specimen DNA difficult to obtain from mixed zooplankton samples. By using a double-tagging method (combination of primer tags and sequencing library tags) to combine ~1000 individual larvae or more per sequencing run on a high-throughput sequencing platform (Illumina MiSeq), we were able to sequence larvae cheaper, faster and with a higher success rate than with Sanger sequencing (Shokralla et al. 2015).

In instances where efficient processing is key, metabarcoding would be the preferred option. In this case, as was done in **Paper II**, bulk zooplankton samples are homogenized, then all DNA extracted, amplified and sequenced. In its current state, metabarcoding of bulk zooplankton samples is considered unquantitative though recent work has shown progress towards getting quantitative estimates (Ershova et al. 2021). In addition, DNA picked up by metabarcoding could have originated from pieces of adult tissue floating in the water column, as food item in

the gut of a planktonic consumer or simply as free DNA (eDNA) from a nearby adult population and therefore not represent planktonic early life stages themselves. While these scenarios are certainly possible, they seem unlikely to account for the bulk of the benthic invertebrate DNA found in zooplankton samples, especially not for the so-called non-local taxa found in **Paper II**, though to my knowledge this has yet to be tested.

#### 3.2.4 Bioinformatics

Sequences obtained from the MiSeq runs were processed in the OBITools package, a free software package designed for DNA metabarcoding data (Boyer et al. 2016) using parameters specifically optimized for the COI fragment amplified by the Leray-XT primer set (Wangensteen et al. 2018). While **Papers I and III** were based on barcoding of individual larvae, the double-tag multiplexing approach mimicked metabarcoding and required similar data processing. First, forward and reverse reads were aligned using function *illuminapairedend* and reads with quality score <40 discarded, so as to only retain high-quality sequences. Function *ngsfilter* was used for assigning reads (demultiplexing) to each larva (**Papers I and III**) or zooplankton sample (**Paper II**) and removing primers. Function *obigrep* filtered reads so that only those with 299-320 base pairs, the expected size of Leray-XT fragment, and no ambiguous bases (anything other than ACGT) were retained and *obiuniq* dereplicated remaining sequences (i.e. combined identical sequences and noted their abundances) within each sample. The Vsearch *uchime\_denovo* function (Rognes et al. 2016) detected and removed chimeras (a sequence made up of a combination of two or more parent sequences wrongly joined during amplification) based on the assumption that parent sequences are more abundant than the chimeric sequences. Sequences were dereplicated again, this time across samples, generating a table of unique sequences with their abundances in each sample. Sequences were then clustered into MOTUs (Molecular Operational Taxonomic Units) using Swarm v2 (Mahé et al. 2015) with maximum distance value of  $d = 13$ , optimized for clustering sequences into species-level MOTUs. The COI gene displays large intraspecific variability making clustering a more relevant approach than denoising which would overestimate species diversity. *Ecotag* (Boyer et al. 2016) then assigned taxonomy by comparing the representative sequence of each MOTU to a custom reference database. When no match greater than 97% was found, *ecotag* assigned taxonomy to a higher taxonomic level based on the last common ancestor of all database sequences having the highest similarity to the query sequence. Finally, the LULU algorithm removed putative pseudogenes and NUMTs (nuclear mitochondrial DNA) by

combining sequence similarity information with sequence co-occurrence patterns (Frøslev et al. 2017). Taxonomy was then further checked in BOLD and NCBI's BLAST for definitive assignment.

### 3.3 Comparison to adult communities

To address research question 3 '*Where do the larvae come from?*', the larval community was compared to the adult community in the Barents Sea (**Paper II**) and Chukchi Sea (**Paper III**). Adult community information was obtained from published datasets for the Barents Sea (Jørgensen et al. 2015; Andrade et al. 2017; Zakharov et al. 2020) and Chukchi Sea (Bluhm et al. 2009; Grebmeier et al. 2015b; Iken et al. 2019) and supplemented in the Barents Sea with species occurrence information from the GBIF (Global Biodiversity Information Facility) online database. For each station, occurrences were combined, irrespective of sampling year, as it is assumed that adult benthic communities are relatively stable on an interannual basis (Grebmeier et al. 2015b).

In the Barents Sea (**Paper II**), all larval taxa collected within one of three areas of interest (Figure 4) were compared to adult occurrences in the same areas. Those for which the adult was found in the same area were considered local and the others non-local. The geographical extent of the three areas of interest were relatively small compared to the size of the overall oceanographic regions in which they each lay. Several alternative options in defining these areas, including based on whole oceanographic regions (e.g. all Barents Sea north of Polar Front, etc.), based in benthic fauna community clusters from published literature, or based on the particle tracking areas of origin (section 3.4) were considered but ultimately the smallest, most conservative option was retained. Acknowledging that 'non-local' taxa for which the adult is located just outside the area of interest have a different significance compared to the taxa for which the nearest adult is known only from distant seas, the distance of each non-local taxon to the closest known adult was measured using function *distGeo* in package Geosphere (Hijmans 2019) in R. In the Chukchi Sea (**Paper III**), stations were not formally grouped into areas of interest but the overlap (or lack thereof) of adult and larval distributions were observed visually from the maps.

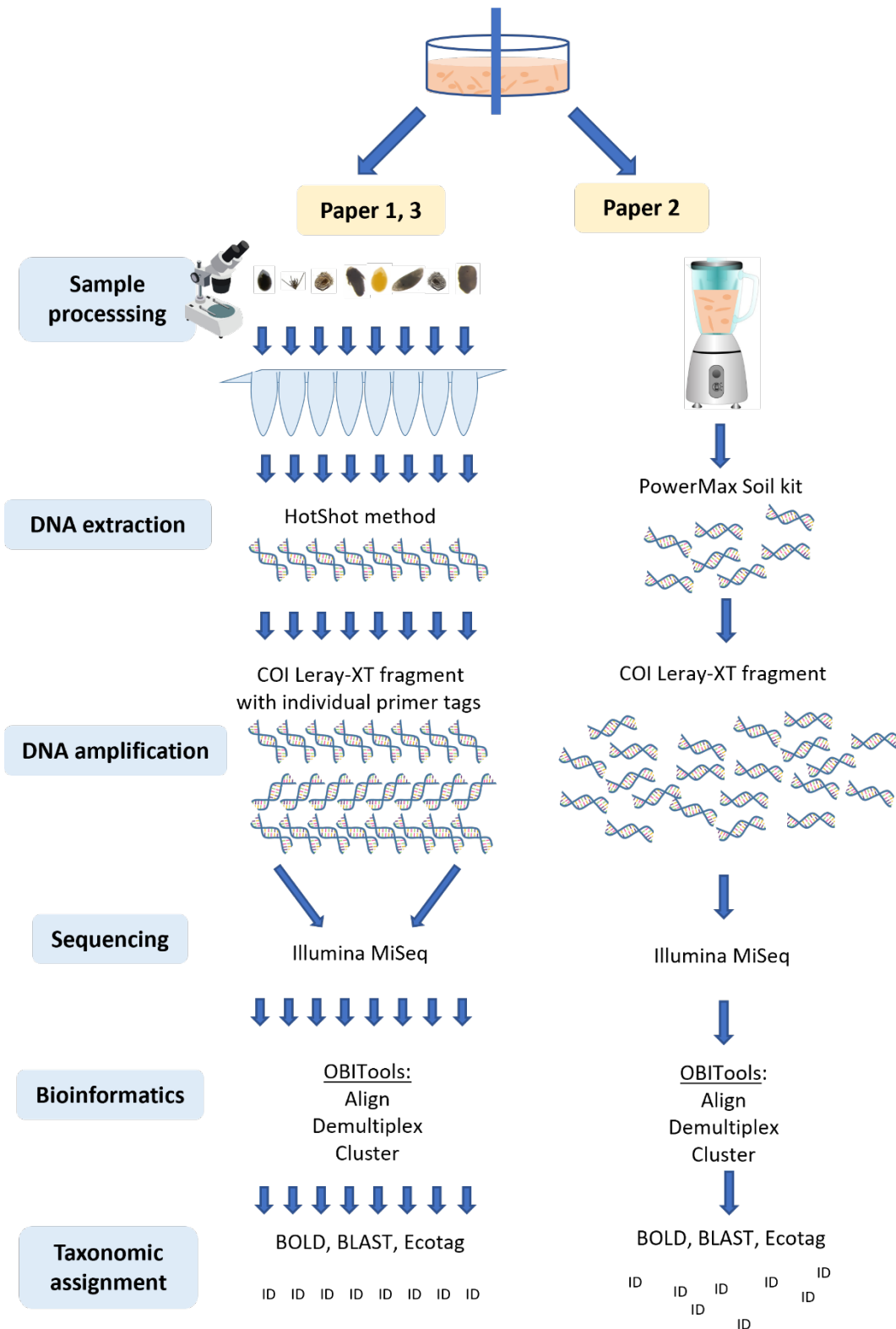


Figure 5: Methodological flow for identification of larvae through DNA barcoding of individual larvae (left, **Papers I and III**) and DNA metabarcoding of bulk zooplankton samples (right, **Paper II**). **Papers I and II** were based on zooplankton samples collected in the Barents Sea while **Paper III** was based on samples collected in the Chukchi Sea.

### 3.4 Particle tracking

Further addressing question 3 ‘*Where do the larvae come from?*’, particle tracking analysis was used to determine the possible areas of origin of larvae found in the Barents Sea and around Svalbard (**Paper II**). Particles were released in a 4 km x 4 km grid across the North Atlantic, Nordic seas and Barents Sea and let drift for three months. If at any time during the 3-month drift period a particle entered one of the three polygons representing the three areas of interest, its release site was considered a possible area of origin. Larval duration differs greatly across taxa, ranging from minutes to months (Shanks 2009), and rarely years (e.g., Strathmann and Strathmann 2007). Within taxa, water temperature can further alter larval duration with colder temperature extending development time (O’Connor et al. 2007). Those larvae with minutes to days-long dispersal phase likely remain close to the bottom and have limited dispersal potential so are not relevant for this particle tracking experiment. Conversely, taxa with years-long dispersal are presumably rare and such long dispersal would be difficult to model with particle tracking analysis due to the additive uncertainty over time. The 3-month drift represents a realistic drift period for a large number of taxa while being sufficiently long to account for dispersal of species beyond the current limits of their adult distributions.

The particle tracking analysis was performed for two periods, one starting on August 23, 2017 and the other on May 10, 2018, to account for the variability in current speeds throughout the year. These dates were chosen so that the end date of each 3-month period coincided with the November and August larval collections, respectively. Based on the results of **Paper I**, November and August were the sampling events with the highest larval abundances as well as high species richness.

Particles were released and maintained at 20 and 130 m throughout the duration of the drift experiments. The 20-m drift was chosen to represent the fast-flowing surface layer while avoiding the top few meters where winds and surface processes impart high temporal variability in current speed and direction. The 130-m drift on the other hand was chosen to represent drift in deeper water masses.



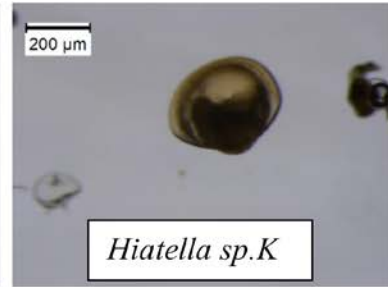
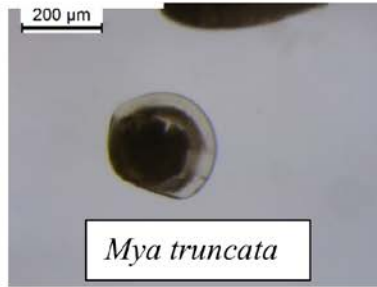
## 4. RESULTS

### 4.1 Meroplankton communities

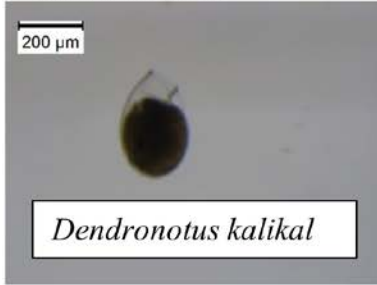
Meroplankton was observed at every sampling event, in both the Barents and Chukchi seas (**Papers I-III**) but abundances varied temporally and spatially, ranging from <100 individuals m<sup>-2</sup> to over 500,000 individuals m<sup>-2</sup>. I expected these Arctic inflow communities to be dominated by echinoderm, mollusc, polychaete and arthropod larvae, and found that both the Chukchi and Barents Sea had high abundances of bivalve, echinoderm and polychaete larvae, partially confirming this original hypothesis, but arthropods were not as dominant as expected in the Barents Sea. For molluscs, gastropods were found on both shelves but in comparatively lower abundances compared to the numerically-dominant bivalve larvae. Cirriped larvae made up a much higher proportion of the total abundance in the Chukchi Sea compared to the Barents Sea where they were never found in high abundance. Decapod crustaceans were also more abundant in the Chukchi Sea samples but likely due, at least in part, to the fact that they were specifically sampled by semi-oblique 505 µm Bongo net there, a more appropriate sampling method for large and strong-swimming crab larvae. In addition to these dominant phyla, both shelves had nemertean, cnidarian and bryozoan larvae though abundances were generally low. Finally, the Barents Sea harboured low numbers of sipunculid larvae which were not observed on the Chukchi shelf though they could have been mistaken for polychaete larvae there.

As expected, due to successful implementation of DNA barcoding, taxon richness was relatively high on both shelves, with 86 MOTUs (representing 72 taxa) in the Barents Sea (91 species-level MOTUs in the metabarcoding study) and 35 MOTUs (bivalves and echinoderms only) on the Chukchi shelf. Some of the species collected are shown in Figure 6. Comparisons of overall taxon richness between the Chukchi and Barents Sea shelves are difficult to interpret as the Chukchi Sea study only barcoded bivalves (20 taxa) and echinoderms (8 taxa) and the two studies differed in spatial and temporal coverage. In the Barents Sea, taxon richness was largely dominated by polychaetes (28 taxa), molluscs (18 gastropod and 7 bivalve taxa) and echinoderms (11 taxa, mostly ophiuroids).

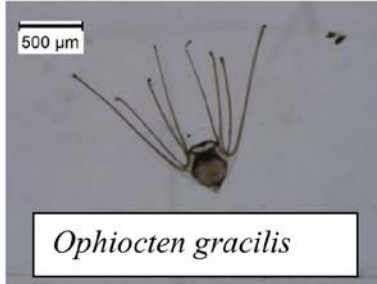
BIVALVIA



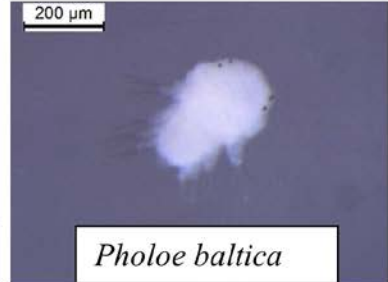
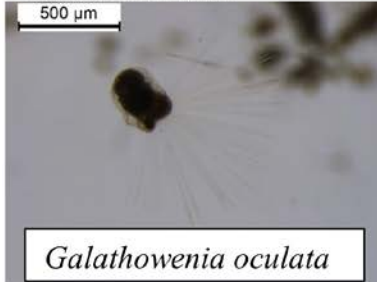
GASTROPODA



OPHIUROIDEA



POLYCHAETA



NEMERTEA



SIPUNCULA



CNIDARIA

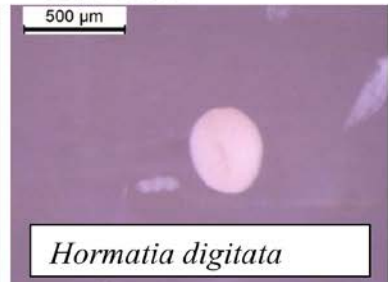


Figure 6: Example larval forms collected in the Barents Sea.

The two shelves shared some of the same species in the larval pool including the bivalves *Ciliatocardium ciliatum*, *Macoma calcarea* and *Mya truncata*, as well as the ophiuroids *Ophiocten sericeum*, *Ophiopholis aculeata* and *Ophiura sarsii*. These species were all present in August on the Barents Sea shelf (though not exclusively in August), around the same time of the year as the Chukchi Sea samplings occurred.

## 4.2 Temporal variability

### 4.2.1 Seasonality

Meroplankton abundance, taxon richness and community composition varied seasonally on the Barents Sea shelf (**Paper I**, Figure 7). Meroplankton was present in the water column year-round but, contrary to my initial hypothesis, total abundance did not peak around the spring bloom but rather in later summer to fall. These overall seasonal patterns were in large part driven by bivalves which dominated abundance in most months, except for April when echinoderms made up most of the larval abundance. Echinoderms and polychaetes were also abundant and followed a similar seasonal pattern to bivalves. Gastropod (mostly nudibranch) larvae peaked in the winter months. Once broken down to species level, however, it was apparent that each species displayed its own seasonality which did not always fit its group's overall patterns. For example, while overall gastropod abundance peaked in November and January, the heterobranch *Diaphana hiemalis* was only present in June.

The seasonal sampling also offered a window into larval duration of various taxa (**Paper I**). While several taxa were only present at one sampling time, indicative of a relatively short larval period, others were present over multiple seasons. The ophiuroid *Ophiocten gracilis* was present in April, June and August, with increasing body size through time, suggestive of a single spawning event followed by a long larval duration. The bivalve *Hiatella* sp. K was present year-round, except in April. Contrary to *O. gracilis*, however, its average size showed no distinct trend over time, suggesting continuous reproduction.

### 4.2.2 Interannual changes

Chukchi Sea meroplankton samples were collected over five summers (**Paper III**). The community composition differed from year to year, though the difference was not significant for species-level assemblages of echinoderms and bivalves. Note that the different spatial

coverage and slightly different timing of sampling across years make interannual comparisons difficult to interpret in this study.

### 4.3 Spatial variability

In the Barents Sea, the meroplankton community was compared between the north (largely cold Arctic Water) and south (largely warm Atlantic Water) of the Polar Front (**Paper I**). Overall, meroplankton abundance was about one order of magnitude greater to the south compared to the north. Out of the 72 taxa identified, 27 were shared between the two locations, whereas 20 were only found to the south and 25 only to the north of the Polar Front, offering partial support to the hypothesis that the meroplankton communities would differ across water masses. However, the spatial differences in the meroplankton communities were overpowered by the seasonal variability and were not statistically significant (Figure 7).

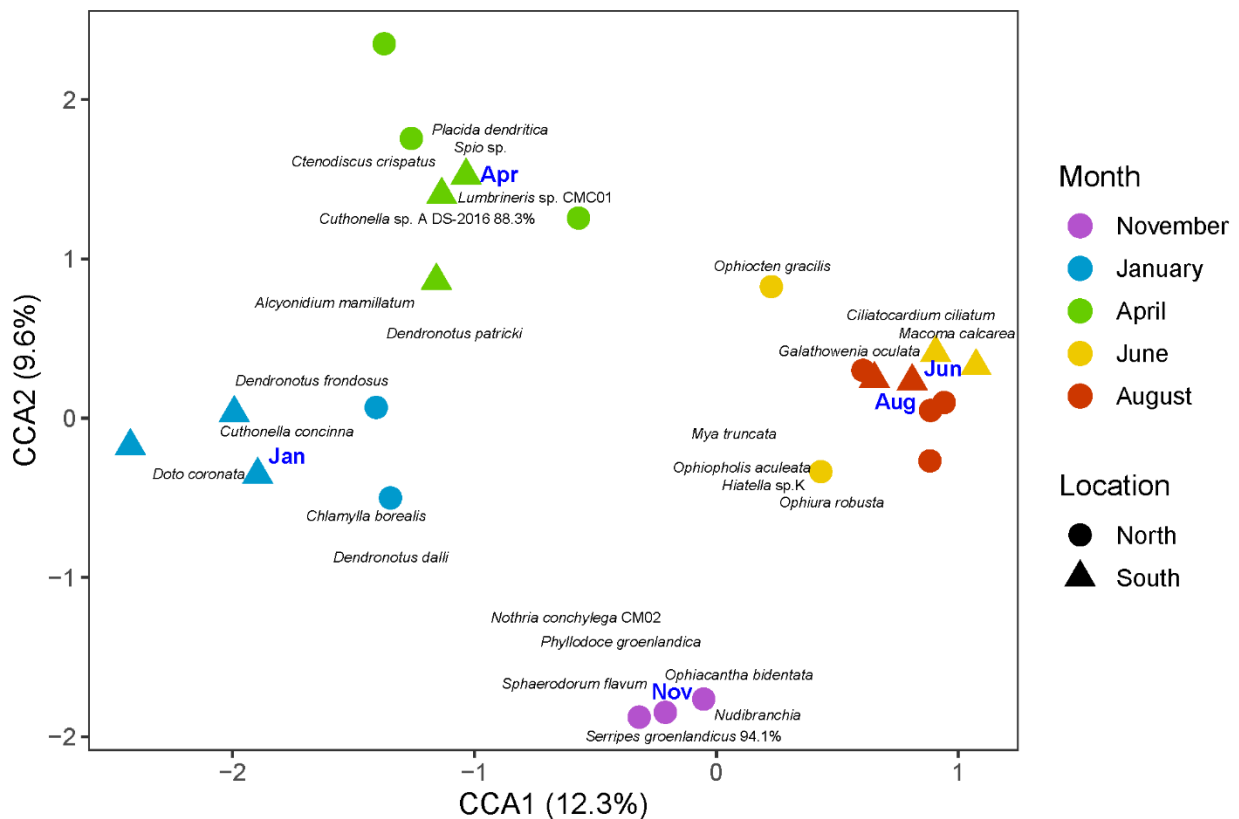


Figure 7: Similarity of Barents Sea meroplankton communities across months (colours), north and south of the Polar Front (symbols) shown in a canonical correspondence analysis (CCA) plot based on fourth-root transformed meroplankton abundance data (**Paper I**). Each point represents a single sample per month, location and depth layer. The first axis explains 12% of the variance while the second explains 9.6%.

Meanwhile, in the Chukchi Sea (**Paper III**), water masses had a stronger influence on meroplankton community structure than temporal (here interannual) variability (Figure 8). Meroplankton abundances, particularly echinoderms and bivalves, were generally highest near the Alaskan Coast or, if away from the coast, in waters influenced by the Alaska Coastal Current.

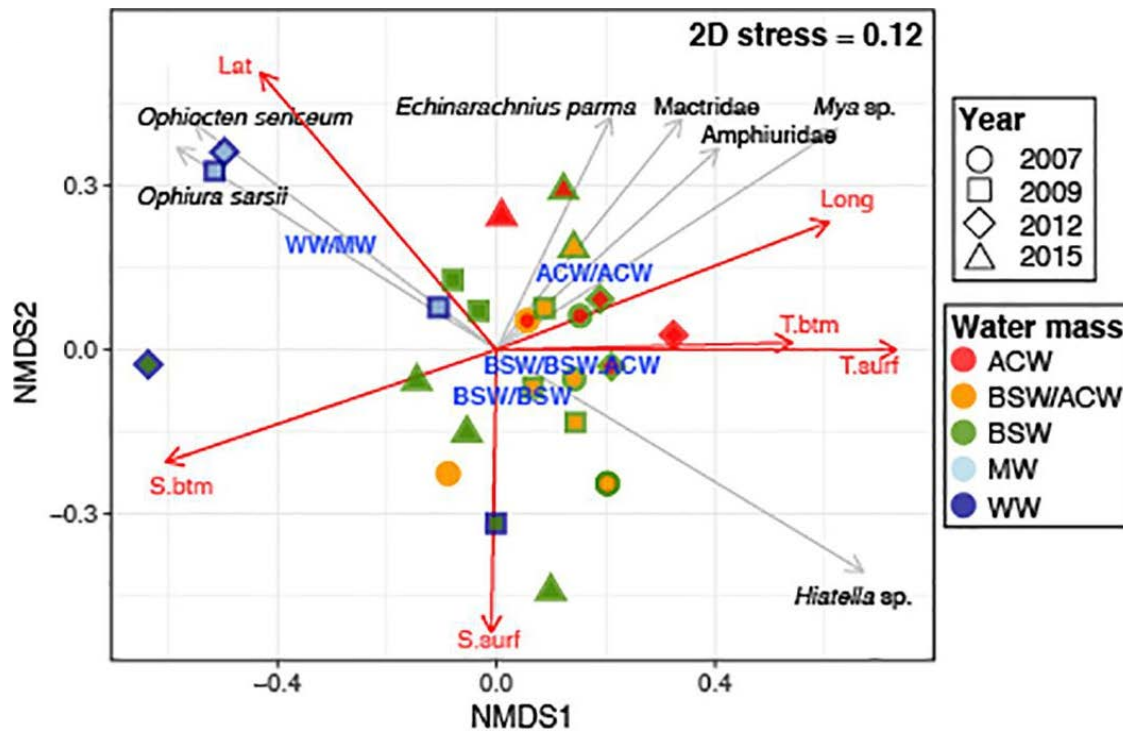


Figure 8: nMDS ordination of log-transformed abundance of larvae of bivalve and echinoderm species at Chukchi Sea stations where barcoding was done (**Paper III**); symbols represent year sampled; outer colour represents bottom water mass; inner colour – surface water; Vectors indicate significant ( $p < 0.05$ ) correlations of taxa abundances (gray arrows)/physical parameters (red arrows) to the ordination, with length reflecting  $R^2$ , and text labels the centroids for each respective water mass. T.btm, bottom water temperature; S.btm, bottom water salinity; T. surf, surface temperature; S.surf, surface salinity; Lat, latitude; Long, longitude. Water masses are listed as Bottom Water Mass/Surface Water mass: BSW, Bering Sea Anadyr Water; ACW, Alaska Coastal Water; BSW/ACW, Bering Sea Anadyr/Alaska Coastal Water (mix or uncertain); MW, Melt Water; WW, Winter water.

#### 4.4 Origins

The potential origins of the larvae collected on the two Arctic inflow shelves were explored to some extent in all three papers but discussed at greater lengths in **Papers II** and **III**, by comparing the larval communities to the adult populations (both papers) and by using particle tracking analysis to identify the possible areas of origin (**Paper II** only). As hypothesized, the larval community of both the Barents and Chukchi Seas represented a mix of local and advected

benthic taxa. In the Barents Sea, about a third of meroplanktonic species, mostly polychaetes and nudibranchs, were not local to the area where they were collected. For the majority of non-local taxa, the nearest adult was in fact found relatively close by in the Barents Sea itself, around Svalbard or along the northern Norwegian coast. For eight taxa, however, the closest adults were found in more distant locations, as far as the Canadian Arctic Archipelago and the Sea of Japan, thousands of kilometres away. In the Chukchi Sea, bivalves, echinoderms and crabs were identified to species. All species identified there as larvae are known from the Chukchi Sea but, except for a few taxa like the anthozoan *Cerianthus* sp. or the echinoid *Echinarachnius parma*, the spatial distribution of larvae and adults within the Chukchi Sea did not overlap tightly. Most of the larval diversity on the Chukchi shelf belonged to taxa that are known to occupy soft bottom shelf habitats such as found predominantly over the Chukchi Sea but a few taxa belonged to shallow water (e.g. *Limecola balthica* – now *Macoma balthica*) and/or hard bottom taxa (e.g. cirripeds) which would presumably have drifted in from the coast (depth and substrate affiliations obtained from the Arctic Traits Database, Degen and Faulwetter 2019). Note that in **Paper III**, *Hiatella arctica* is listed as a coastal species, which it often is, but it can actually also be found in soft bottoms and in deeper waters typical of the shelf.

Particle tracking analysis showed largely regional-scale connectivity in the Barents Sea given a 3-month drift period (**Paper II**, Figure 9). The majority of particles found north or south of the Polar Front originated within the same region or nearby, largely in the northern or southern Barents Sea, respectively. Of all three areas of interest, the one northwest of Svalbard had the broadest probable area of origin, reaching as far south as 69°N along the coast of Norway. Together, the particle tracking and comparison to adult records show a sizeable connection from the Norwegian coast to the Barents Sea and Svalbard.

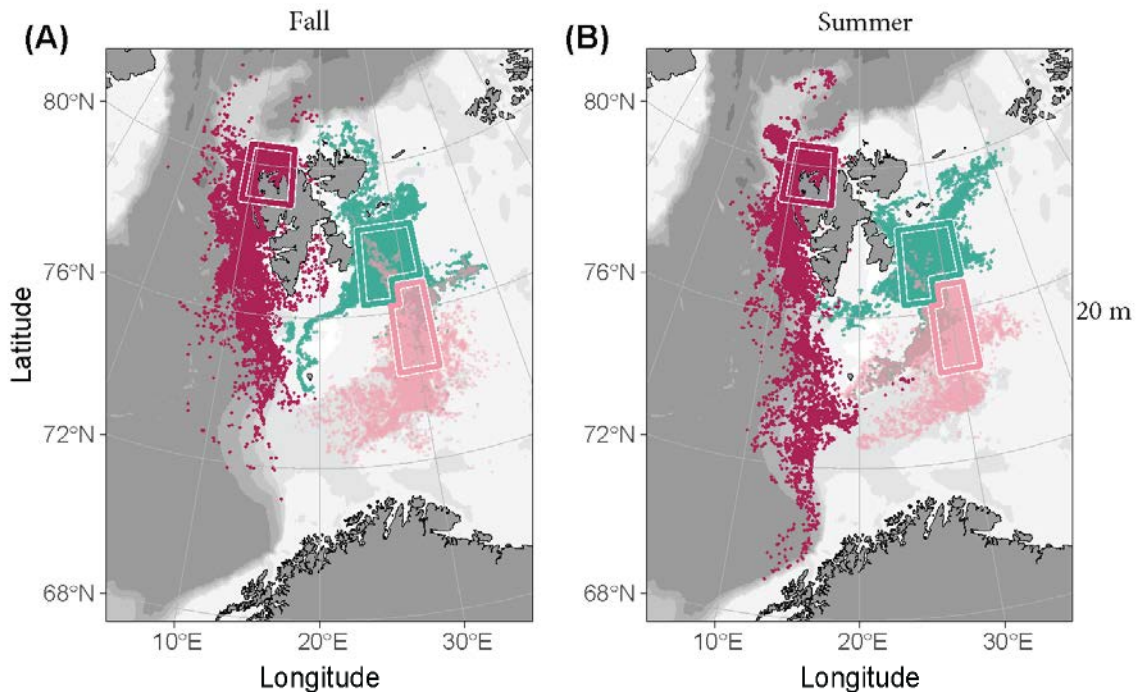


Figure 9: Possible origins of particles (coloured dots) drifting into one of three areas of interest (coloured polygons): north of Svalbard (red) and in the Barents Sea north of Polar Front (green), and south of Polar Front (light pink), anytime during a 3-month period starting on (A) 23.08.2017 (fall) and (B) 10.05.2018 (summer) at 20 m (**Paper II**).

## 5. DISCUSSION

### 5.1 Advancements to the field

Meroplankton studies have long been limited by the inability to identify larvae, and even more so embryos, to species level, restricting the range of ecological questions that could be answered using meroplankton surveys. The three papers presented here relied heavily on identification of larvae to species level for describing the larval community, its seasonality and spatial variability in greater detail than ever achieved before, and, for the first time, evaluating the origin of the larvae on a species-by-species basis. The methods developed here have proven extremely effective, obtaining upwards of 93% success rate for the majority of larval phyla examined, and are cheap, fast and simple to use compared to ‘standard’ molecular methods. I therefore strongly encourage the use of HotShot extraction method coupled with amplification and sequencing of the Leray-XT fragment of the COI gene for future meroplankton studies.

The biggest remaining challenge to identifying larvae using molecular methods is the incompleteness of DNA reference databases. A larval DNA sequence is only useful for identification if it finds a match in the databases, though even sequences without direct matches in the database can give some information on diversity and broad taxonomic affiliation. Even as the use of molecular methods for species identification rapidly gains momentum, investment into taxonomic identification and sequencing of adult voucher specimens to enhance DNA reference databases is often overlooked and underfunded. In collaboration with experts at the University Museum in Bergen and the Norwegian Barcode of Life (NorBOL, [www.norbol.org](http://www.norbol.org)), I have contributed 150 new adult specimens belonging to 44 species to the Barcode of Life Database ([www.boldsystems.org](http://www.boldsystems.org)). These new sequences facilitated my own larval barcoding efforts and will hopefully be useful to the broader research community for years to come.

Another key contribution of this thesis is the first look into seasonality of meroplankton communities on an Arctic shelf. A few seasonal studies exist in (relatively) easily-accessible fjords but to date none had been undertaken on any Arctic shelf. Seasonal sampling offshore is no small undertaking and it was fortuitous that different research expeditions happened to visit roughly the same region of the Barents Sea at different times of the year in 2017 and 2018 and that they all welcomed me onboard! The resulting dataset offered unprecedented insights into the larval communities both by capturing a greater proportion of the total diversity than would have been achieved by a summer-only collection and by elucidating seasonal changes in community composition.

## **5.2 Larval communities**

### **5.2.1 Species richness**

Thanks to a broad spatial and temporal coverage as well as species-level identification, this thesis has uncovered the highest known diversity of meroplanktonic larvae on the two Arctic inflow shelves. Larval taxon richness greatly surpassed what had previously been recorded in the Barents Sea (Schlüter and Rachor 2001), Chukchi Sea (Hopcroft et al. 2010) or anywhere else in the Arctic such as in the Kara Sea (Fetzer and Arntz 2008). While diversity was higher than in previous studies in the Arctic, it was approximately comparable or slightly lower to that found in the Antarctic (Stanwell-Smith et al. 1999; Bowden et al. 2009). The two polar oceans are now considered to have similar levels of benthic invertebrate species richness (Piepenburg 2005) which seems to be reflected in the meroplankton richness. Compared to lower latitude



area, however, the Arctic seems to have anywhere from comparable to considerably lower species richness, depending on regions/studies (e.g., Lindeque et al. 2013; Schroeder et al. 2020). Differences in taxonomic resolution and in spatial and temporal coverage make it difficult to compare larval diversity between different regions and across studies directly. Given the improvements in effectiveness and accessibility of molecular tools demonstrated here, however, their use will likely become more widespread, facilitating comparisons across studies and regions.

Shared methodologies for taxonomic identification of larval echinoderms and bivalves on the Barents and Chukchi seas permit a broad-level comparison of their diversity across the two shelves. Larval echinoderm species diversity was similar on both shelves and most taxa were ophiuroids. Bivalve diversity however was notably higher on the Chukchi shelf compared to the Barents. In an inventory of the adult benthic invertebrates of the Arctic seas, Sirenko (2001) lists 96 bivalve species on the seafloor of the Barents Sea and 74 for the Chukchi Sea (though sampling effort had admittedly been lower on the Chukchi shelf) so the higher larval diversity in the Chukchi is likely not a simple reflection of the adult diversity on the seafloor.

### 5.2.2 Community composition

Together, the studies presented here showed a shelf meroplankton community numerically dominated by cirripeds (only in the Chukchi Sea), echinoderms, bivalves and polychaetes. These taxa are typically abundant in Arctic meroplankton though which of those are most abundant varies across studies. In this thesis, as well as in other Arctic (Schlüter and Rachor 2001; Kuklinski et al. 2013; Stübner et al. 2016; Weydmann-Zwolicka et al. 2021) and sub-Arctic studies (Silberberger et al. 2016; Michelsen et al. 2017), bivalves are consistently abundant. The larvae of *Hiatella arctica* dominated the bivalve community in both the Barents and Chukchi seas but it is now apparent that *H. arctica* is a species complex (Layton et al. 2016). The larvae found here on either side of the Arctic in fact belonged predominantly to two now separately recognized species: *Hiatella* sp. K on the Barents Sea shelf and *Hiatella* sp. L (Layton et al. 2016) on the Chukchi Sea shelf.

The meroplankton communities in the Barents and Chukchi seas varied spatially and temporally though patchiness (Kersten et al. 2019) can make the detection of potential spatial patterns difficult. On the Barents Sea shelf (**Paper I**) meroplankton was more abundant south of the Polar Front and the communities differed to some extent across locations (**Papers I and II**)

though there was no significant association between meroplankton communities and water masses. A study in the Fram Strait and on the shelf west and north of Svalbard, however, did show significant correlations between larval community composition and water masses (Meyer-Kaiser et al. 2022). In the Chukchi Sea, the meroplankton assemblage also varied significantly between different water masses and was most abundant and diverse in the Alaska Coastal Water (**Paper III**).

Meroplankton assemblages in the Chukchi Sea also varied somewhat from year to year though the difference was not statistically significant for species-level echinoderm and bivalve assemblages (**Paper III**). Another broad-level zooplankton study in the Chukchi Sea found the meroplankton community to be more variable across years compared to the holoplankton community (Questel et al. 2013). While communities change from year to year, however, it is seasonality that often drives the bulk of the change in meroplankton communities (Highfield et al. 2010).

### 5.2.3 Thorson's rule

The works presented here show a previously-unrecognized diversity of planktonic larval invertebrates in the Arctic (**Papers I, II and III**) which adds to previous evidence (Marshall et al. 2012) countering the idea that this strategy is disadvantageous in polar areas (Thorson 1950; Mileikovsky 1971). Still, the ~100 (all phyla combined) and ~35 taxa (echinoderms and bivalves only, other taxa were not barcoded) identified here in the plankton of the Barents and Chukchi seas, respectively, represent a small portion of the ~4600 benthic taxa known to occur in the Arctic (Bluhm et al. 2011) or more specifically of the >3000 taxa and >1000 taxa that inhabit the Barents and Chukchi seas, respectively (most but not all benthic, Sirenko 2001). More spatially- and temporally-extensive surveys of meroplankton will undoubtedly increase the tally. When broken down by taxonomic group and location, it appears that long-lived planktonic larvae are especially common in ophiuroids and more prevalent over the Chukchi shelf (Figure 10) though again lower sampling efforts for adult benthic invertebrates on the Chukchi shelf (Sirenko 2001) could bias this comparison. It should be noted that Thorson's predictions were based largely on his observations of life history patterns in gastropods (Pearse and Lockhart 2004) and indeed, except for nudibranchs and sacoglossans, no gastropods were identified in the larval pool here. Note, however, that this thesis was not specifically designed to test Thorson's rule. A systematic review of reproductive strategies across benthic taxa over

a broad latitudinal range such as that by Marshall et al. (2012) is better equipped to adequately determine whether planktonic larval development is proportionally rarer in high-latitude environments, as predicted by Thorson (Thorson 1950; Mileikovsky 1971). Results from such a review do not lend support to Thorson’s original theory, at least not in the northern hemisphere.

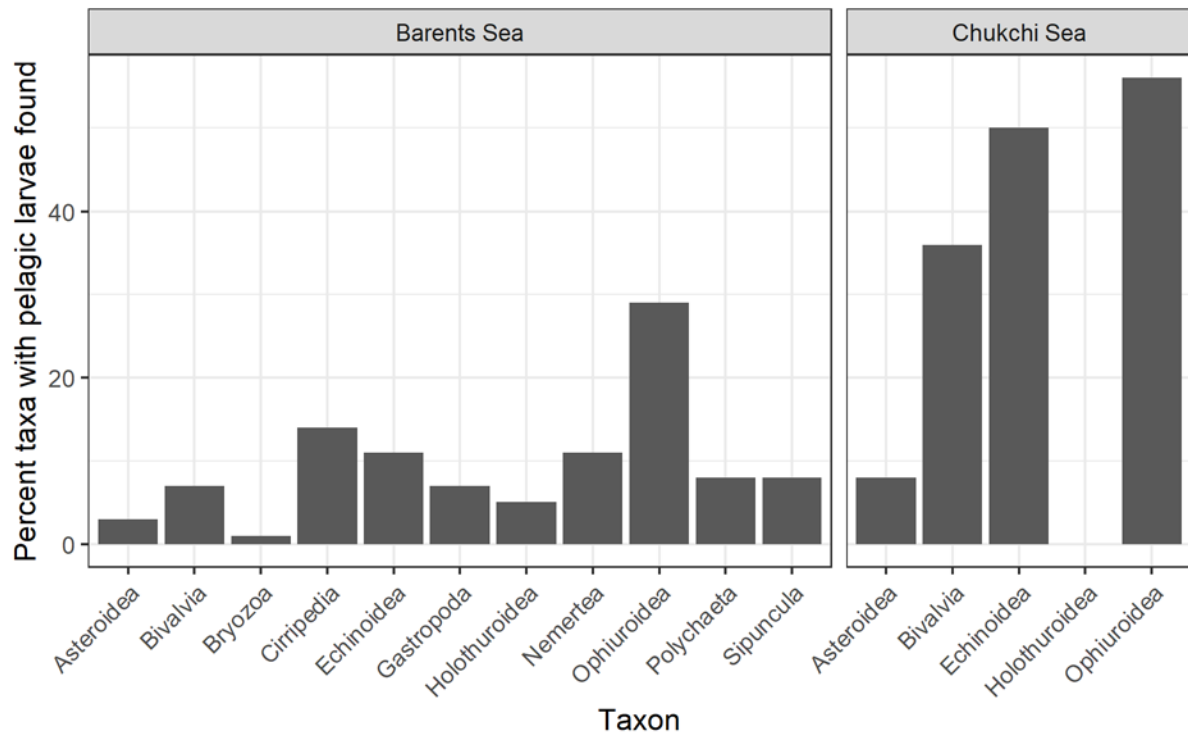


Figure 10: Percent of the species richness listed in Sirenko (2001) found as planktonic larvae in **Paper I** (Barents Sea) and **Paper III** (Chukchi Sea) by taxonomic groups. Note that this is a very rough estimate based on the total number of taxa only, not on a species-specific match between the two datasets. No holothuroid larvae were found on the Chukchi shelf.

Thorson’s rule can be viewed more broadly by incorporating nutritional mode instead of focusing solely on the presence or absence of planktonic development (Clarke 1992). This modified rule predicts an overrepresentation of non-feeding forms such as lecithotrophic larvae in polar areas based once again on the presumed unreliability of food availability in these systems. In the studies presented here, the majority (in terms of abundance and diversity) of larval forms collected were planktotrophic (based on larval morphotype, data not shown). It should be noted, however, that the sampling scheme used here is inherently biased towards collecting long-lived larvae which are largely planktotrophs by nature. Short-lived taxa could easily appear in the intervals between sampling events and have therefore been more likely to be missed. Indeed, a review across a wide geographical scale confirmed the prediction of fewer

feeding larvae at high latitudes (Marshall et al. 2012). Nonetheless, my work suggests that planktotrophy is a relatively common nutritional mode, even in polar regions.

### 5.3 Seasonality

#### 5.3.1 Seasonality across taxa

Seasonality is a well-known determinant of meroplankton community composition. It was the biggest driver of community patterns in the seasonal study of the Barents Sea here (**Paper I**) as well as in a Svalbard fjord (Weydmann-Zwolicka et al. 2021) and elsewhere (e.g. in the English Channel, Highfield et al. 2010). On the Antarctic coast, Sewell et al. (2011) showed seasonality in meroplankton abundance but not in richness. In the Barents Sea, larval abundance varied by a factor of about 100 between the lowest and highest points of the (sampled parts of) year. Contrary to my original hypothesis, the peak in larval abundance occurred in late summer and fall (**Paper I**). This finding contrasts to most Arctic coastal studies where larval abundance peaks in the spring or early summer (Kuklinski et al. 2013; Stübner et al. 2016). The sampling scheme in the Chukchi Sea study presented here (**Paper III**) could not resolve seasonality but a long-term sediment trap in the northern Chukchi Sea found a peak in meroplankton in October (Lalande et al. 2020). As a sediment trap collects sinking particles, the larvae collected were either on their way to the seafloor and therefore at the end of their planktonic phase at the time of collection or they were caught while still actively drifting. These two scenarios would imply different seasonality but either way, it is clear that meroplankton are still abundant in the water column in the fall on the Chukchi shelf also.

The spring peak in meroplankton abundance in coastal areas is often driven in large part by a few highly-abundant taxa, notably cirripeds (Kuklinski et al. 2013). Larval cirripeds appeared in low numbers on the Barents Sea shelf (**Paper I**) samples but were quite prevalent on the Chukchi Sea shelf (**Paper III**, Lalande et al. 2020). Interestingly, the single specimen that was found in the Barents Sea samples in **Paper I** was a cyprid (late stage) collected in January north of the Polar Front. Metabarcoding data from **Paper II** (not shown) did, however, find cirriped DNA in most samples despite the low numbers found in manually-sorted subsamples in **Paper I**. Many taxa, including echinoderms and bivalves, peak later in the year, even for the coastal areas where overall meroplankton abundance reaches its highest abundance in the spring (Kuklinski et al. 2013; Weydmann-Zwolicka et al. 2021). Meroplankton abundance is generally low in winter in Svalbard and northern Norwegian fjords (Stübner et al. 2016; Michelsen et al.

2017; Weydmann-Zwolicka et al. 2021) and in one study on Svalbard was altogether absent (Kuklinski et al. 2013). In the Barents Sea, winter (January) meroplankton was also less abundant than most other seasons but it was very diverse with larvae of seven phyla present in the water column (**Paper I**).

Not only is seasonality in the plankton specific to each taxon but timing and duration of larval presence also vary geographically. For several bivalve taxa, including *Ciliatocardium ciliatum*, *Mya truncata* and *Macoma calcarea*, for example, the timing of larval occurrence seemed to be delayed in the Barents Sea north of the Polar Front (first occurrence in August) compared to south of the Polar Front (first occurrence in July, **Paper I**) and further to the White Sea (first occurrence in June, Günther and Fedyakov 2000) or to a Svalbard fjord (May, Brandner et al. 2017). It should be noted, however, that first appearance in the samples does not necessarily mean that a larva was only recently released as larvae that had spawned earlier could have been advected in from elsewhere as is in fact shown in this thesis.

### 5.3.2 Mismatch with phytoplankton bloom

Conventional wisdom dictates that seasonality of meroplankton should be strongly linked to the spring phytoplankton bloom in high-latitude environments, an idea discussed by Thorson himself (Thorson 1936). The seasonal study presented here strongly challenges that idea for an Arctic shelf environment. A correlation between the spring production peak and meroplankton abundance does exist in some systems, including a Svalbard fjord (Stübner et al. 2016), a northern Norwegian fjord (Michelsen et al. 2017) and the English Channel (Highfield et al. 2010). Similarly to my findings from the Barents Sea, however, Antarctic meroplankton communities are not strongly associated with the peak in primary production (Pearse et al. 1991), nor are those on the shelf off of northern Norway (Silberberger et al. 2016) or those collected in a sediment trap on the northern Chukchi Sea shelf (Lalande et al. 2020). Though it is much too early to conclude definitively, it seems that meroplankton on polar ocean shelves could be less tightly linked to the phytoplankton bloom than their counterparts in nearshore environments. Note, however, that even in Arctic nearshore environments, a few highly-abundant taxa (e.g. cirripeds) tend to drive the spring peak in meroplankton while most taxa appear in the plankton later in the summer, though in many cases not as late as was observed here on the shelf.

Most larvae found here on both shelves, including those collected outside of phytoplankton bloom periods, belonged to larval types that are typically planktotrophic (ophioplutei, veligers, etc.). They should therefore be feeding while in the plankton, even when chlorophyll concentrations are low. The consequences of malnourishment in larvae can be dire, leading to delayed development or death though cold temperatures might confer some resistance to starvation by lowering metabolism (Anger and Dawirs 1981). Considering the wide phylogenetic, morphological and size ranges of meroplanktonic larvae, larval diet is not one-size-fits-all. Some larvae are selective feeders. In feeding experiments, cirriped larvae, for example, preferentially select diatoms (Turner et al. 2001) which is consistent with their dominance in times of phytoplankton blooms in coastal areas. Decapod larvae are omnivorous but select dinoflagellates when available (Fileman et al. 2014). Larvae likely select their prey in part on the basis of size (2-30  $\mu\text{m}$  for several bivalves, Lindeque et al. 2015). In addition, some Antarctic asteroid larvae feed on bacteria (bacteriotrophy, Rivkin et al. 1986) and some larvae may even absorb dissolved organic matter directly from the water around them (osmotrophy, Levin and Bridges 1995). While the seasonality of microbial communities over the Barents Sea shelf is not currently resolved, bacteria, picoplankton and nanoflagellates are available in fall and winter in Svalbard fjords (Iversen and Seuthe 2011; Marquardt et al. 2016). If such a microbial community was found over the Arctic shelves as well, then they could offer a potential food source to sustain the rich meroplankton community found there in the fall and winter.

In species for which the larval stage can sustain itself on other food sources, it may be other life stages that are timed to coincide with the bloom. For some of the taxa collected here, the adults themselves may require the energy from the sunken sea ice or phytoplankton bloom production to fuel reproduction. This reproductive strategy is termed ‘income breeding’ (as opposed to ‘capital breeding’ where reproduction is fueled by stored energy, Drent and Daan 1980) and could account for the months-long lag between peak in phytoplankton availability (May-July, Dong et al. 2020; Dalpadado et al. 2020) and peak in larval abundances (August-November, **Paper I**) on the Barents Sea shelf. In an interannual comparison of zooplankton communities in the Chukchi Sea, where non-cirriped meroplankton abundances peaked after the phytoplankton bloom, Questel et al. (2013) found much higher meroplankton abundance in a year of higher primary production, speculating that the increased nutrition could have fuelled more reproduction on the benthos that year. Indeed, while the abundances of bivalves,

echinoderms and polychaetes spiked that year, barnacle larvae numbers remained relatively stable supporting the idea that these typically late-spawning bivalves, echinoderms and polychaetes are in majority income breeders on the Arctic inflow shelves whereas cirripeds fit the capital breeder profile. In other taxa, the algal bloom might rather coincide with the settlement of juveniles on the seafloor. The larvae of many nudibranch taxa found here, for example, appeared in the water column in January. Assuming a larval duration of a few months such as in *Dendronotus frondosus* (Sisson 2005), juveniles might settle in time to feed on the fresh carbon flux sinking to the seafloor from the overlying algal bloom. These scenarios are highly hypothetical, however, as very little is known about reproductive timing and its association with food availability in Arctic benthic invertebrates. The larval timings observed here contribute one piece of the puzzle to which adult reproduction and juvenile settlement timing and dietary requirements must be added to form a complete picture of seasonality of life cycles on Arctic inflow shelves.

#### **5.4 Connectivity to sub-Arctic seas**

The strong advective nature of the Barents Sea and its connection to the Atlantic Ocean were reflected in the occurrence of larvae of some sub-Arctic species over the Barents Sea shelf. For example, larvae of the nudibranchs *Dendronotus yrjargul*, *Doto maculata*, *Coryphella gracilis* and *Bohuslania matsmichaeli*, whose adult's northernmost distribution limits are along the southern coast of Norway, have presumably drifted 1000-2000 km before being collected in the Barents Sea (**Papers I and II**). According to the particle tracking analysis (**Paper II**), a larva released in Skagerrak, close to the only known adult population of *B. matsmichaeli* (Korshunova et al. 2018), would require more than a year to drift to the Barents Sea north of the Polar Front where it was found. On the Pacific side, however, all larval taxa identified have been previously recorded on the Chukchi shelf as adults. Some taxa whose larval distribution were listed as non-overlapping with that of the adult in **Paper III** now (some four years after data analysis for that paper) have occurrence records in GBIF which better overlap with the larvae. Some taxa still have few recorded occurrences as adults in the Chukchi Sea (e.g. *Mytilus trossulus*, *Ophiura maculata*, **Paper III**, GBIF) and were not historically considered as inhabitants of the Chukchi Sea (Smirnov 1994). Nudibranch larvae were not identified to species on the Chukchi shelf, so it is not yet possible to say whether boreal nudibranchs drift into the Chukchi Sea from the south similarly to what is observed in the Barents Sea. On both inflow shelves, however, the larval and adult distributions did not always match at a local scale

indicating that larvae can travel substantial distances. Overall, despite the strong advective inflow, results shown here indicate largely regional-scale larval connectivity, with most larvae likely having originated from within the Barents or Chukchi seas.

Many of the taxa collected over the Barents or Chukchi shelves belonged to typically coastal communities (**Papers II and III**). Several nudibranch larvae identified on the Barents Sea shelf as well as larvae of cirripeds and the bivalve *Hiatella arctica* on the Chukchi Sea shelf, for example, are usually found in shallow and/or rocky habitats. Perhaps the most extreme example is a nudibranch species whose adult population is only known to inhabit very shallow (5-7m depth) brackish waters of an inner fjord in southern Norway (Korshunova et al. 2018). The likelihood of larvae from this species, or from most other coastal species, to settle and survive on the Barents Sea or Chukchi Sea shelves is low. Either larvae will die from the inhospitable conditions on the shelf or they will keep drifting, waiting for a settlement cue indicating a suitable habitat for settlement. Indeed, larvae of many taxa are selective in their settlement sites based on habitat requirements of the adults (Snelgrove et al. 1999). If these coastal or boreal expatriate taxa fail to find suitable habitats on the Arctic shelves, however, they will become part of the ‘trails of death’ (Wassmann et al. 2015) whereupon the advected biomass will be consumed or sink to the seafloor, contributing to the success of other species if not their own.

## **5.5 Impacts of climate change**

The Arctic Ocean is in a period of immense change (Huntington et al. 2020). Combined, the effects of climate change and borealization (Polyakov et al. 2020) may have big impacts on the benthic communities of the Arctic shelves (Ingvaldsen et al. 2021) which are already in transition (Grebmeier 2012). Together, these changes will impact the composition (Oziel et al. 2020), intensity (Palmer et al. 2014) and timing of phytoplankton blooms (Oziel et al. 2017; Ardyna and Arrigo 2020), including perhaps more fall blooms (Ardyna et al. 2014). This change in bloom phenology could have big consequences on the benthic invertebrates and on their reproductive cycles. Whether the parents rely on the food supply to fuel reproduction or the larvae feed directly on the phytoplankton, a change in phytoplankton phenology will likely alter the timing and/or success of reproduction. In addition, with conditions becoming increasingly boreal in nature, the larvae of more southern expatriates may be able to settle and grow on the Arctic inflow shelves in the future. These new taxa could compete with the local fauna and alter the ecosystem. Finally, these environmental changes could affect the speed of development and



lifespan in the plankton while at the same time changing current flow speeds, altering the dispersal potential of larvae. We will not be able to detect the impacts of these environmental changes, however, unless we have strong baseline knowledge of the system as it is now against which to compare future changes.

## **6. CONCLUSION AND FUTURE DIRECTIONS**

In the last 200 years, we have learned that some benthic invertebrate taxa release larvae into the water column to drift with the currents, that this drift can impact genetic connectivity across populations, that there is a wide range of morphology, feeding modes and larval durations, that the larvae respond to environmental cues around them, and so much more! This thesis contributes another piece of the puzzle, shedding light on the diversity, seasonality and connectivity of larvae on Arctic inflow shelves, regions that will be strongly affected by climate change and borealization in the years to come. We now know that these shelf communities are much more diverse than we had previously realized, that peak abundance and diversity come outside of the phytoplankton bloom, mostly in the fall, and that some taxa are able to drift onto the Arctic inflow shelves from elsewhere though most of the larvae originated within the shelves themselves (Figure 11). The blurry picture of the shelf meroplanktonic communities is coming into sharper focus.

In many ways, this thesis raises more questions than it answers. The high abundance and diversity of planktotrophic larvae in the fall in particular raises many questions. First, is this seasonal pattern a recurring feature of all Arctic shelves? Of polar shelves in general? And what are the larvae eating in the fall? We may now be able to use metabarcoding of gut content to obtain detailed information on larval diet in the wild (e.g., Lindeque et al. 2015). Information on timing of adult reproduction and juvenile occurrence on the seafloor will also help complete the picture of seasonal cycles and how they relate to food availability.

The molecular tools for identifying larvae to species level are now well in place and can be used on a broader scale. These tools can make meroplankton studies more accessible, more comparable and more informative. Incorporating these tools into existing zooplankton time series would be an efficient way to survey the meroplankton communities. Time series can track changes in meroplankton communities over time (e.g. in the North Sea, Kirby et al. 2008) and detect newcomers. International collaborative synthesis of meroplankton communities

across the Arctic seas as has been recently achieved for the adult benthic communities (Jørgensen et al. 2022) would be greatly helpful for discerning spatial patterns. In addition, using population genetics tools could more clearly pinpoint the origin of larvae and define connectivity between boreal seas and the Arctic shelves. One important area of focus in the coming years, however, should be to populate the DNA barcode databases for as many species as possible, associated with robust taxonomic expertise to identify the animals from which the source sequences originated. This will make every future study that much more meaningful. Amazing initiatives like the Barcode of Life's BIOSCAN are making big strides in that direction (<https://ibol.org/programs/bioscan/>).

Possible new avenues for research are endless. Let's see what the next 200 years of larval research will teach us!

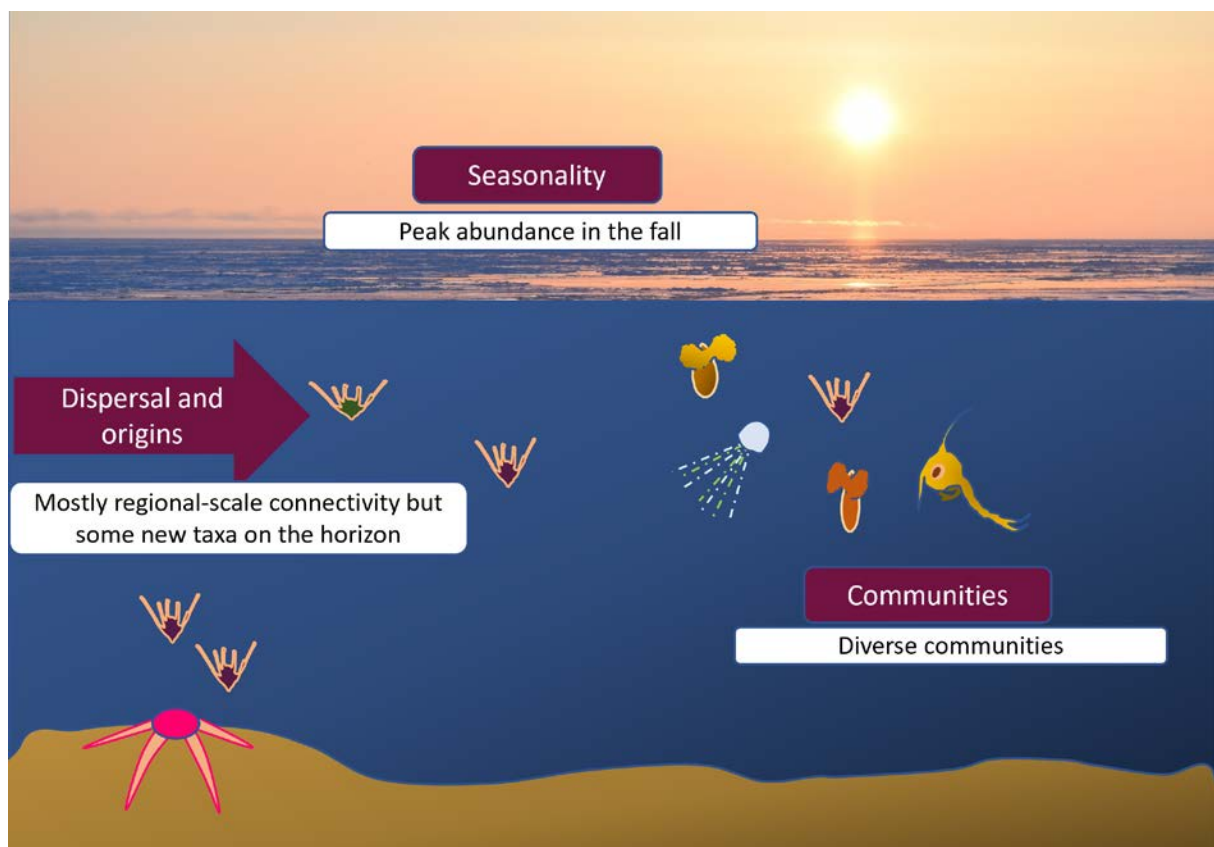


Figure 11: Main take-home messages from the PhD thesis.

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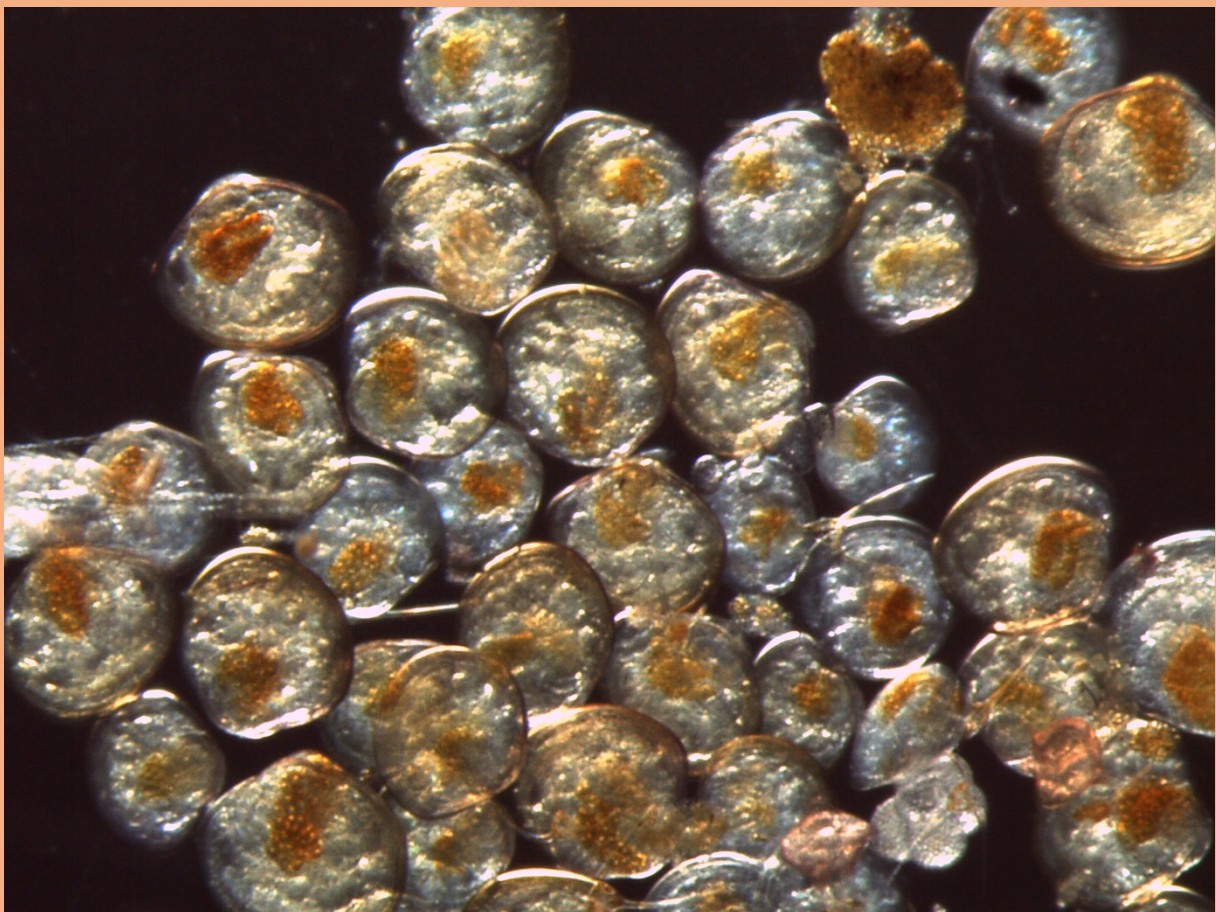
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# Paper I

Descôteaux R, Ershova E, Wangensteen OS, Præbel K, Renaud PE, Cottier F, Bluhm BA (2021) Meroplankton diversity, seasonality and life-history traits across the Barents Sea Polar Front revealed by high-throughput DNA barcoding. *Front Mar Sci* 8:677732, <https://doi.org/10.3389/fmars.2021.677732>







# Meroplankton Diversity, Seasonality and Life-History Traits Across the Barents Sea Polar Front Revealed by High-Throughput DNA Barcoding

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## OPEN ACCESS

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### Specialty section:

This article was submitted to  
Marine Ecosystem Ecology,  
a section of the journal  
Frontiers in Marine Science

**Received:** 08 March 2021

**Accepted:** 03 May 2021

**Published:** 28 May 2021

### Citation:

Descôteaux R, Ershova E,  
Wangensteen OS, Præbel K,  
Renaud PE, Cottier F and Bluhm BA  
(2021) Meroplankton Diversity,  
Seasonality and Life-History Traits  
Across the Barents Sea Polar Front  
Revealed by High-Throughput DNA  
Barcoding. *Front. Mar. Sci.* 8:677732.  
doi: 10.3389/fmars.2021.677732

In many species of marine benthic invertebrates, a planktonic larval phase plays a critical role in dispersal. Very little is known about the larval biology of most species, however, in part because species identification has historically been hindered by the microscopic size and morphological similarity among related taxa. This study aimed to determine the taxonomic composition and seasonal distribution of meroplankton in the Barents Sea, across the Polar Front. We collected meroplankton during five time points seasonally and used high-throughput DNA barcoding of individual larvae to obtain species-level information on larval seasonality. We found that meroplankton was highly diverse (72 taxa from eight phyla) and present in the Barents Sea year-round with a peak in abundance in August and November, defying the conventional wisdom that peak abundance would coincide with the spring phytoplankton bloom. Ophiuroids, bivalves, and polychaetes dominated larval abundance while gastropods and polychaetes accounted for the bulk of the taxon diversity. Community structure varied seasonally and total abundance was generally higher south of the Polar Front while taxon richness was overall greater to the north. Of the species identified, most were known inhabitants of the Barents Sea. However, the nemertean *Cephalothrix iwatai* and the brittle star *Ophiocten gracilis* were abundant in the meroplankton despite never having been previously recorded in the northern Barents Sea. The new knowledge on seasonal patterns of individual meroplanktonic species has implications for understanding environment-biotic interactions in a changing Arctic and provides a framework for early detection of potential newcomers to the system.

**Keywords:** meroplankton, larvae, seasonality, DNA barcoding, Barents Sea, life history traits

## INTRODUCTION

In the Arctic, and around the world's oceans, benthic invertebrates play important roles in carbon cycling and remineralization of nutrients (Renaud et al., 2007a), as prey for fish (Eriksen et al., 2020), birds (Merkel et al., 2007), and mammals (Dehn et al., 2007), as well as supporting important fisheries (e.g., the Northern shrimp *Pandalus borealis*, Garcia, 2007), and subsistence harvesting

(Rapinski et al., 2018). The Barents Sea, located in the Atlantic gateway to the Arctic, is home to over 3,000 benthic invertebrate taxa, making it one of the most diverse regions of the Arctic (Piepenburg et al., 2011; Renaud et al., 2015).

Most studies on the benthic invertebrates of the Barents Sea have focused on the adult stage (Carroll et al., 2008; Cochrane et al., 2009; Jørgensen et al., 2015; Zakharov et al., 2020). For a large proportion of benthic invertebrates, however, the larval phase constitutes the main vector for dispersal, having important implications for population connectivity (Cowen and Sponaugle, 2009), species conservation and management of marine protected areas (Krueck et al., 2017), dispersal of invasive species and understanding of the impacts of environmental change on benthic communities (Levin, 2006; Renaud et al., 2015). Compared to adults, planktonic stages are relatively short-lived, with a presence in the water column from hours to months (Shanks, 2009), though this becomes longer in colder temperatures, sometimes more than doubling in duration with a drop of a few degrees (O'Connor et al., 2007). Therefore, repeated sampling over the course of a year is required to capture as much of the diversity as possible. Such seasonal sampling of Arctic meroplankton has mostly been done in fjordic environments to date (Kuklinski et al., 2013; Stübner et al., 2016; Brandner et al., 2017; Michelsen et al., 2017). There, most meroplanktonic groups peak in abundance in the spring/early summer, around the time of the phytoplankton bloom, presumably to maximize food availability for the larvae. The few studies that focus on Arctic offshore locations only sampled during a short time window, together covering only the period from May to September (Clough et al., 1997; Schlüter and Rachor, 2001; Fetzer and Arntz, 2008). A peak in larval abundance coinciding with a peak in local primary production, as occurs in Arctic and Antarctic fjords and coasts (Bowden et al., 2009; Arendt et al., 2013; Michelsen et al., 2017; Presta et al., 2019) as well as in lower latitude regions (Highfield et al., 2010), is often assumed but has not yet been corroborated with seasonally resolved sampling on Arctic shelves.

The spring phytoplankton bloom is broadly found to occur in May in the southern Barents Sea, when sufficient light and stratification of the water column favor bloom development. In ice-covered waters of the northern Barents Sea, however, the phenology of the phytoplankton bloom is more variable, occurring anytime from May to July depending on timing of sea ice retreat (Dalpadado et al., 2020; Dong et al., 2020). In these seasonally ice-covered waters, an ice algal bloom as well as an under-ice phytoplankton bloom can contribute primary production to the system prior to ice melt, extending the duration of the productive period (Syvertsen, 1991; Leu et al., 2015; Ardyna et al., 2020). Even with these additional sources of production in ice-covered waters, however, total primary production in the northern Barents Sea is substantially lower than in the southern Barents Sea (Slagstad et al., 2011; Hunt et al., 2013).

The Barents Sea is an Arctic inflow shelf which receives large inputs of Atlantic Water (Carmack and Wassmann, 2006; Jakobsen et al., 2011). As this Atlantic Water flows north and eastward, it eventually meets colder and fresher Arctic Water flowing south (Oziel et al., 2016; **Figure 1**). The boundary area between these two major water masses is termed the Polar Front

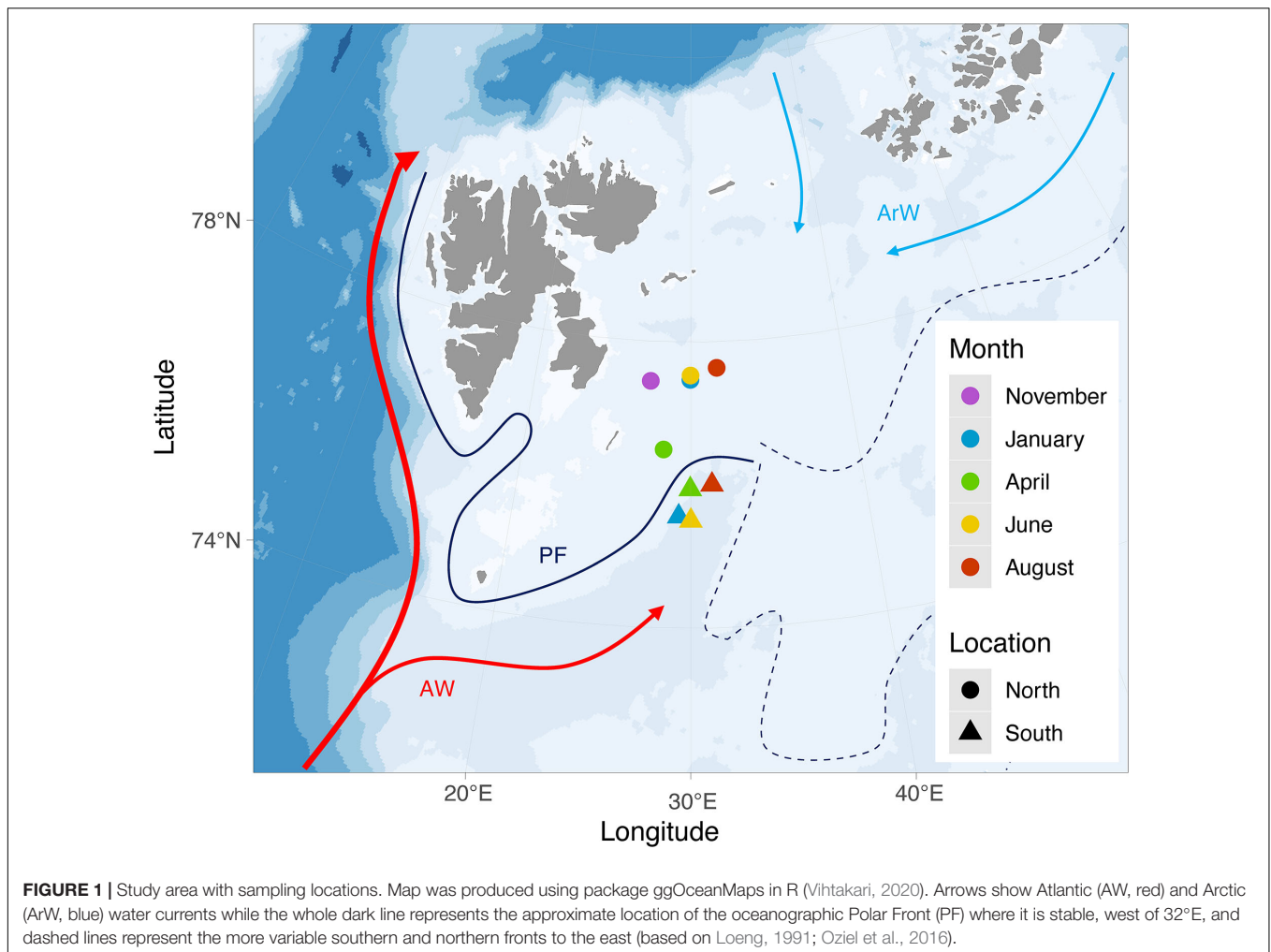
(Oziel et al., 2016). The front is relatively narrow and its location constrained largely by topography, remaining relatively constant through time. East of approximately 32° E the Polar Front splits into a southern and a northern front, the locations of which are typically more variable than the front west of 32°E (Oziel et al., 2016), though Barton et al. (2018) noted that the location of the northern front is also well constrained by topography. As Atlantic Water cools and mixes with other water masses in the Barents Sea, it becomes denser and sinks, forming Barents Sea Water (Oziel et al., 2016) which can then penetrate under Arctic Water north of the Polar Front (Lind et al., 2012). Atlantic Water entering the Barents Sea from the south can supply high abundances of biota, including zooplankton, onto the Arctic shelf (Wassmann et al., 2015; Hunt et al., 2016), potentially including planktonic life stages of more southern benthic species. Arctic inflow shelves such as the Barents Sea are thus likely to increasingly receive boreal species extending their range into the Arctic as the climate continues to warm (Renaud et al., 2015). At this time, we do not know which species have the capacity to reach the Arctic during their larval stage.

Benthic invertebrate early life stages can be very difficult, and sometimes even impossible, to identify to species or genus using morphological characteristics alone. Consequently, most zooplankton studies either group taxa into an all-encompassing “meroplankton” category or identify them to broad taxonomic resolution such as phylum or class levels (e.g., Hirche et al., 2011; Gluchowska et al., 2016). Even in studies that focus specifically on meroplankton, species-level identification is scarce due to the common lack of distinguishing features across closely-related taxa (e.g., Bowden et al., 2009; Highfield et al., 2010). The use of DNA barcoding to identify meroplankton, while promising, initially achieved relatively low success rates (Heimeier et al., 2010). However, recent advances in methods specifically designed to barcode meroplankton have enabled the identification of hundreds of individuals, and dozens of taxa, with high success rate (Ershova et al., 2019).

Such species-level identification opens up possibilities to obtain detailed community composition and its variability in time and space. In addition, species-level identification can contribute important information on larval biological traits such as timing of larval release as well as larval duration for a large number of species simultaneously. Historically, much of the information on larval duration of individual species relied on laboratory rearing of larvae (Shanks, 2009). As such, the information was limited to a subset of species, each experiment studying a single or a few species at once, and could have potentially been biased by laboratory conditions (Shanks, 2009). Finally, species-level identification also has the potential to detect non-native species as they drift into the Arctic through water currents, perhaps serving as an early warning system for harmful alien species, or species of potential ecosystem impact.

The objective of this study was to determine the taxonomic composition and seasonality of the meroplankton community in the Barents Sea, north and south of the Polar Front. By using a high-throughput DNA barcoding method to identify early life stages, we aimed to achieve high taxonomic resolution yielding species-specific information on seasonality, larval duration, and





association with water masses, as well as identifying greater taxon richness than previously realized, including identification of potential newcomers. We hypothesized that seasonality differs across taxa but that most species peak in abundance around the spring phytoplankton bloom and remain in the water column for up to a few months. We also predicted that the meroplankton community differs in composition between north and south of the Polar Front, in association with different water masses, and that the larval assemblage represents a mix of larvae from local Barents Sea species as well as some more southern species advected into the Barents Sea. Implications of these findings in the context of invasibility of the Arctic shelves are discussed.

## MATERIALS AND METHODS

### Field Sampling

Meroplankton samples were collected at stations north and south of the Polar Front during a series of seasonal cruises in November 2017 and in January, April, June and August 2018 (Table 1 and Figure 1). Due to cruise logistics, no samples were collected south

of the Polar Front in November 2017. In addition, extensive sea ice blocked northward travel in April, so sampling north of the Polar Front occurred at slightly lower latitude in April compared to the other time points. At each station and sampling month, a CTD cast (Seabird SBE 911 or SBE 911+) preceded zooplankton sampling to determine the depth distribution of water masses based on the vertical profiles of temperature, salinity and density (Table 1). Temperature, salinity, and fluorescence measurements (as a proxy for chlorophyll *a* concentration) data were obtained from the CTD casts for further analysis. Zooplankton samples were collected from each water layer individually using a 64- $\mu\text{m}$  closing WP2 (0.255 m<sup>2</sup> mouth opening, Hydro-Bios) or Multinet (0.25 m<sup>2</sup> mouth opening, Hydro-Bios) towed vertically at 0.5 m/s, retrieving two to four samples per station and covering the entire water column from ~10 m above the seafloor to the surface. In April, when phytoplankton was highly abundant, 180  $\mu\text{m}$  mesh was used instead of 64  $\mu\text{m}$  to prevent clogging. The 180  $\mu\text{m}$  mesh was also used to sample the surface layer in June at the northern location after the upper portion of the 64  $\mu\text{m}$  mesh ripped open during sampling of lower layers, leaving only the outer 300  $\mu\text{m}$  mesh toward the net opening (see Table 1 for further details). Zooplankton samples were concentrated on a 64  $\mu\text{m}$  sieve and

**TABLE 1** | Sampling stations and gears for collection of meroplankton north and south of the Barents Sea Polar Front from November 2017 to August 2018.

	Date	Latitude (DD.DDD° N)	Longitude (DD.DDD° E)	Bottom depth (m)	Net, mesh size (μm)	Depth layer (m)	Water mass	% Sample quantified	No of ind. barcoded
North Polar Front	23 Nov., 2017	77.444	27.415	170	WP2, 64	160–100	ArW	5.7	56
						100–50	ArW	3.0	65
						50–0	SW	4.2	88
	10 Jan., 2018	77.470	29.970	202	WP2, 64	191–130	ArW/BSW	15.6	29
						130–80	ArW	13.8	40
						80–0	ArW	8.8	48
	29 Apr., 2018*	76.491	28.366	150	Multinet, 180	140–120	BSW	10.9	32
						120–80	BSW	11.3	40
						80–40	ArW/BSW	4.0	64
	27 Jun., 2018	77.531	29.979	205	WP2, 64 and 180**	40–1	ArW	4.0	72
						195–130	ArW	23.5	8
						130–18	ArW	12.7	24
11 Aug., 2018	77.634	31.689	164	Multinet, 64	15–0	SW	8.5	27	
					145–140	BSW	13.2	3	
					140–110	BSW	8.5	16	
					110–30	ArW/SF	3.8	48	
South Polar Front	Nov, 2017	75.558	29.320	335	WP2, 64	30–0	SW	6.7	21
						320–250	BSW	17.1	30
	12 Jan., 2018	75.558	29.320	335	WP2, 64	250–200	BSW/AW	13.2	22
						200–0	AW	8.8	88
						305–200	BSW	11.0	48
	30 Apr., 2018	75.940	29.966	315	Multinet, 180	200–40	BSW	10.9	77
						40–0	BSW	9.9	96
						345–45	AW/BSW	2.0	88
	02 Jul., 2018	75.500	30.000	360	WP2, 64	45–0	AW	0.8	193
						310–30	AW/BSW	1.1	248
09 Aug., 2018	76.000	31.223	320	Multinet, 64	30–0	AW	1.4	160	

\*In April 2018, the extensive sea ice cover prevented sampling from the same latitude as in other months for the location north of the Polar Front.

\*\*In June 2018, at the north location sampling 18–130 m layer, the inner mesh of the zooplankton net ripped open near the top opening, leaving only the outer 300 μm mesh intact. The net was replaced by the 180 μm net for the 15–0 m layer. It is unclear whether the net was already ripped during sampling of the 185–130 m layer.

Water masses are abbreviated as follows: AW, Atlantic water; ArW, Arctic water; BSW, Barents Sea water; SW, surface water.

immediately transferred into 96% ethanol for preservation. The ethanol was changed after ~24 h to maintain optimal conditions for DNA preservation.

## Sorting and Quantification

In the laboratory, a quantitative subsample from each zooplankton sample was sorted under a Leica M205C dissecting microscope at 20–100 × magnification to quantify and isolate individual meroplankton specimens for barcoding. Subsamples of 0.8–23.5% of total sample volume (Table 1), depending on meroplankton density, were collected using a 5-mL pipette with the tip cut off to prevent clogging from large zooplankton. All meroplanktonic individuals in the subsample were visually classified into morphotypes largely corresponding to broad taxonomic levels (e.g., Bivalvia, Nudibranchia, Ophiuroidea, etc.) and counted. For each morphotype, the number of individuals counted in the subsample was multiplied by the subsampling factor to estimate the numbers in the whole sample. Abundance values (individuals/m<sup>3</sup>) of each morphotype in each sample were then calculated by dividing the number of larvae by the volume of seawater filtered for each sample. For each sample, up to ~30 individuals from each morphotype were photographed (for reference and for measurements of body size) using a Leica MC170 HD microscope camera, then soaked in MilliQ water prior to DNA extraction.

## High-Throughput DNA Barcoding

Each photographed individual was then transferred individually into 20 µL Alkaline Lysis Reagent (25 mM NaOH, 0.2 mM EDTA) using sterilized tools, and heated at 95°C for 30 min to extract the DNA. Bivalve and gastropod larvae were crushed with the flat blade of a micro-scalpel prior to extraction. The DNA extracts were then pH-neutralized by adding 20 µL Neutralization Buffer (40 mM Tris-HCl) and kept at 4°C until amplification. This extraction method (HotShot extraction, Truett et al., 2000; Meissner et al., 2013) is ideal for very small organisms like meroplankton because it minimizes DNA loss by eliminating all cleaning and transfer steps.

We used a high-throughput multiplexing approach (after Ershova et al., 2019) to DNA-barcode a large number of larvae (Shokralla et al., 2015). By using a double-tagging strategy, we were able to sequence ~1,000 individuals simultaneously. First, a ~313 base pair fragment of the mitochondrial cytochrome C oxidase I (COI) gene was amplified by PCR using individually-tagged Leray-XT primers, a highly degenerate primer set (Geller et al., 2013; Wangensteen et al., 2018). The availability of reference sequences as well as its ability to assign taxonomy to species level (Hebert et al., 2003; Andújar et al., 2018) make this marker ideal for meroplankton identification. Each PCR reaction consisted of 10 µL polymerase (AccuStart II PCR ToughMix or AmpliTaq Gold), 0.16 µL bovine serum albumin 20 µg/µL, 5.84 µL nuclease-free water, 1 µL individually-tagged forward primer (5 µM, mlCOIintF-XT 5'-GGWACWRGWTGRACWITITAYCCYCC-3'), 1 µL individually-tagged reverse primer (5 µM, jgHCO2198 5'-TAIACYTCIGGRTGICCRARAAYCA-3') as well as 2 µL undiluted DNA template for a total reaction volume of 20 µL.

The PCR protocol consisted of a denaturation step for 10 min at 95°C followed by 35 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 1 min, and a final extension of 5 min at 72°C (Wangensteen et al., 2018). The same tag was used on the forward and reverse primers to enable detection of chimeras. A total of 96 different primer tags were available, so sets of 96 individually-tagged PCR amplicons were pooled into libraries. Libraries were cleaned (fragments below 70 base pairs removed) and concentrated using MinElute columns and final DNA concentration measured using a Qubit fluorometer with broad-range dsDNA BR Assay Kit (Qubit). Each library was then prepared for sequencing using the NEXTflex PCR-free DNA sequencing kit (BIOO Scientific), according to manufacturer instructions, omitting the second bead cleaning round in Step B to improve yield. This library preparation protocol includes ligation of Illumina adapters as well as a 6-base library tag (BIOO Scientific). Each pool of 96 samples received a different library tag so that DNA from each larva was recognizable through its unique combination of the primer tag and the library tag. Prior to sequencing, each library was quantified by qPCR using the NEBNext Library Quant Kit (New England Biolabs). Libraries were sequenced on an Illumina MiSeq platform with v2 or v3 2 × 250 bp kits and spiked with 1% PhiX used as an internal control to calculate error rates.

## Bioinformatics

All sequences obtained from the MiSeq runs were processed in the OBITools software suite (Boyer et al., 2016) as in Ershova et al. (2019). The forward and reverse reads were aligned and annotated for reads with alignment quality scores > 40 using the function `illuminapairedend`. Contigs were assigned to the corresponding larvae (demultiplexed) using `ngsfilter`, which also removed primer sequences. Only sequences with 300–320 base pairs and no ambiguous bases were retained for clustering in Swarm v2 (Mahé et al., 2015) with local clustering threshold  $d = 13$ . Singletons were discarded. Initial taxonomic assignment was performed with `Ecotag` (Boyer et al., 2016) against a local reference database (Wangensteen et al., 2018) and then checked manually in the Barcode of Life database (BOLD<sup>1</sup>) and NCBI database BLAST<sup>2</sup> for definitive assignment. Due to the nature of high-throughput sequencing, most larval samples contained reads from several taxa (possibly food items, contamination, etc.). The sequence with the most abundant reads within each sample was assumed to correspond to the larva itself. All samples with either less than 10 reads total or with fewer than 1,000 reads and, at the same time, less than 75% of reads assigned to the most abundant sequence were considered unreliable and were not included in further analysis. We then compared the taxonomic assignments from DNA barcoding to the initial morphological identification. Individuals, for which the two did not match at even a coarse taxonomic level, as well as non-meroplanktonic taxa, were omitted from subsequent analysis. The remaining sequences were considered successful. A sequence match of >98% to the reference database was considered an “exact” match

<sup>1</sup><http://boldsystems.org>

<sup>2</sup><https://blast.ncbi.nlm.nih.gov/Blast.cgi>

(Leray et al., 2016). For the larvae with <98% sequence match, the taxon name of the closest match was retained, but the % match was listed alongside for clarity. Accepted taxonomic names and classification were obtained from the World Register of Marine Species (WoRMS<sup>3</sup>).

## Larval Body Size Measurements

For species that were detected in the samples at multiple time points, body size was measured to help differentiate between species with continuous reproduction (average body size relatively constant throughout the year) from species with more discrete reproductive timing but long larval duration (average body size increases through time). For the purpose of this analysis, temperature effects were not considered. Body size measurements were made using the photographs of each individual obtained prior to DNA extraction. Measurements were conducted in ImageJ software (Abramoff et al., 2004) with the Straight Line tool (Segmented Line when needed), using the scale bar on each photograph to calibrate the measurements. In echinoderm pluteus larvae, the length of the postero-dorsal arm was used while in bivalve veliger larvae, the anterior-posterior length was measured (Shanks, 2001).

## Data Analysis

Temperature and salinity data from the station CTD casts were manually de-spiked and binned by 1 m (or 1 db). Salinity data were calibrated to Autosal salinities when available (June–July and August 2018). Each sample from a given water layer was assigned to a water mass based on its mean salinity (S) and temperature (T) values: Atlantic Water (AW:  $S > 34.8$ ,  $T > 3^{\circ}\text{C}$ ), Arctic Water (ArW:  $S < 34.7$ ,  $T < 0^{\circ}\text{C}$ ) and Barents Sea Water (BSW:  $S > 34.8$ ,  $T \leq 2^{\circ}\text{C}$ , Oziel et al., 2016). Where mean temperature and salinity values fell between the defined cores of two water masses, the water was considered to be a mixing product of the two. When the surface layer did not fit in the standard water mass definitions but was much fresher than underlying layers, it was called Surface Water (SW).

Density values for each species detected by DNA barcoding were obtained by multiplying the number of each morphotype in each sample by the percentage of the barcoded larvae corresponding to each sequence-based identification. For example, if a sample contained 50 ophioplutei/m<sup>3</sup> and from that sample 10% of all barcoded ophioplutei matched with *Ophiura sarsii*, then we estimated the density of *O. sarsii* in that sample to be 5 individuals/m<sup>3</sup>. We then multiplied the density in each sample by the depth range of that same sample and added all depth layers within one location to obtain a depth-integrated abundance value expressed in individuals/m<sup>2</sup>. Larvae that were not successfully identified through DNA barcoding were not included in subsequent analysis so listed abundances represent a conservative estimate of total abundances. Meroplankton diversity and community analyses were conducted with package *vegan* in RStudio, v. 1.4.1106 (Oksanen et al., 2019) and visualized using package *ggplot2* (Wickham, 2016). A species accumulation curve (function *specaccum*) was used to determine

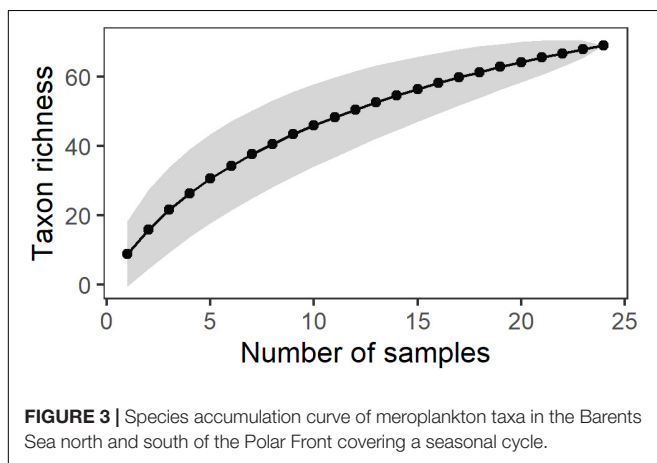
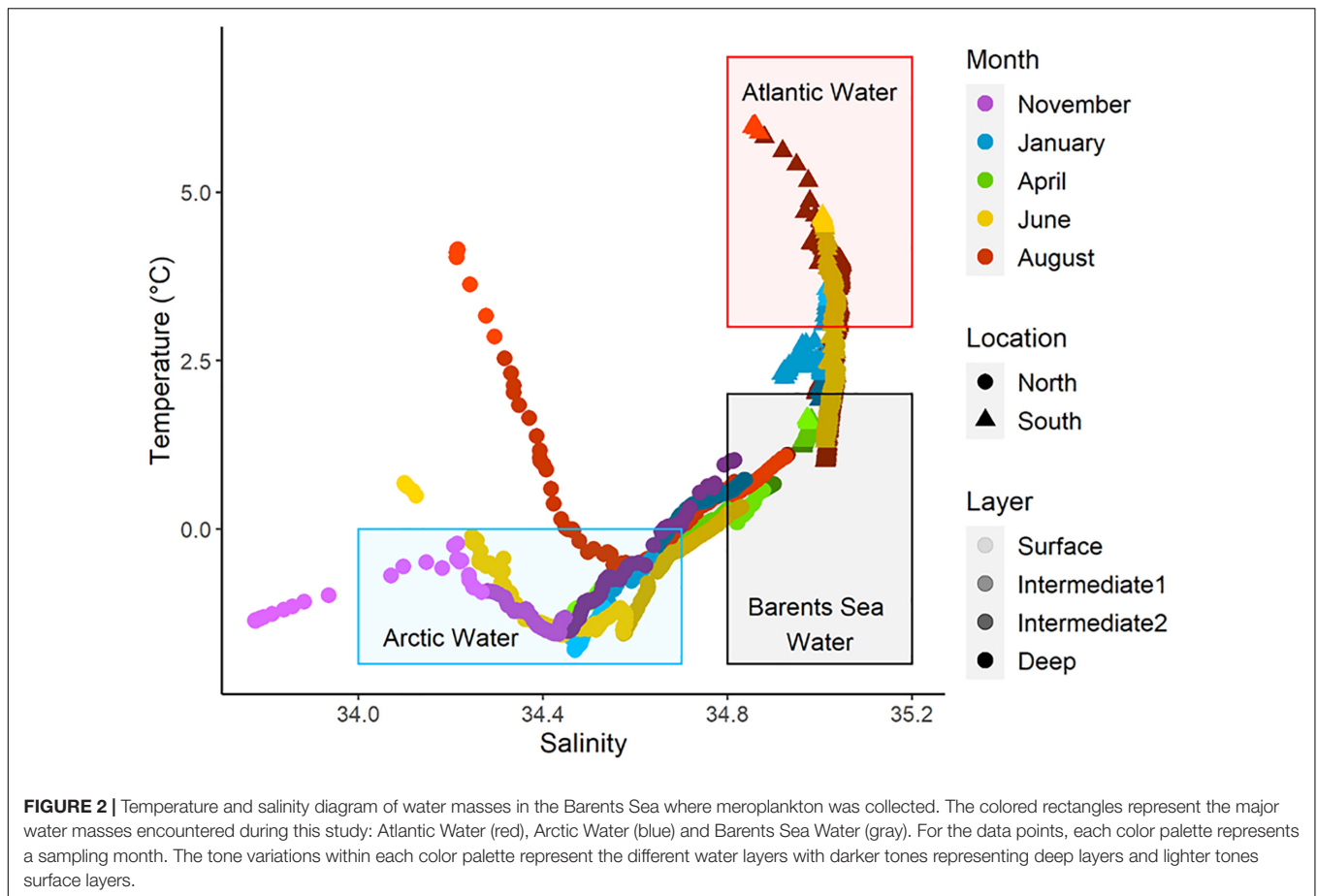
whether our sampling covered the full diversity of the region. A heatmap of species abundances was created to visualize seasonal patterns. Meroplanktonic taxa in the heatmap were clustered according to the seasonal and geographical distribution using the UPGMA (unweighted pair group method with arithmetic mean) clustering method. To test the difference in meroplankton communities across months and locations, we did separate analyses of “size” (total abundance) and “shape” (community composition, Greenacre, 2017). The difference in total abundance across locations and months was tested using Kruskal–Wallis tests performed separately for location and month. The unequal variance across locations and months prevented the use of an ANOVA. The difference in taxon richness across location and month, however, was tested using a two-way ANOVA without an interaction term. Spatial and seasonal patterns in community structure were explored using Canonical Correspondence Analysis (CCA) of the fourth-root transformed taxa abundances. Explanatory variables included water mass type (Atlantic, Arctic, Barents Sea and Surface Waters; Table 1), location (north vs. south of the Polar Front), sampling month (November, January, April, June, and August), as well as average water column temperature, salinity, and fluorescence (as a proxy for chlorophyll *a* concentrations). The best subset of constraining variables was selected by stepwise selection using the *ordistep* function. The significance of the overall model and individual terms was calculated using permutation tests (*anova.cca* function) at a significance level of  $p < 0.05$ . The correlations of the individual species abundances to the resulting ordination were obtained using the function *envfit*, and only species that were found to be significantly driving the ordination ( $p < 0.05$ ) are shown on the biplots.

## RESULTS

### Oceanography

Water mass characteristics contrasted sharply between the north and south of the Polar Front during all seasons (Figure 2 and Table 1). North of the Polar Front, deep waters consisted of the relatively cold and saline Barents Sea Water throughout the year while shallower layers consisted mostly of Arctic Water. The characteristics of the surface layer fluctuated seasonally. A colder and fresher water layer overlaid the Arctic Water in November, but was not present in January. Surface temperatures began to increase again in June and reached upward of 4°C by August. While sampling north of the Polar Front in April occurred further south than in the other months (Figure 1), the water mass characteristics showed a clear similarity to the more northern sampling location of other months, remaining well within characteristics of Arctic Water at the surface (Figure 2). The deep waters south of the Polar Front also consisted mostly of Barents Sea Water, except in January where they were typically 1–2°C warmer. Upper layers in the south consisted entirely of Atlantic Water, except in April when the whole water column was well mixed with characteristics of the Barents Sea Water mass. Together, these observations suggest that our two sampling locations were positioned on either side of the

<sup>3</sup>www.marinespecies.org/



oceanographic Polar Front, but shared water mass characteristics in deeper layers.

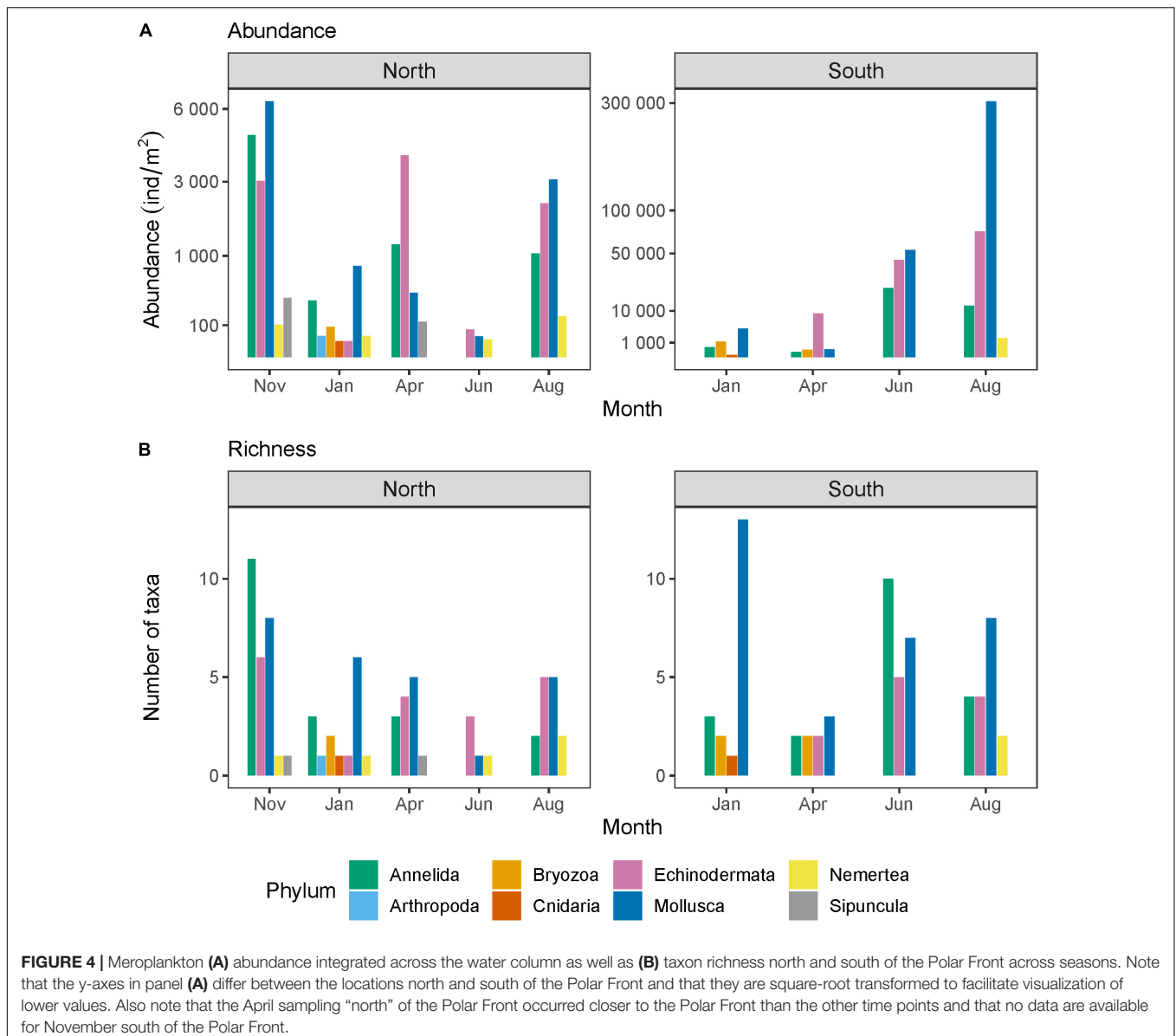
### Success Rate of DNA Barcoding

We had good quality amplification of the DNA extracts, even without the cleaning steps found in most extraction methods, perhaps due to the small size of the samples. A total of 1,672 individuals was barcoded, with 1,192 (71%) resulting in successful

DNA-based identification (**Supplementary Data Sheet 1**). The success rate of DNA barcoding was very high for the majority of taxa: Echinodermata (94%), Bivalvia (93%), Bryozoa (95%), and Nemertea (93%). Annelida had 87% success and the unsuccessful 13% were caused by insufficient reads (6%), a sequence with no match in the DNA databases (3%) or by a DNA-based identification which did not match the visual identification (4%). Gastropoda had the lowest success rate (59%), most often due to the insufficient number of DNA reads in the sequencing process (35%). Approximately 86% of MOTUs (molecular operational taxonomic units) had an “exact” match (>98%) in BOLD or GenBank (**Supplementary Table 1**). Of those “exact” matches, 87% of unique sequences were identified to species, 8% to genus, 3% to family, and 2% to order level.

### Meroplankton – Overall Diversity

Swarm 2.0 clustered the barcoded individuals into 86 different meroplankton MOTUs, assigned to 72 taxa, belonging to eight phyla (**Supplementary Table 1**). Taxon richness ranged from 5 to 27 taxa per sampling event (integrated over the whole water column). The species accumulation curve (**Figure 3**) did not reach an asymptote, indicating that we did not capture the full diversity of the system. Mollusca, Echinodermata, and Annelida dominated the meroplankton community in terms of taxon richness, but we also found some Nemertea, Bryozoa,



Sipuncula, Cnidaria, and Arthropoda (Supplementary Table 1 and Figure 4).

### Meroplankton Seasonality – Abundance

Meroplankton abundance fluctuated seasonally but patterns varied across taxa. Meroplankton was present in the water column year-round but, contrary to our initial hypothesis, total abundance did not peak around the spring bloom (Figure 4). In fact, for the location north of the Polar Front, abundance was at its lowest in June, with values around 150 individuals/m<sup>2</sup>. Abundance increased in August and peaked in November when it was estimated at 14,671 individuals/m<sup>2</sup>. Abundance was low in January (1,369 individuals/m<sup>2</sup>) and reached a secondary peak in April reaching about half of November values (5,736 individuals/m<sup>2</sup>). At the location south of the Polar Front, overall abundance was at its lowest in January

(3,149 individuals/m<sup>2</sup>) and gradually increased through the year, reaching its highest value in August (391,137 individuals/m<sup>2</sup>, no samples were taken in November). Total meroplankton abundance also differed on either side of the Polar Front with overall much greater abundance to the south (3,149–391,137 individuals/m<sup>2</sup>) compared to the north (150–14,671 individuals/m<sup>2</sup>), though this difference was not statistically significant ( $p = 0.206$ ), perhaps due to the low power of the test.

These overall seasonal patterns were in large part driven by molluscs (mostly bivalves) which dominated abundance in most months, except for April when echinoderms made up most of the larval abundance, both north and south of the Polar Front (Figure 4A). Echinoderms and annelids were also abundant and followed a similar seasonal pattern to bivalves, except that annelids peaked in June at the location south of the Polar Front and echinoderms peaked in April at the location north of the

Polar Front. Gastropod larvae peaked in the winter months, in November to the north and in January to the south of the Polar Front (Figure 5). Nemerteans were present all months except April north of the Polar Front but only in August to the south. Sipunculids were only found north of the Polar Front, in November and April. Bryozoans, cnidarians, and arthropods were mostly present in January but only in low numbers.

## Meroplankton Seasonality – Taxon Richness

Taxon richness was significantly greater to the north ( $p = 0.002$ ). Out of the 72 taxa identified, 27 taxa were shared between the two locations whereas 20 were only found to the south and 25 only found to the north of the Polar Front (Figure 6 and Supplementary Table 1). Taxon richness also changed significantly through time ( $p = 0.013$ ). North of the Polar Front, species richness was highest in November (27 taxa), thereafter decreasing gradually until reaching its minimum in June of the following year (five taxa, Figure 4B). The pattern appeared more erratic to the south, with overall high taxon richness throughout the year (>18 taxa each month) except in April when richness was lower (9 taxa).

Taxon richness was dominated by annelids with 28 taxa represented, all of which polychaete worms (Supplementary Table 1). During most months, only 2–4 polychaete taxa were found at each location, but a large rise in diversity occurred in November in the north and in June in the south when 11 and 10 taxa were identified, respectively. As with abundance, molluscs made up a large proportion of species richness at both locations at most sampling events. While bivalves made up the bulk of the mollusc abundance, however, gastropods were much more diverse with 18 different taxa identified (compared to seven bivalve taxa, Supplementary Table 1), almost all of which were nudibranchs. The bivalve *Hiatella* sp. K made up a large proportion of Mollusca abundance in all months, except in April when it was not observed (Figure 5A). Only one sea urchin larva (*Strongylocentrotus pallidus*), one sea star (*Ctenodiscus crispatus*), and one sea cucumber (*Molpadia borealis*) were found so that brittle star larvae made up the bulk of echinoderm abundance and diversity (11 species total, Figure 5C). The brittle star *Ophiocten gracilis* was extremely abundant and made up >80% of echinoderm abundance during all months when ophiuroids were found south of the Polar Front. It was also present to the north, but its relative contribution to the echinoderm abundance was lower there.

## Larval Duration

Most taxa were present at a single time point, but a few species were found during most of the year (Figures 5, 6). The bivalve *Hiatella* sp. K showed no distinct trend in size through time, suggesting continuous reproduction (Figure 7). The size-frequency histogram for the brittle star *O. gracilis*, on the other hand, showed a gradual increase in size through time from April to August, suggestive of a long-lived planktonic phase originating from a discrete spawning event. The bivalve *Mya truncata*, and the ophiuroids *Ophiopholis aculeata* and *Ophiocten sericeum* were

also present in the water column throughout most of the year (Figures 5, 6), but we had insufficient numbers to generate informative size histograms.

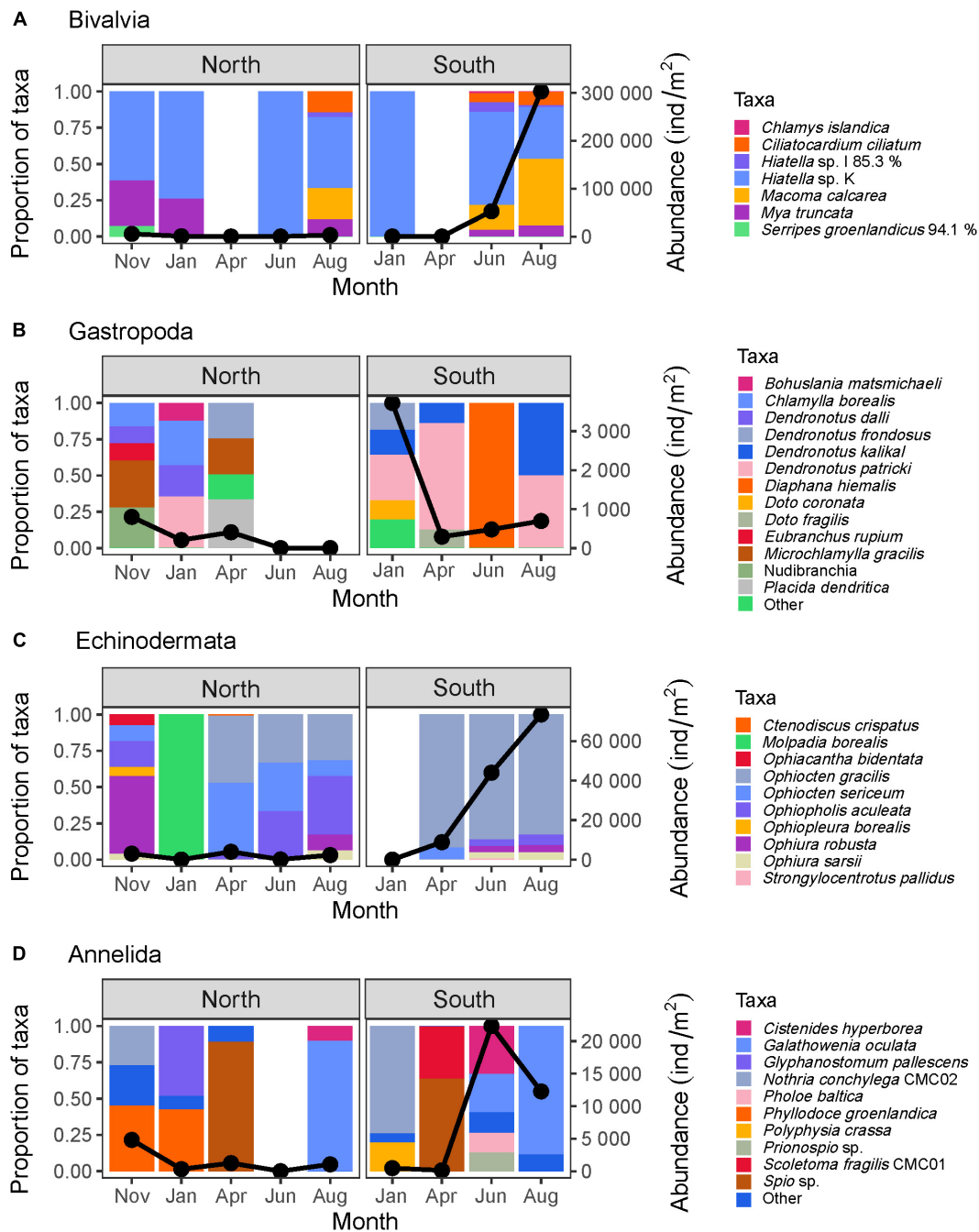
## Meroplankton Community Patterns

The final CCA model was significant ( $p < 0.01$ ) and included only sampling month as a constraining factor, suggesting that seasonal differences overpowered any variation between locations or water masses (Supplementary Table 2). No strong differences between North/South locations were observed on the ordination, though visually, there seemed to be a North/South difference within months in January and June (Figure 8). The CCA explained 34.2% of total inertia in the data, with the first two axes accounting for 22%. The samples collected in November, January, and April each formed their own distinct groups on the ordination, while the communities in June and August were very similar in species composition (Figure 8). In November, the community was characterized by the polychaetes *Sphaerodorum flavum*, *Nothria conchylega*, and *Phyllodoce groenlandica* as well as Nudibranchia indet, the ophiuroid *Ophiacantha bidentata* and the bivalve *Serripes groenlandicus* 94.1%. The community in January was almost entirely characterized by nudibranchs. Nudibranchs were still important in April in addition to the seastar *C. crispatus* and the polychaetes *Spio* sp. and *Lumbrineris* sp. During June/August the bivalves *Macoma calcarea*, *Ciliatocardium ciliatum*, *Mya truncata*, and *Hiatella* sp. K as well as the ophiuroids *Ophiura robusta*, *Ophiopholis aculeata*, and *O. gracilis* and the polychaete *Galathowenia oculata* characterized the meroplankton community.

## DISCUSSION

### Success of DNA Barcoding

We implemented a simple, relatively cheap and highly effective method (Ershova et al., 2019) to efficiently sequence hundreds of DNA-barcodes of meroplankton individuals from eight phyla. The HotShot extraction method (Truett et al., 2000) has proven to be extremely effective in extracting DNA from meroplankton, yielding higher success rates (71% overall but >93% for Echinodermata, Bivalvia, Nemertea, and Bryozoa) than earlier studies using different extraction methods [35% in Heimeier et al. (2010); 49% in Brandner et al. (2017); 68% in Walczynska et al. (2019)]. The benefits of the HotShot extraction method include that no membrane binding of DNA and no transfer of extract are needed, and it can be scaled to high-throughput (Meissner et al., 2013). Our lower success rate for DNA barcoding of gastropods compared to the other taxa may be due to incomplete crushing of the shell or the fact that most gastropod larvae were very small (~100  $\mu\text{m}$ ) and may have provided insufficient material for DNA extraction. In addition, the majority of these were suspected to be young *Limacina helicina* (a pelagic gastropod, not meroplankton) based on the morphological resemblance to successfully-barcoded individuals of the same species, and these can be especially difficult to DNA-barcode (Elizaveta Ershova, IMR, personal communication). Therefore, the success rate



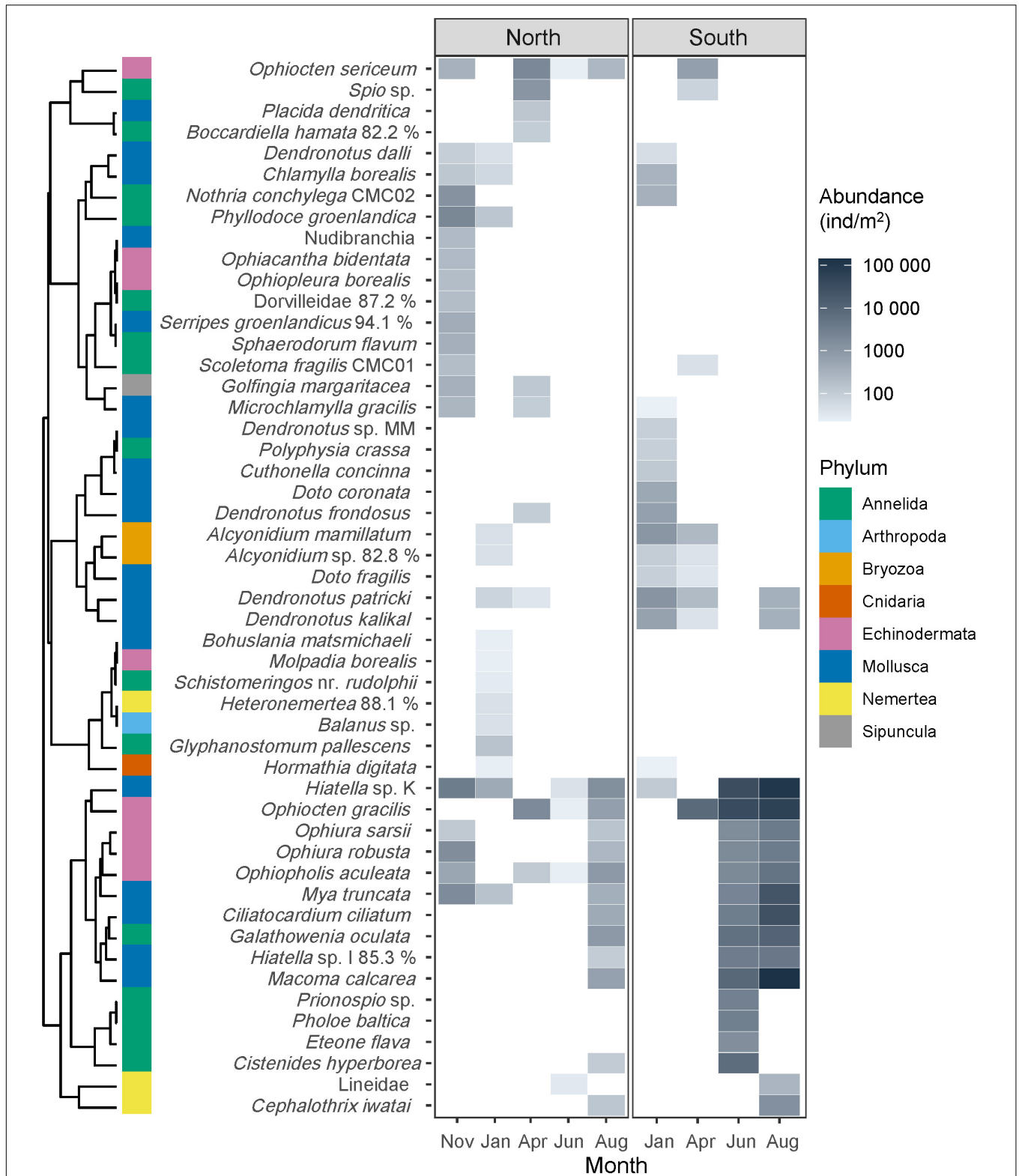
**FIGURE 5** | Composition of the four most abundant meroplanktonic higher taxa: **(A)** Bivalvia, **(B)** Gastropoda, **(C)** Echinodermata, and **(D)** Annelida expressed as proportional abundance (colored bars, left y-axis) as well as total abundance (black line, right y-axis) for the locations north and south of the Polar Front. For Gastropoda and Annelida, taxa that did not make up at least 10% of the group's abundance for at least one sampling point were combined in a "Other" category for ease of reading. Some taxa are named with letters or numbers instead of a species name (e.g., *Hiatella* sp. K and *N. conchylega* CMC02). These represent currently un-named but genetically-distinct lineages.

of DNA barcoding of strictly-meroplanktonic gastropods was probably much higher than reported here.

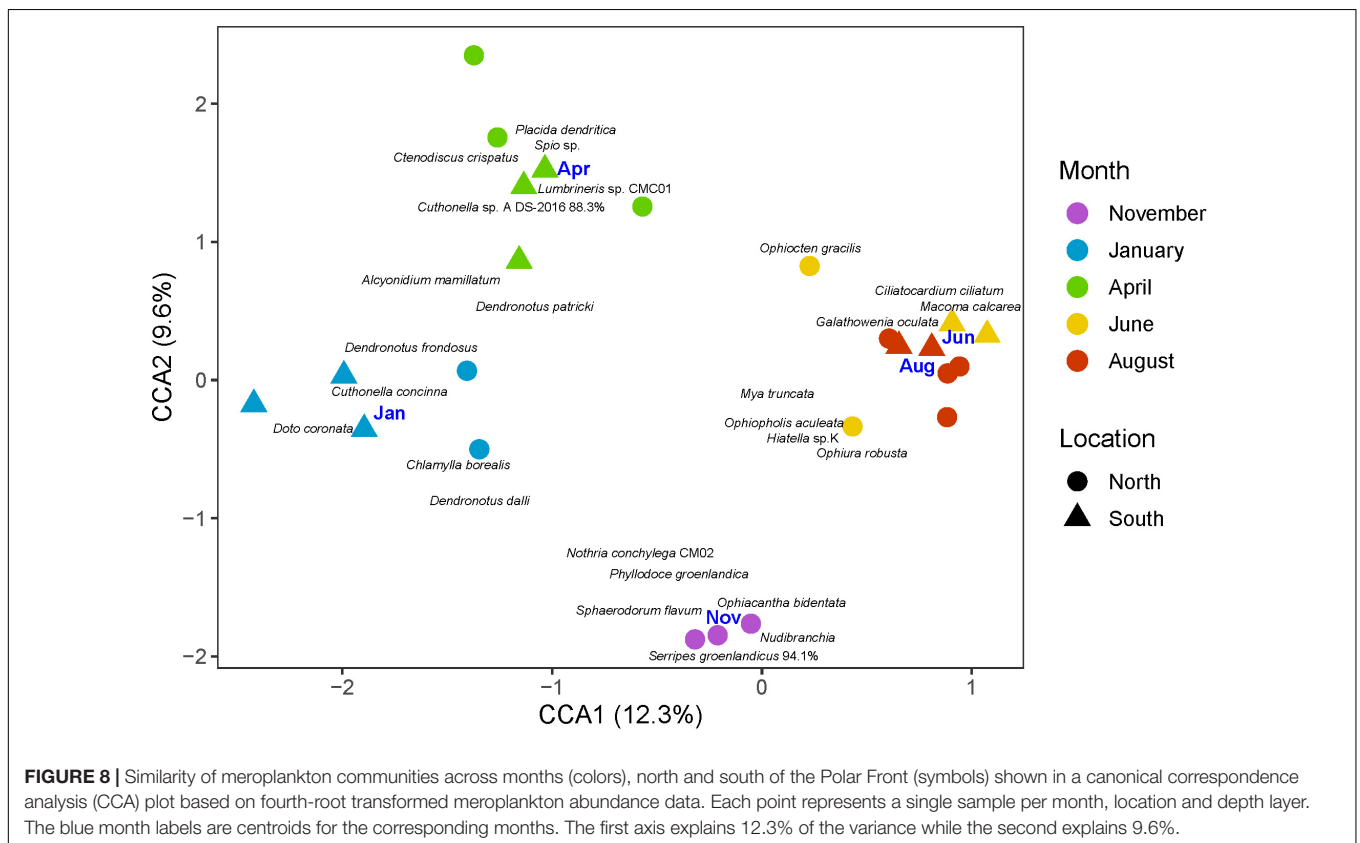
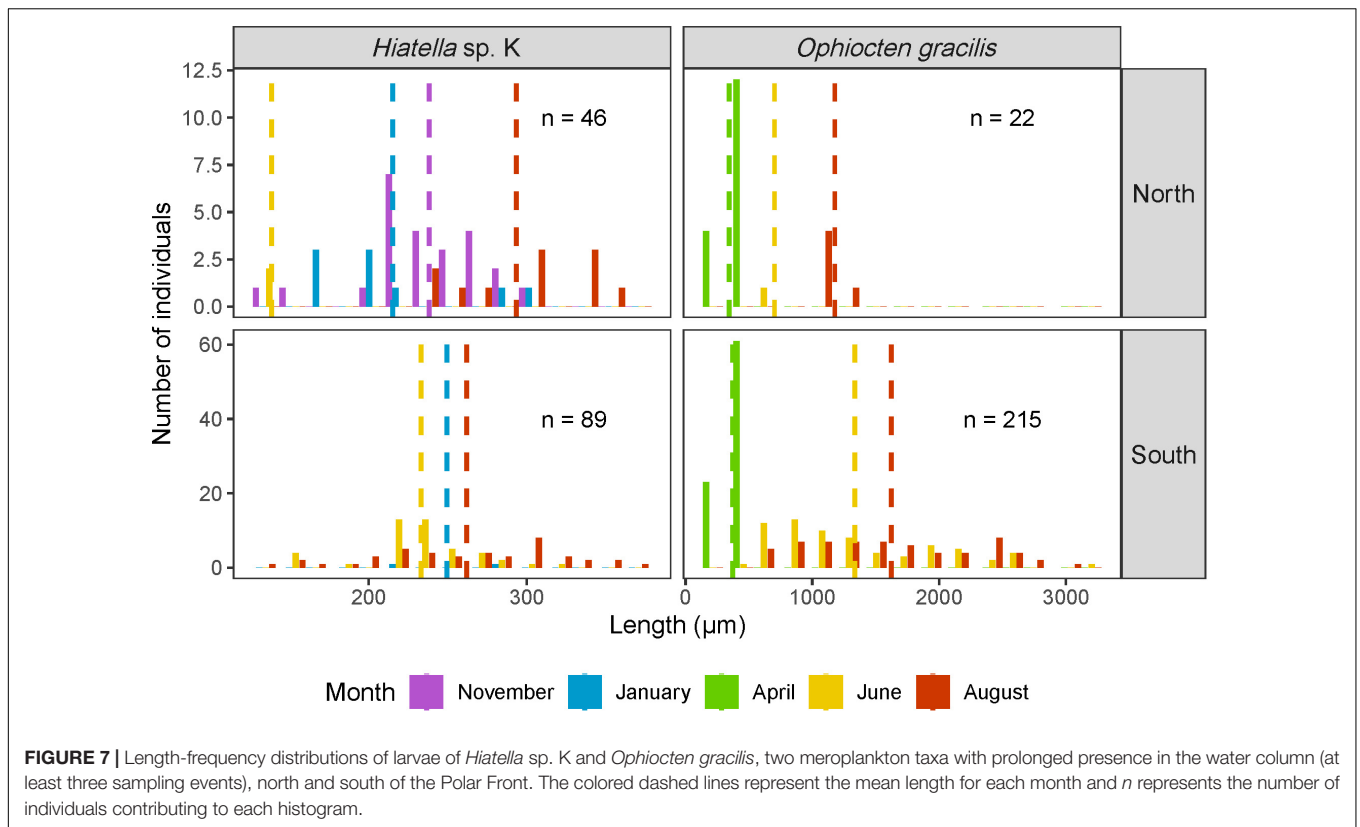
In addition to effective DNA extraction, amplification, and sequencing, successful identification of larvae through DNA barcoding requires a curated barcode database such

as BOLD against which to compare the larval sequences (Hebert et al., 2003). Important efforts in recent years (e.g., Layton et al., 2014, 2016) have added many additional species to the databases, but marine invertebrates in general remain under-represented (Weigand et al., 2019). In our study, 14% of all





**FIGURE 6 |** Heatmap of meroplankton species abundance integrated over the entire water column (individuals/m<sup>2</sup>) north and south of the Polar Front. For readability, only taxa that contributed at least 1% of total abundance of at least one sampling event are included (for all taxa, see **Supplementary Table 1**). Taxa are clustered according to their seasonal and geographical distribution with colors indicating phylum. Note that no samples were taken south of the Polar Front in November 2017.



sequences did not find an “exact” match (>98% sequence match) in BOLD or GenBank. Of the sequences that did get a match >98%, about a tenth was not identified to species level in the barcode database itself. Therefore, the addition of more boreal-Arctic and Arctic species to the DNA barcode databases as well as taxonomic expertise for proper identification of these organisms would further improve our ability to identify meroplankton. Our very high success rate of meroplankton identification clearly demonstrates, however, that DNA barcoding of the COI gene marker is already a highly effective way of identifying meroplankton and will continue to improve as barcode databases become more complete.

## Meroplankton Biodiversity

DNA barcoding enabled identification of meroplankton at a much higher taxonomic resolution than was achieved through morphological identification. We therefore captured a greater taxon richness than reported in most other seasonal high-latitude studies using morphological identification (Schlüter and Rachor, 2001; Fetzer and Arntz, 2008; Kuklinski et al., 2013; Silberberger et al., 2016; Michelsen et al., 2017). Bowden et al. (2009) found an even higher diversity of meroplankton taxa in the Antarctic, but they sampled more frequently throughout the year and had more stations compared to the present study, possibly capturing a larger proportion of the overall meroplankton community.

In our study, molluscs, echinoderms, and annelids dominated the meroplankton community in terms of taxon richness. While annelids and echinoderms are often amongst the most diverse groups in high-latitude meroplankton assemblages (Schlüter and Rachor, 2001; Michelsen et al., 2017), to our knowledge our study is the first to identify such a diverse larval nudibranch fauna. Echinoderms, molluscs, and annelids also dominated the meroplankton community in abundance, similarly to other studies in high-latitude regions (Schlüter and Rachor, 2001; Sewell and Jury, 2011). These phyla are also abundant and taxon-rich on the seafloor in the Barents Sea (Carroll et al., 2008; Cochrane et al., 2009; Jørgensen et al., 2015). Other taxa that are abundant on the seafloor in our study region (Zakharov et al., 2020), however, were notably rare or absent in our samples. First, species without a pelagic early life stage or those which remain in the benthic boundary layer just above the seafloor would not have been captured by our sampling method. A majority of polar prosobranchs, for instance, rely on direct development (Clarke, 1992) explaining why so few non-nudibranch gastropods were caught here, despite being present on the seafloor (Jørgensen et al., 2015). Additionally, our seasonal sampling may have missed species with a short-lived planktonic stage of less than a few weeks (Shanks, 2009) such as the tunicate *Styela rustica* (Khalaman et al., 2008). Large and mobile taxa such as crab larvae may have been able to avoid the slow-moving fine-mesh nets used in this study (Porter et al., 2008) or been too rare to be captured in a single net tow. Finally, our offshore location may explain the relative paucity of coastally dominant cirripeds [but see Basedow et al. (2010)].

While the majority of taxa found in this study are known inhabitants of the Barents Sea (Sirenko et al., 2001), a few species have, to our knowledge, never been recorded there.

Notably, the nudibranch *Bohuslania matsmichaeli* (99.68% DNA match) is currently only known from one fjord in southern Norway (Korshunova et al., 2018). The presence of its larva in the Barents Sea suggests either that its propagules can disperse over very large distances or that the geographical range of the adults extends further north than currently known, although a third possibility of transport by ship ballast water cannot be excluded. Note, however, that our observation of this species here is only based on one barcoded larva. The brittle star *O. gracilis* (100% DNA match), the larvae of which were found in this study in very large numbers, has also not been recorded in the northern Barents Sea, but is commonly found on the shelf and continental slope along the coast of Norway [Sirenko et al., 2001; Global Biodiversity Information Facility (GBIF), 2019b]. While its primary distribution is along bathyal depths (Gage and Tyler, 1981), several adults have been collected from shallower locations [Global Biodiversity Information Facility (GBIF), 2019b] so the species' ability to settle and survive on the Barents Sea shelf in the future seems unlikely but possible. It is also possible that adults of *O. gracilis* are currently found on the northern Barents Sea shelf but have been confused for the closely-related *O. sericeum*. Finally, we barcoded several individuals of the nemertean *Cephalothrix iwatai* (99% DNA match), a deep-sea species previously only recorded in the Sea of Japan [Chernyshev, 2013; Global Biodiversity Information Facility (GBIF), 2019a]. It is, however, highly unlikely that the larvae have drifted into the Barents Sea from the Sea of Japan. Therefore, one can presume that *C. iwatai* is more widely distributed than previously thought or that our specimens belong to a closely-related species which has not yet been barcoded. So far, conditions on the Barents Sea shelf have likely prevented some of these and other more southern species from settling onto the seafloor and growing to a reproductive stage, at least not in sufficient numbers to grow a detectable population. However, climate change and Atlantification of the Barents Sea (Polyakov et al., 2017, 2020) may increase the flow of Atlantic Water onto the Barents Sea shelf (Oziel et al., 2020), potentially transporting more propagules north as well as making conditions more favorable for some non-native species to settle and grow there as has been suggested for other species (e.g., Berge et al., 2005; Geoffroy et al., 2018; Renaud et al., 2019).

## Seasonality

Larval abundance and composition varied sharply throughout the year. Contrary to our initial hypothesis, larval abundance peaked in August (south) and November (north) rather than in the spring when food would have presumably been most abundant for the larvae. There was, however, a secondary peak in larval abundance in April north of the Polar Front. In 2018 in our study area, the phytoplankton bloom peaked in early May, first north of the Polar Front, then, about 1 week later, to the south (Marie Porter, SAMS, personal communication). Thus, our April sampling captured the beginning and our June sampling captured the end of the bloom. A mooring study at 81°N on the northern coast of Svalbard similarly found the bloom to begin in early May (Henley et al., 2020). At first glance, this asynchrony of the bulk of the meroplanktonic community with

the peak in primary production appears to contrast with studies in Svalbard fjords where overall meroplankton abundance peaks in spring/summer around the phytoplankton bloom (Kuklinski et al., 2013; Stübner et al., 2016), as well as in fjords in mainland Norway (Michelsen et al., 2017), in Greenland (Arendt et al., 2013), in the Antarctic and sub-Antarctic (Bowden et al., 2009; Presta et al., 2019), and off of the coast of the United Kingdom (Highfield et al., 2010). However, seasonality differs across taxa and a peak in abundance around the phytoplankton bloom is often driven by a few highly abundant taxa such as cirriped larvae (Węśławski et al., 1988; Highfield et al., 2010), which were not captured in large numbers in our study. Indeed, larvae of molluscs, echinoderms, polychaetes, and other taxa are often found later in the year than cirripeds (Węśławski et al., 1988; Highfield et al., 2010), although generally not as late as the August/November peak found here. Consistent with our findings of a late summer/fall peak (August/November) in abundance, a sediment trap study in the Chukchi Sea showed a peak in meroplankton export to the seafloor in September/October (Lalande et al., 2020). Additionally, a seasonal study of meroplankton on the continental shelf off of mainland Norway showed a peak in total abundance in July to September (Silberberger et al., 2016), consistent with our peak in August and November.

A combination of factors may explain the mismatch between the meroplankton peak and the phytoplankton bloom. First, for some of the species we collected, the pelagic larva can rely on a maternally-derived yolk sac for nutrition so it does not need to eat while in the plankton (e.g., lecithotrophic larvae of *Capitella capitata*, Méndez et al., 2000). For these larvae, availability of food in the plankton is irrelevant so there would be no need to time larval release with the phytoplankton bloom, at least not for the sake of the larval stage survival itself. Most taxa that we collected, however, are planktotrophic and would presumably require food to survive while in the plankton (Arctic Traits Database<sup>4</sup>, Degen and Faulwetter, 2019). These feeding larvae may rely on different food sources (Boidron-Métairon, 1995) than the dominant diatom taxa such as *Thalassiosira* spp. and *Chaetoceros* spp. present during the spring bloom in the study region (Hegseth, 1998; Wassmann et al., 1999). A wide range of other planktonic organisms including dinoflagellates, cercozoans, and fungi feed copepods outside of bloom periods in the Arctic (Cleary et al., 2017) and may feed meroplankton as well. Bivalve larvae, for instance, can feed on ciliates or dinoflagellates (Lindeque et al., 2015), which are present year-round in an Arctic fjord (Marquardt et al., 2016). Metabolic rate decreases with decreasing temperature in polar invertebrates (e.g., Heilmayer and Brey, 2003) so meroplankton in cold high-latitude waters may require less food to survive. Given that larval occurrence of most species in our study did not coincide with the spring bloom, it may be the adults instead which take advantage of the abundant food available during the bloom to fuel reproduction (e.g., Witte, 1996; Renaud et al., 2007b). For these income breeders, there would be a time lag between peak food availability and peak larval

abundance (Kuklinski et al., 2013), just as we have observed in this study. In addition, some of the larvae may have been released around the spring bloom in a different location and taken several months to reach our study area. Finally, larvae released in the fall may benefit from the darker conditions to escape visual predators, though this theory remains to be tested.

Some potential biases in our sampling need evaluating. While the exact location of sampling differed slightly across months, especially for the location north of the Polar Front, we consider artifacts of this in the data minimal since the temperature and salinity profiles indicated clear differences between the north and the south of the Polar Front across all seasons. While the November sampling occurred closest to land which may partially explain the higher abundances at that time (Bluhm et al., 2018), August sampling, which also had very high meroplankton abundances, occurred furthest away from land. It is therefore likely that the August/November meroplankton peak remains valid. A secondary peak in abundance also occurred in April to the north of the Polar Front. This peak, largely driven by the ophiuroids *O. gracilis* and *O. sericeum* as well as the polychaete *Spio* sp., may represent a real surge in abundance just prior to the phytoplankton bloom (in May, Dalpadado et al., 2020; Dong et al., 2020). However, it could also be an artifact of the closer proximity to the Polar Front compared to other months, given that plankton often accumulate in patches around fronts (Trudnowska et al., 2016) and adult benthic invertebrates on the seafloor also occur in higher densities near the Polar Front (Carroll et al., 2008). However, *O. gracilis* does not occur on the northern Barents Sea shelf at all, so higher adult densities are unlikely to explain the April peak north of the Polar Front. Given that the zooplankton net mesh size was larger in April (180  $\mu\text{m}$ ) and, at the northern location, in June (180 and 64/300  $\mu\text{m}$ , see **Table 1** note for details), smaller larvae (e.g., earliest stages of *Hiatella arctica* measure around 120  $\mu\text{m}$ , Flyachinskaya and Lesin, 2006; Brandner et al., 2017) could have been under-sampled and abundances under-estimated compared to other months. Nonetheless, it is unlikely that an abundant species would have been missed entirely as some individuals would have presumably been caught in the net through aggregations of other plankton such as phytoplankton clusters. Finally, zooplankton distribution can be spatially patchy, with plankton concentrations sometimes varying by more than one order of magnitude over a distance of a few kilometers (e.g., Trudnowska et al., 2016) and meroplankton is no exception (Kersten et al., 2019). Taking multiple replicates of each net sample would help obtain a more robust estimate of species abundance and composition at a particular location. In this study, as is often the case during oceanographic surveys on shared research vessels, sampling time was limited, allowing for collection of only one replicate per sample. We recommend that future studies put a strong emphasis on replication of net samples.

The meroplankton community varied greatly throughout the year with most species present at one or two consecutive sampling events, suggesting a larval period of at most a few months for most species (assuming at least some local retention). However, some taxa (the ophiuroids *O. gracilis*, *O. sericeum*, and

<sup>4</sup><https://www.univie.ac.at/arctictraits/>

*O. aculeata*, the bivalves *Hiatella* sp. K and *M. truncata*, and the nudibranchs *Dendronotus patricki* and *Dendronotus kalikal* were present through most of the year. Our data suggest that the extended presence in the water column can be explained in some taxa by a long-lived pelagic phase [e.g., *O. gracilis*, also observed in Gage and Tyler (1981)]. On the continental slope off of the United Kingdom, *O. gracilis* spawns in late winter and early spring and settlement of the post-larvae to the seafloor occurs in summer (Tyler and Gage, 1982). Similarly, we observed a pulse of small larvae in April and on average larger larvae as well as several post-larvae in August, suggestive of a similarly long larval phase in the Barents Sea compared to the coast of the United Kingdom, even though we would have expected a longer larval phase in our region as colder waters tend to extend larval period (O'Connor et al., 2007). Contrary to *O. gracilis*, the average size of *Hiatella* sp. K showed no obvious pattern over time, suggesting continuous reproduction (or at least multiple reproductive events) throughout the year, which is consistent with prior observations (Garcia et al., 2003; Brandner et al., 2017). In addition, several species identified in our study, such as the polychaetes *Bylgides groenlandicus* and *N. conchylega* as well as several nudibranchs, were identified in the meroplankton but, to our knowledge, a pelagic life stage has never been explicitly recorded in these species (**Supplementary Image 1**). Data from this study therefore contributes valuable, although coarse, species-level information on reproductive strategy, timing, and duration.

## Role of the Polar Front

While seasonality drove the bulk of the differences in community composition among samples, there were some differences between the sampling locations north and south of the Polar Front as well. Specifically, about two thirds of the taxa were found only on one side of the front or the other and the taxon richness was significantly greater to the north, partially supporting our hypothesis of different meroplankton communities across the front. Note, however, that the difference in community structure was not statistically significant as per the multivariate analysis. The distinctiveness of zooplankton communities across fronts has been demonstrated in various systems worldwide (Perry et al., 1993; Coyle and Pinchuk, 2005; Powell et al., 2015), including in the Barents Sea where the Polar Front has been interpreted to act as a barrier to larval dispersal (e.g., Schlüter and Rachor, 2001). The benthic macrofaunal communities differ substantially across the Barents Sea Polar Front (Carroll et al., 2008), potentially due to different larval supplies or, vice versa, supplying larvae of different species on either side of the front. Indeed, in our study, early life stages of polychaetes (a predominantly macrofaunal group) differed greatly in species composition across the Polar Front. Of the 28 polychaete taxa identified in the meroplankton, only seven were shared between north and south, but with all phyla combined, about a third of the taxa were shared. This observation is congruent with the benthic megafaunal communities (including most echinoderms and some molluscs) on the seafloor below, which differ somewhat between the two locations but are overall more similar to each other compared to other regions of the Barents Sea (Jørgensen et al., 2015;

Zakharov et al., 2020). Importantly, while our two locations were positioned on either side of the Polar Front, with surface waters differing greatly in temperature and salinity, deeper layers were similar between the two locations. The Barents Sea water mass, which spanned the deeper layers of both the north and south locations, potentially acted as a corridor for transport of larvae between our two locations. Other oceanographic features (particularly eddies, Porter et al., 2020) may also facilitate exchange of water, and therefore of meroplankton, across the oceanographic Polar Front. Eddies have been shown to transport larvae in other regions of the world (e.g., Madagascar, Ockhuis, 2016). The branch of Atlantic Water that wraps around the western coast of Svalbard and enters the Barents Sea from the north (Lind et al., 2012) is of relatively low volume and has limited penetration southward into the Barents Sea, so is unlikely to constitute a significant supply of meroplankton to our sampling site north of the Polar Front. Therefore, our study suggests that the Polar Front may not act as an impervious barrier to larval dispersal, yet maintains some level of community differentiation.

Despite some differences in faunal characteristics across the Polar Front, there was little impact of water mass on the meroplankton community composition. The same taxa were often found at all depths, in water layers of different properties, within a given station. While another meroplankton study in the Barents Sea showed strong associations of meroplankton communities with water masses (Schlüter and Rachor, 2001), another study in the Ross Sea found no significant differences across water masses once other factors, such as depth and region, were accounted for Gallego et al. (2014). A possible explanation could be that some taxa may actively migrate within the water column as a means of regulating their range of dispersal (Largier, 2003). Alternatively, we may have captured either the upward trajectory of newly-released larvae or the downward journey of settling propagules. It therefore appears that early life stages of some of these taxa may be able to withstand relatively large variations in temperature and salinity, at least for short periods of time (Costlow and Bookout, 1969).

## CONCLUSION

Our new knowledge gained from the combination of high taxonomic and seasonal resolution opposes the previous notion of tight coupling of the spring bloom to larval abundance, and instead shows taxon-specific seasonal patterns with most species occurring in late summer and fall. This study also highlights the value of high-throughput DNA barcoding as a means of identifying meroplankton to high taxonomic resolution. In addition to providing species-specific information on reproductive timing, etc., this method enabled the identification of species that are non-native to the study area. Boreal species which already have a means of reaching the Arctic inflow shelves during their larval phase may be among the first to expand their range into the Arctic as the climate continues to warm. Regular monitoring of the meroplanktonic community on the inflow shelves using high-throughput DNA barcoding may play a key role in early detection of these newcomers.

## DATA AVAILABILITY STATEMENT

The raw sequencing datasets presented in this study are publicly available in the Sequence Read Archive (SRA) repository of NCBI Bioproject name: PRJNA725248; <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA725248>. The rest of the data, as well as photos of meroplankton individuals and the bioinformatics and data analysis R codes can be found at Dryad Digital Repository <https://doi.org/10.5061/dryad.n8pk0p2vf>.

## AUTHOR CONTRIBUTIONS

RD, EE, PR, and BB conceived the study. RD, EE, OW, and KP designed the molecular protocol. FC and RD collected and interpreted the physical data. RD and EE collected the zooplankton samples. RD, EE, and OW performed the laboratory work. OW ran the bioinformatics and RD performed data analysis. RD wrote the manuscript with contributions from EE, OW, KP, PR, FC, and BB. All authors have read and approved the manuscript.

## FUNDING

This research has been jointly funded by UiT the Arctic University of Norway and the Tromsø Research Foundation under the project “Arctic Seasonal Ice Zone Ecology,” project number 01vm/h1 as well as by the Fram Centre Flagship “Climate Change in Fjord and Coast” grant number 272019 and the Fonds de Recherche Nature et Technologies du Québec (file

number 270604). The ArcticPRIZE project (NE/P006302/1 – United Kingdom Natural Environment Research Council) and the Nansen Legacy project (Norwegian Research Council project 276730) contributed ship time for sampling. The UiT library covered the costs of open access publication. The work of EE was done within the framework of the state assignment of IO RAS (theme no. 0128-2021-0007).

## ACKNOWLEDGMENTS

We would like to thank the chief scientists, crew, and fellow scientists onboard the RV *Helmer Hanssen*, RV *Kronprins Haakon*, and RRS *James Clark Ross* for their support during our sampling efforts. We would also like to thank ArcticPRIZE project and the Nansen Legacy project for contributing CTD data and discussion fora. We are grateful to Helena Michelsen (UiT), Kristine Cerbule (UiT), and Julie Bitz-Thorsen (UiT) for their assistance during laboratory work. We would also further like to thank Emily Venables (SAMS) for processing the CTD data of the January, April, and June cruises, to Randi Ingvaldsen (IMR) for general discussions on oceanography of the Barents Sea and to Michael Greenacre (University Pompeu Fabra) for advice on statistical analyses.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.677732/full#supplementary-material>

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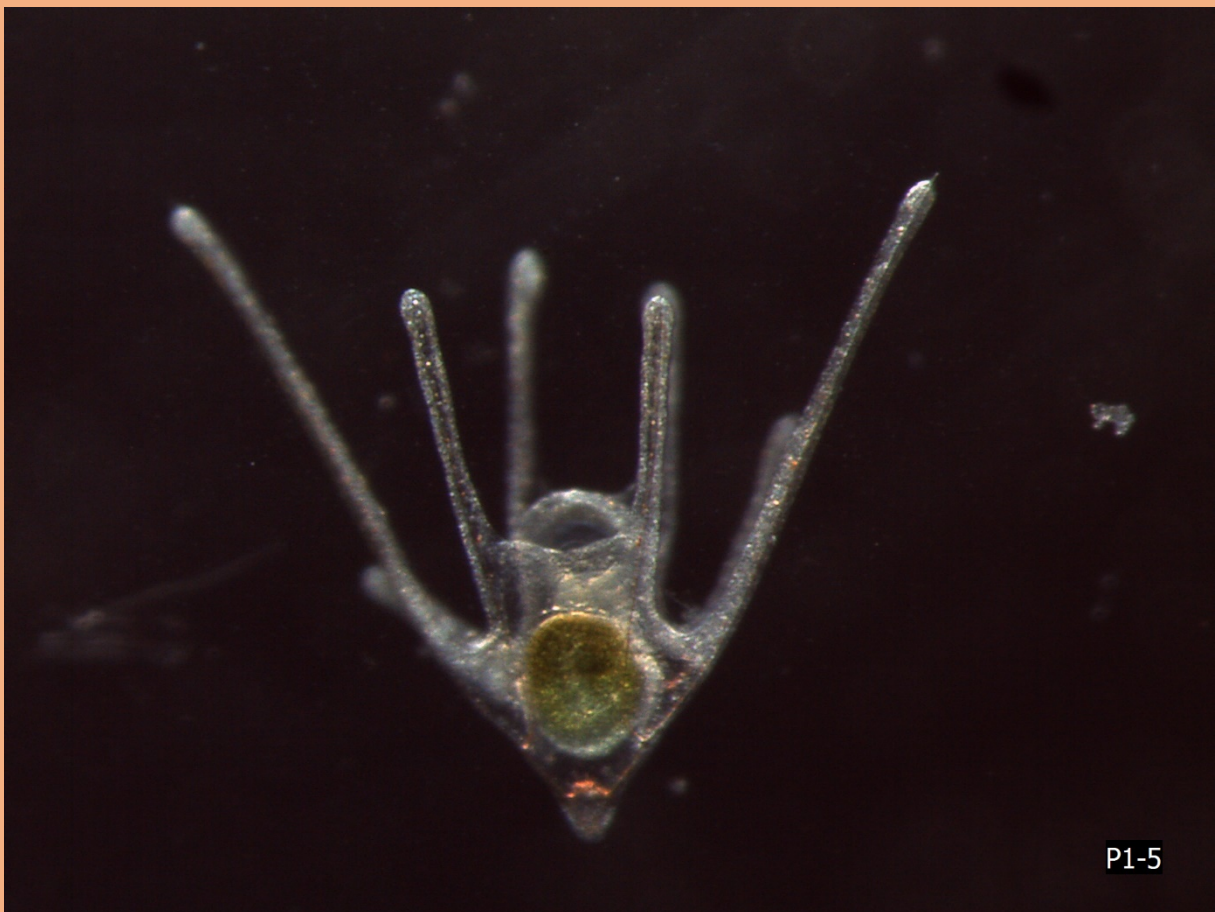
**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Paper II

Descôteaux R, Huserbråten M, Jørgensen LL, Renaud PE, Ingvaldsen, RB, Ershova EA, and Bluhm BA. Origin of marine invertebrate larvae on an Arctic inflow shelf. [accepted with minor revisions in Marine Ecology Progress Series]





1 Origin of marine invertebrate larvae on an Arctic inflow shelf

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13  
14 Abstract

15 Many benthic invertebrate taxa possess planktonic early life stages which drift with water  
16 currents and contribute to dispersal of the species, sometimes reaching areas beyond the current  
17 ranges of the adults. Until recently, it had been difficult to identify planktonic larvae to species  
18 level due to lack of distinguishing features, preventing detection of expatriate species. Here we  
19 used DNA metabarcoding of the COI gene to obtain species-level identification of early life  
20 stages of benthic invertebrates in zooplankton samples from the Barents Sea and around Svalbard  
21 where, regionally, large volumes of warm Atlantic Water enter the Arctic from the south. We  
22 compared the larval community in the water column to the adult community on the seafloor to  
23 identify mismatches. In addition, we implemented particle tracking analysis to identify the  
24 possible areas of origin of larvae. Our results show that 30-45% of larval taxa, largely  
25 polychaetes and nudibranchs, were not local to the sampling area though most were found nearby  
26 in the Barents Sea. In the particle tracking analysis, some larvae originating along the Norwegian  
27 coast were capable of reaching the northwest coast of Svalbard within 3 months, but larvae found  
28 east of Svalbard had a more constrained possible area of origin which did not extend to the  
29 Norwegian coast. This study highlights largely regional-scale larval connectivity in the Barents  
30 Sea but demonstrates the potential for some long-lived larval taxa to travel to Svalbard and the  
31 Barents Sea from further south.

32  
33 Keywords: Meroplankton, Larval dispersal, Barents Sea, Arctic benthos, Species distributions,  
34 Climate change

## 36 1. INTRODUCTION

37 Climate change and Atlantification of the Arctic are transforming the Barents Sea shelf  
38 ecosystem (Ingvaldsen et al. 2021). The Barents Sea is already warmer now compared to recent  
39 decades (Lind et al. 2018, Skagseth et al. 2020) and is expected to continue warming, with  
40 surface waters reaching up to 5°C above the long-term average by the end of the century  
41 (Drinkwater et al. 2021), although some models predict a more modest change (Long & Perrie  
42 2017). The Barents Sea is an Arctic inflow shelf (Carmack & Wassmann 2006) where warm and  
43 saline Atlantic Water flowing northwards meets cold and relatively fresh Arctic Water flowing  
44 towards the south (Fig. 1, Oziel et al., 2016). The convergence of these two water masses occurs  
45 at the Polar Front, the location of which is strongly tied to the bathymetry, at least in the western  
46 Barents Sea where the front largely follows the northern edge of Bear Island Trough and Hopen  
47 Trench (Oziel et al. 2016). The Arctic-dominated northern Barents Sea is seasonally ice-covered  
48 but is predicted to become ice-free year-round by the end of the century (Onarheim & Årthun  
49 2017). Since the flow is strongly linked to the topography, the flow regimes will likely persist.  
50 However, the loss of sea ice facilitates enhanced vertical mixing, so that some of the surface heat  
51 can be redistributed towards deeper layers (Polyakov et al. 2017, Lind et al. 2018). As such, the  
52 seafloor will not be sheltered from surface heating and is expected to warm concurrently, albeit at  
53 a slower pace (Renaud et al. 2015). Models predict bottom water temperatures on Arctic shelves  
54 to increase on average by  $2.3\text{ °C} \pm 1.0\text{ °C}$  by 2100 (Heuzé et al. 2015) with the biggest changes,  
55 up to 6°C in some regions, occurring in the Barents Sea (Renaud et al. 2019).

56  
57 As a result of changing environmental conditions, many species are expected to move poleward.  
58 The Arctic, and the Barents Sea in particular, will become increasingly habitable for a range of  
59 potential aquatic invasive species (Goldsmid et al. 2020). In addition, the surface speed of the

60 Atlantic Water has increased in recent years, potentially strengthening the bio-advection of  
61 southern species into these regions (Oziel et al. 2020). Barents Sea benthic communities comprise  
62 a mix of Arctic, boreal (Atlantic) and boreal-Arctic taxa (Jørgensen et al. 2019, Zakharov et al.  
63 2020) which has shifted with climate with boreal taxa becoming relatively more abundant in  
64 warmer periods, both on glacial timescales as well as interannual timescales in the recent past  
65 (Blacker 1965, Jørgensen et al. 2019). In recent decades, Barents Sea fish communities have  
66 started transitioning into a more boreal assemblage with more warm-water species moving north  
67 and the range of typical Arctic species retracting (Renaud et al. 2012, Berge et al. 2015, Fossheim  
68 et al. 2015). In the Barents Sea, the new, more boreal, fish assemblage is characterized by larger,  
69 faster-growing and more omnivorous/generalist taxa compared to the previous more Arctic  
70 community (Frainer et al. 2017) with potential important consequences on trophic connections in  
71 the ecosystem (Kortsch et al. 2015). While most benthic invertebrates are not as mobile as fish, at  
72 least at the adult stage, some cases of northward expansions have also been reported, such as blue  
73 mussels expanding their range to the Svalbard coast (Berge et al. 2005), several gastropod taxa  
74 extending into the Barents Sea (Zakharov & Jørgensen 2017) and Barents Sea peracarids  
75 assemblages shifting to increasingly boreal composition (Zimina et al. 2019). These changes in  
76 species composition can alter ecosystem functioning.

77  
78 For many benthic invertebrate taxa, the adult stage has limited mobility, so it is up to the  
79 planktonic early life stages (from here on, referred to as meroplankton) to disperse. For some, the  
80 dispersal stage is short, lasting from minutes to a few hours, resulting largely in recruitment to the  
81 parent population, while others disperse for up to several months (Shanks 2009) and, in rare  
82 cases, years (Strathmann & Strathmann 2007). In a previous seasonal study in the Barents Sea,  
83 most meroplanktonic taxa were found at discrete time points suggesting a larval phase of no more

84 than a few months, though a minority of taxa showed evidence of longer dispersal periods  
85 (though still shorter than a year, Descôteaux et al. 2021). Those taxa with potential for long-  
86 distance dispersal may be better adapted to finding new habitat following periods of change  
87 (Wares & Cunningham 2001, Hardy et al. 2011). Unfortunately, larval duration is only known for  
88 a very small subset of benthic invertebrate taxa, and cold Arctic temperatures may extend the  
89 larval period as well (O'Connor et al. 2007). Without this information, it may be difficult to  
90 predict which taxa have the potential for long-distance dispersal and hence which boreal species  
91 currently have the means of reaching the Arctic Ocean within their larval phase. Instead, one can  
92 look directly in the Barents Sea for larvae of boreal origin. Such a strategy relies on species-level  
93 identification of larvae which has historically been challenging because of the general lack of  
94 species-specific features at the larval stage for most taxa. New molecular methods, however, now  
95 enable reliable identification of planktonic early life stages of benthic invertebrates (Ershova et  
96 al. 2019, Descôteaux et al. 2021).

97  
98 Here, we aimed to determine the likely origin of benthic invertebrate larvae that are found in the  
99 Barents Sea and around Svalbard. We used metabarcoding of zooplankton samples, a fast and  
100 efficient way to process large sample volumes, to identify the larval community in three  
101 oceanographically different areas of the Barents Sea and Svalbard (Fig. 1). The areas north of  
102 Svalbard and south of the Polar Front are highly influenced by inflowing Atlantic Water whereas  
103 the area north of the Polar Front (east of Svalbard) is more Arctic in character (Lind &  
104 Ingvaldsen 2012, Smedsrud et al. 2013, Renner et al. 2018). We then compared the larval pool  
105 with the adult community on the seafloor to evaluate which species are local and which may be  
106 drifting in from other seas. Compared with other Arctic Seas, the Barents Sea benthos is  
107 relatively well studied (e.g., Cochrane et al. 2009, Jørgensen et al. 2015, Zakharov et al. 2020),



108 offering a robust dataset against which to compare the larval community. We hypothesized that  
109 the majority of the collected larvae belongs to taxa that are known to inhabit the region. We then  
110 implemented particle tracking analysis to highlight which regions could realistically supply  
111 larvae to the Barents Sea within a 3-month drift. We hypothesized that the areas north of  
112 Svalbard and south of the Polar Front would receive particles (larvae) from the Norwegian coast  
113 but that the area north of the Polar Front would be more isolated. This information can provide  
114 some indication of which boreal taxa currently have the means of reaching the Barents Sea during  
115 their larval stage and therefore which may be some of the first to settle once conditions there  
116 become favorable for establishment of a new population. Finally, based on our previous  
117 observation of a larva of *Bohuslania matsmichaeli* in the Barents Sea (Descôteaux et al. 2021), a  
118 nudibranch species otherwise only known from one fjord in southern Norway (Korshunova et al.  
119 2018), we performed an additional particle tracking analysis where particles were released from a  
120 single point in southern Norway and allowed to drift for a year. We hypothesized that a few of  
121 those particles would reach the Barents Sea and Svalbard within a year, though fewer in the  
122 northern Barents Sea north of the Polar Front where the influence of the northward-flowing  
123 Atlantic Water is comparatively low.

124

125

## 126 2. MATERIALS AND METHODS

### 127 2.1 Collection and preparation of meroplankton samples

128 Zooplankton samples were collected in August, September and November 2017 as well as  
129 January, April, June and August 2018 in three regions: north of Svalbard (11 samples), and east  
130 of Svalbard north (28 samples) and south (22 samples) of the Polar Front (Fig. 1). Samples were  
131 collected using a WP2 or Multinet (Hydro-Bios) with 64 or 180  $\mu\text{m}$  mesh, depending on

132 availability and bloom conditions, from near bottom to surface (Table S1) and preserved in 96%  
133 ethanol. While smaller larvae could be missed, and therefore total biodiversity underestimated, by  
134 using the 180  $\mu\text{m}$  mesh, abundant phytoplankton would clog the 64  $\mu\text{m}$  mesh preventing its use  
135 in times of bloom. Whole zooplankton samples were split in half using a Motodo box-type  
136 plankton splitter. While most samples were complete prior to splitting, some had a subsample  
137 removed for use in a different research project resulting in metabarcoded fractions varying from  
138 0.19 to 0.5 of the initial sample (Table S1). One of the two fractions was used for metabarcoding  
139 (below) while the other was kept as a voucher for future reference. The portion of the  
140 zooplankton sample dedicated to metabarcoding was blended in a high-power blender  
141 (Nutribullet Max 1200) for at least 1 minute, until the sample appeared homogeneous. The  
142 blended sample was then transferred into a jar to settle overnight. The next day, the sample was  
143 decanted to remove as much of the supernatant ethanol as possible before transferring to a 50-mL  
144 Falcon tube. The samples in Falcon tubes were centrifuged at 3000 g for 5 minutes to further  
145 separate the ethanol from the sample. The ethanol was then decanted out and discarded and the  
146 remaining sample weighed inside its Falcon tube (wet weight). For particularly large samples,  
147 some of the material was removed to keep weight below 10 g for subsequent DNA extraction, as  
148 per manufacturer recommendation. The plankton splitter, blender container, jars, etc. were all  
149 soaked in 10% bleach for a few minutes and then rinsed in tap water in between samples to  
150 prevent cross-contamination.

151

## 152 2.2 DNA metabarcoding

153 DNA was extracted from the decanted zooplankton samples using the DNeasy PowerMax Soil  
154 Kit (Qiagen) according to manufacturer protocol, except that in step 4, the tubes were shaken in a  
155 shaking incubator (Infors HT, Microtron) at 65 °C for 2 hours at 200 rpm and, in step 18, the

156 DNA was eluted in 3 mL of solution C6 instead of 5 mL and run over the spin column twice to  
157 increase DNA concentration. The PowerMax Soil Kit was selected for its ability to process large  
158 sample volumes so as to obtain an extract as representative of the whole sample as possible  
159 though its ability to extract efficiently across all meroplanktonic phyla remains to be confirmed.  
160 We amplified the Leray-XT fragment of the COI gene (~313 base pairs), selected based on its  
161 ability to amplify across all our phyla of interest and to discriminate across species as well as on  
162 the completeness of its reference databases (Andújar et al. 2018, Wangenstein et al. 2018). Use  
163 of this fragment is extremely effective for identification of meroplankton (Ershova et al. 2019,  
164 Descôteaux et al. 2021). Each PCR reaction consisted of 10  $\mu$ L AmpliTaq Gold polymerase, 0.16  
165  $\mu$ L bovine serum albumin 20  $\mu$ g/ $\mu$ L, 5.84  $\mu$ L nuclease-free water, 1  $\mu$ L individually-tagged  
166 forward primer (5  $\mu$ M, mlCOIintF-XT 5'-GGWACWRGWTGRACWITITAYCCYCC-3'), 1  $\mu$ L  
167 individually-tagged reverse primer (5  $\mu$ M, jgHCO2198 5'-  
168 TAIACYTCIGGRTGICCRAARAAYCA- 3') as well as 2  $\mu$ L undiluted DNA template for a total  
169 reaction volume of 20  $\mu$ L. The PCR protocol consisted of a denaturation step for 10 min at 95 °C  
170 followed by 35 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min and a final  
171 extension of 5 min at 72°C (Wangenstein et al. 2018). The same tag was used on the forward and  
172 reverse primers to facilitate detection of chimeras. All samples were combined into a library, then  
173 cleaned (fragments below 70 base pairs removed) and concentrated using MinElute columns. The  
174 final DNA concentration was measured using a Qubit fluorimeter with broad-range dsDNA BR  
175 Assay Kit (Qubit). The NEXTflex PCR-free DNA sequencing kit (BIOO Scientific) was used to  
176 prepare the library for sequencing according to manufacturer instructions, omitting the second  
177 bead cleaning round in Step B to improve yield. The library was quantified by qPCR using the  
178 NEBNext Library Quant Kit (New England Biolabs) and sequenced on an Illumina MiSeq

179 platform with v3 2 x 250 bp kit spiked with 1% PhiX used as an internal control to calculate error  
180 rates.

181

### 182 2.3 Bioinformatics

183 Bioinformatics were carried out using the OBITools v1.01.22 pipeline (Boyer et al. 2016).

184 Paired-end sequences were aligned with *illuminapairedend*, and sequences with a score of < 40

185 were removed. Samples were identified via primer tags, and primer sequences were removed

186 from the data using *ngsfilter*. Unique reads of length between 299 and 320 were selected using

187 *obigrep* and *obiuniq*, and chimeras were identified and removed from the dataset using the

188 *uchime\_denovo* algorithm (Edgar et al. 2011) from vsearch v1.10.1 (Rognes et al. 2016).

189 SWARM 2.1.13 (Mahé et al. 2015) was used to cluster the sequences into Molecular Operational

190 Taxonomic Units (MOTUs) using a distance value of d=13. A preliminary taxonomic assignment

191 was done using *ecotag* (Boyer et al. 2016) against DUFA-Leray v.2020-06-10, a custom

192 reference database (publicly available from [github.com/uit-metabarcoding/DUFA](https://github.com/uit-metabarcoding/DUFA)), which

193 includes Leray fragment sequences extracted from BOLD/Genbank and in-house generated

194 sequences. Likely pseudogene sequences were then removed from the resulting dataset using the

195 algorithm LULU (Frøslev et al. 2017).

196

197 Only the MOTUs that made up at least 0.01% of at least one sample were retained for further

198 analysis. These sequences were then searched in BOLD (Barcode of Life database, Ratnasingham

199 & Hebert 2007) for taxonomic identification using package *bold* (Chamberlain 2021) in R (R

200 version 4.2.1, R Core Team 2021). When BOLD did not produce a match > 97%, we used the

201 Ecotag assignment or identified the sequence using NCBI's BLAST (Basic Alignment Search

202 Tool, Altschul et al. 1990). Sequences for which no match >97% was found, for which the match  
203 was not at species-level and those belonging to fish, holoplanktonic taxa (e.g. copepods,  
204 chaetognaths, pteropods, etc.) or suspected contaminants (terrestrial or freshwater taxa) were not  
205 included in subsequent analyses. Our analysis focused exclusively on benthic invertebrate taxa  
206 with planktonic early life stages.

207

#### 208 2.4 Comparison to adult community

209 Adult (and settled juvenile) benthic invertebrate community data for each of our three areas of  
210 interest were compiled from large datasets including Norwegian-Russian Ecosystem Surveys  
211 (Jørgensen et al. 2015) and from an Akvaplan-niva dataset (Andrade et al. 2017). The Norwegian  
212 Institute of Marine Research, the Polar branch of VNIRO (Russian Federal Research Institute of  
213 Fisheries and Oceanography) and the Murmansk Marine Biological Institute have been  
214 conducting annual trawl surveys of the Barents Sea megafauna since 2005 (Jørgensen et al. 2015,  
215 Zakharov et al. 2020). For this paper, we used data from the 2010, 2012, 2013 and 2015  
216 samplings on the Norwegian side of the Barents Sea shelf which totaled 694 sampling events and  
217 805 taxa. The Akvaplan-niva dataset consists of a compilation of macrofauna abundance data  
218 collected by Van Veen grab at 138 stations across the Barents, Pechora and Kara Seas from 1992-  
219 2005, including 1380 taxa, most of which were identified to species level. The boundaries of each  
220 area of interest encompass the three geographical clusters of zooplankton sampling (Fig. 1). The  
221 list of meroplanktonic taxa found in each of the three areas from all seasons and depths combined  
222 was then compared to the list of adults compiled above. Meroplanktonic taxa that were not found  
223 as adults in one of those datasets were then investigated further in the GBIF database (Global  
224 Biodiversity Information Facility) for each of the three areas of interest using package *rgbif* in R  
225 (Chamberlain et al. 2022, GBIF.org 2022a). Taxa which still did not match any adult records

226 were then investigated further in the literature to ensure that no occurrence records were missed  
227 (additional occurrence records were found in Kukliński 2002, Hansen et al. 2019, Dvoretzky &  
228 Dvoretzky 2021). Those taxa for which no evidence of the adults living in the same area was  
229 uncovered were considered as ‘non-local’. We counted the number of samples in which the taxon  
230 was present (frequency of occurrence) as a measure of prevalence. Finally, we calculated the  
231 distance of the closest known adult record (GBIF.org 2022b) to the center of each of the three  
232 areas of interest using function *distGeo* in package *geosphere* (Hijmans 2019) in R. This function  
233 calculated the shortest distance between two points on a WG84 ellipsoid (a highly accurate  
234 representation of the earth surface) but did not account for land or any other obstacle, resulting in  
235 an underestimation of the true minimum distance travelled. The boundaries of our three areas of  
236 interest were relatively small compared to the size of the overall oceanographic regions in which  
237 they each lay (see Fig. 1). Accordingly, in our interpretation, we placed less emphasis on the taxa  
238 for which the nearest known adult resides just outside the boxes but within the same  
239 oceanographic regions compared to the taxa for which the nearest known adult is known only  
240 from distant seas.

241

## 242 2.5 Particle tracking

243 A particle tracking analysis was used to determine the possible areas of origin of larvae found in  
244 the Barents Sea and around Svalbard. The hydrodynamic model used to represent the ocean  
245 currents in the study area was based on the Regional Ocean Modeling System (ROMS), a free-  
246 surface, hydrostatic, primitive equation ocean general circulation model (Shchepetkin &  
247 McWilliams 2005). ROMS was run with a horizontal resolution of 4 km x 4 km in an orthogonal,  
248 curvilinear grid covering parts of the North Atlantic, all the Nordic seas and the Barents Sea (see  
249 Lien et al. 2013 for details on model set-up and e.g. Lien et al. 2014 for a similar application).

250

251 To model the advection of particles in the horizontal plane we applied the fourth order Runge-  
252 Kutta scheme LADiM (the Lagrangian Advection and Diffusion Model, Ådlandsvik 2021)  
253 coupled with the velocity fields from ROMS. Here particles were released in a regular grid (every  
254 4 km) across the model domain, initialized at 20 and 130 m on August 23<sup>rd</sup> 2017 (fall) and May  
255 10<sup>th</sup> 2018 (summer), and drifted at the depth set at initiation until the end of the drift period (three  
256 months). If at any time during the 3-month drift period a particle entered one of the three  
257 polygons representing the three areas of interest, its release site was considered a possible area of  
258 origin. Three months represents a conservative estimate of the larval duration for the majority of  
259 benthic invertebrates (Shanks 2009) though unfortunately larval period is unknown for most taxa  
260 in our region. The times of release were chosen so that the end of the 3-month drift would  
261 coincide with our previous observation of peak larval abundance (as well as high species  
262 richness) in August and November (Descôteaux et al. 2021). Drifts at 20 and 130 m accounted  
263 for differences in water mass and current properties through the water column.

264

265 Finally, to explore possibilities for long-range dispersal of particles, a separate drift experiment  
266 was initialized from a single point in Skagerrak, south of Norway/Sweden. One thousand  
267 particles released each day of January 2018 (for a total of 31000 particles) were allowed to drift  
268 for a year (until December 31, 2018) at 20 m depth. We then checked whether any of the particles  
269 entered one of the three areas of interest during their year-long drift period.

270

271

## 272 3. RESULTS

## 273 3.1 Meroplankton diversity

274 Metabarcoding uncovered a total of 2480 MOTUs across all zooplankton samples, of which 643  
275 made up at least 0.01% of at least one sample. Of those, a total of 213 obtained > 97% similarity  
276 match in BOLD, Ecotag or BLAST, 171 (80%) of which were identified to species level.  
277 Approximately half of the sequences identified to species level belonged to benthic taxa (91  
278 sequences) while the rest (80 sequences) belonged to non-meroplanktonic taxa. Only  
279 meroplanktonic taxa identified to species level with > 97% sequence match were retained for  
280 further analysis.

281

## 282 3.2 Match to benthos settled at seafloor

283 We found a total of 42 meroplanktonic species with a mismatch to the adult communities on the  
284 seafloor. Possible non-local taxa made up about a third of the meroplanktonic taxon richness at  
285 each of the three areas of interest except south of the Polar Front where it reached 45% (Fig. 2).  
286 In all three areas, the majority of taxa not represented as adults belonged to polychaetes and  
287 nudibranchs, but we found taxa belonging to six phyla (Annelida, Arthropoda, Bryozoa,  
288 Echinodermata, Mollusca and Nemertea, Table 1, Fig. 2). The adults of the majority of these taxa  
289 are found relatively close by in the Barents Sea itself, around Svalbard or along the northern  
290 Norwegian coast, but seven taxa are only known to occur in more distant locations (Fig. 3, 4).  
291 The closest known adults of *Serripes laperousii* for instance reside in the Canadian Arctic while  
292 *Cephalothrix iwatai* is only known from the Sea of Japan (Chernyshev 2013), over 5000 km  
293 away in a straight line. Other notable examples include the nudibranchs *Dendronotus elegans* and  
294 *Dendronotus patricki* found along the Kara Sea coast of Novaya Zemlya, *Dendronotus elegans*  
295 from the White Sea as well as *Doto maculata*, *Coryphella gracilis* and *Dendronotus yrjargul*



296 further south along the Norwegian coast (Fig. 3A). For the area north of Svalbard, most taxa had  
297 adults residing on the shelf west of Svalbard or along the northern Norwegian coast (Fig. 3B).  
298 The adults of most larvae in the area north of the Polar Front on the other hand were found  
299 around Svalbard, in the northern Barents Sea or along the northern Norwegian coast. The closest  
300 adults to several taxa south of the Polar Front were also found on the northern Norwegian coast,  
301 but none came from around Svalbard. Instead, most were located in the southwestern Barents  
302 Sea. Distance to closest adult ranged from 77 km to 5730 km, with two peaks in number of taxa  
303 around 100 and 1000 km (Fig. 3C).

304

### 305 3.3 Particle tracking

306 The particle tracking analysis revealed different possible areas of larval origin depending on time  
307 of release, drift depth and location (Fig. 5). East of Svalbard, the majority of particles found north  
308 of the Polar Front originated within the same region or from nearby (largely within 300 km,  
309 maximum around 500 km), both in fall and summer. Some particles could have come from as far  
310 south as Bear Island in the fall at 20 m depth (Fig. 5A) but not at 130 m depth (Fig. 5C). In  
311 summer, particles originating from that area, both shallow and deep, also moved north along Bear  
312 Island Trough and Hopen Trench (Fig. 5B, 5D). Several particles also drifted in from the north,  
313 around the northeastern coast of Svalbard in fall (Fig. 5A, 5C) and in from the northeast in  
314 summer, particularly at 20 m (Fig. 5B, 5D). Most particles found south of the Polar Front  
315 originated from well within the Barents Sea itself, largely within Hopen Trench or along the  
316 southern and northern edges of Bear Island Trough. Some particles crossed the Polar Front from  
317 the north but only at 20 m. Of all three areas of interest, the one northwest of Svalbard had the  
318 broadest probable area of origin, especially during summer (Fig. 5). Indeed, some particles  
319 released along the coast of Norway around 69°N (> 1000 km away) were able to drift north of

320 Svalbard within the 3-month drift period, following the West Spitsbergen Current flowing north  
321 along the shelf break. In the fall, however, particles originated no further south than  $\sim 72^\circ\text{N}$   
322 (Fig.5A, C). In both seasons, the majority of particles flowed northward along the shelf break. In  
323 this region, depth of release had minimal impact on the distribution of particles.

324  
325 The majority of particles released in the Skagerrak in January 2018 at 20 m for a 1-year drift  
326 followed the coastline of mainland Norway and eventually that of the Kola Peninsula in Russia  
327 (Fig. 6). A minority of particles, however, diverged away from the coast and headed northward  
328 along the western edge of the Barents Sea then west of Svalbard along the slope. Out of the  
329 31,000 particles released, only two eventually drifted into the area northwest of Svalbard within  
330 one year, with one particle arriving on day 313 and the second on day 328 after release. The two  
331 particles took very similar trajectories except for a section along mid-Norway, from around  $63^\circ\text{N}$   
332 to  $67^\circ\text{N}$ , where one stayed close to shore while the other drifted further offshore along the slope  
333 before their tracks came together again. A single particle reached the area south of the Polar Front  
334 on the Barents Sea shelf. This particle followed a similar trajectory to that of the previous two  
335 particles, except that it took a marked detour offshore around  $62^\circ\text{N}$  before heading back inshore  
336 then northward along-shore like the others. This particle diverged westward around  $72^\circ\text{N}$ ,  
337 following the Bear Island Through and Hopen Trench into the area south of the Polar Front,  
338 arriving on day 308 after release. No particles reached the area north of the Polar Front in the  
339 Barents Sea within the one-year drift period (Fig.6).

340

341

342

343

## 344 4. DISCUSSION

345

## 346 4.1 Origin of larvae

347 The Barents Sea, as an Atlantic gateway to the Arctic, has the potential to be the first introduction  
348 point of new species to the Arctic, along with the Chukchi Sea on the Pacific side (Ershova et al.  
349 2019). Given that the mass transport from the south is much greater on the Atlantic side  
350 compared to the Pacific (Hunt et al. 2013), boreal imports may be particularly frequent in the  
351 Barents Sea. In this study, however, we found largely regional-scale larval connectivity, similarly  
352 to another meroplankton study further south along the Norwegian coast (though particles there  
353 were only drifting for 28 days, Silberberger et al. 2016). We demonstrated that 30-45% of  
354 meroplanktonic taxa were not local to the individual areas where they were collected, but most  
355 were native to the Barents Sea and Svalbard as a whole. This contrasts with the adjacent Kara Sea  
356 where 47% of larval taxa were not known to the region at the time (Fetzer & Arntz 2008). For  
357 many species, those whose closest adult was found around Svalbard or on the Barents Sea shelf, a  
358 3-month drift would be sufficiently long to supply the larvae into our three areas of interest. For  
359 the six taxa for which the closest adult was located along the northern Norwegian coast (Fig. S1),  
360 however, a larval duration greater than three months would be required to account for our  
361 observations. Indeed, in this region, the Norwegian/Murman Coastal Current would entrain  
362 larvae released nearshore northeastward along the coast into the Russian Arctic (Sakshaug et al.  
363 2009, Fig. 1). It is therefore likely that more southern specimens of the species (e.g. found along  
364 the continental slope of the Norwegian Sea) would supply the larval pool to the Barents Sea and  
365 around Svalbard despite being located further away. Though such dispersal would be more  
366 realistic, it would still take longer than three months.

367

## 368 4.2 Pelagic larval duration

369 The larval duration period is known only for a small subset of benthic taxa with a meroplanktonic  
370 stage. In a review across taxonomic groups, Shanks (2009) found larval drifts up to 3-4 months,  
371 though most taxa had much shorter durations. In the Shanks (2009) review, most taxa dispersing  
372 for weeks or more belonged to Mollusca, Echinodermata, Polychaeta and Crustacea. In our study,  
373 most taxa identified in fact belonged to these groups. Our choice to run particle tracking analysis  
374 for 3 months was based on the fact that few taxa are known to disperse for longer (though see  
375 Descôteaux et al. 2021). Teleplanic larvae have the ability to survive as a larva for years  
376 (Strathmann & Strathmann 2007), but they are presumably rare. According to our particle  
377 tracking analysis, larvae of the nudibranch *Bohuslania matsmichaeli* (to date only known from  
378 southern Norway/Sweden, Korshunova et al. 2018) would require more than a year to reach the  
379 northern Barents Sea where they have been previously recorded (Descôteaux et al. 2021).

380  
381 Many living organisms do not behave as passive particles and tend to drift slower than models  
382 would suggest (Shanks 2009). Several taxa with weeks to months-long larval duration disperse  
383 less than a kilometer in that period while passive dispersal would have predicted tens to hundreds  
384 of kilometers covered (Shanks 2009). Indeed, by remaining close to the seafloor where currents  
385 are slower, by vertically-migrating, or even, for more active taxa, by swimming against the  
386 currents, larvae may be retained closer to their area of origin (Shanks 2009). Vertical migration  
387 behaviour has been observed in some species of decapod crustaceans (Queiroga & Blanton  
388 2004), barnacles (Bonicelli et al. 2016), molluscs (Rawlinson et al. 2004) and other groups. Our  
389 particle tracking analysis did not take into account any of these possible behaviours, so likely  
390 represents a maximum possible distance of origin for a 3-month drift.

391

## 392 4.3 Potential drivers of larva-adult mismatches

393 A mismatch between larval and adult distribution can be artificial, created by an incomplete  
394 picture of the benthic diversity in a region or differences in species identification. Thanks in part  
395 to an extensive annual survey carried out since 2006 there is comparatively strong knowledge of  
396 the benthic megafauna (here defined as fauna caught in trawls) of the Barents Sea shelf  
397 (Jørgensen et al. 2015, Zakharov et al. 2020). Information on benthic macrofauna (those animals  
398 caught on a 0.5 or 1-mm sieve) also exists for the study region (Denisenko 2001, Carroll et al.  
399 2008, Cochrane et al. 2009, Carroll & Ambrose 2012, Kędra et al. 2013), though not as spatially  
400 and temporally extensive as for the megafauna. The absence of adults of a particular species, and  
401 consequent mismatches between larval and adult distributions, is therefore more likely to be  
402 driven by lack of data (or different taxonomic naming) in the macrofaunal taxa (largely  
403 polychaetes and bivalves) compared to the larger megafauna. Some taxa like nudibranchs  
404 (discussed below) may be too rare to be reliably caught by grabs or box cores used to sample  
405 macrofauna and too small to be retained in the trawl nets used to sample megafauna. Except for a  
406 few comparatively well-studied fjords, the benthic fauna of shallow coastal waters of Svalbard is  
407 poorly resolved (Renaud et al. 2015), again potentially leading to artificial mismatches between  
408 adult and larval distributions.

409  
410 The lack of data on adult nudibranch distributions could partially explain the dominance of this  
411 group in our non-local larval fauna. For some of these taxa, the adult could inhabit the Barents  
412 Sea shelf but have remained undetected. The nudibranch *Dendronotus patricki* was first  
413 discovered near a whalefall at 1820 m depth in the Pacific Ocean off of California (Stout et al.  
414 2011) and more recently found in the Arctic Kara Sea at 216 m depth (Ekimova et al. 2019).  
415 Given its affinity for deeper soft bottom habitats (Ekimova et al. 2021), this species could

416 realistically inhabit the Barents Sea shelf. Many nudibranch (and other heterobranch gastropods)  
417 taxa identified here, however, appear to have a shallow coastal distribution (including  
418 *Microchlamylla gracilis*, now accepted as *Coryphella gracilis*, Korshunova et al. 2017,  
419 *Cuthonella concinna*, *Eubranchius rupium* and *Placida dendritica* Svensen & Moen 2020) and  
420 would therefore be unlikely to inhabit the Barents Sea shelf. Indeed, in our study, the nearest  
421 known adults to most non-local nudibranch taxa were found in coastal regions, especially along  
422 the northern Norwegian coast (Fig 3A, 4). In these taxa, a long dispersal stage seems likely. Most  
423 nudibranch species have planktotrophic development, and many require a cue from the adult prey  
424 (e.g. hydroids) to settle (e.g., Sisson 2005), potentially leading to long dispersal duration when  
425 such cues are lacking. Our data (not shown here) suggest that several of the so-called non-local  
426 nudibranchs were present as larvae in the Barents Sea during most of the year. Assuming one  
427 single reproductive period per year, as for many nudibranchs (including *Dendronotus yrjargul*,  
428 Korshunova et al. 2021a), their extended presence in the Barents Sea could indicate a long  
429 dispersal phase. In the laboratory, larvae of *Dendronotus frondosus* metamorphose after 63-86  
430 days at 10 °C (Sisson 2005). Larval duration lengthens with decreasing temperature (O'Connor et  
431 al. 2007) so that larval duration in the Barents Sea, where our modeled larvae experienced sea  
432 surface temperatures between -2 and 10.4°C (Barents Sea temperature ranges 0-8°C in Barton et  
433 al. 2018), could reach well beyond durations observed at higher temperatures in the laboratory.  
434 We therefore hypothesize that the dominance of nudibranch taxa in the non-local pool is in part  
435 driven by their potential for long-distance dispersal in addition to being biased by our lack of  
436 knowledge of true geographical distribution of individual taxa. A third unexplored possibility,  
437 that nudibranch larvae have a greater tolerance to low temperatures and reduced mortality when  
438 entering the Arctic compared to other groups, cannot be excluded. Indeed, nudibranch larvae  
439 figure prominently in winter zooplankton samples in the Barents Sea (Hirche & Kosobokova

440 2011, Descôteaux et al. 2021). Recent taxonomic and molecular work has highlighted the  
441 complexity and diversity of the Arctic nudibranch fauna (Ekimova et al. 2015, Korshunova et al.  
442 2021a,b), and our study emphasizes the need for continued taxonomic, molecular and ecological  
443 studies on this group.

444

#### 445 4.4 Very distant taxa

446 The nemertean *Cephalothrix iwatai* was highly abundant in the larval pool, appearing in the  
447 majority of samples in all three areas of interest. To date, the adults of the species are only known  
448 from the Sea of Japan in the north Pacific, living in deep soft sediments (Chernyshev 2013). The  
449 larvae of palaeonemerteans (to which *C. iwatai* belongs) are usually planktotrophic (Maslakova  
450 2010) though other authors have speculated that a shorter-lived lecithotrophic larval form exists  
451 (Fernández-Álvarez & Machordom 2013). Larval *Cephalothrix* sp. can survive up to 8 weeks in  
452 the absence of food, but it is unknown how long they could survive if provided with adequate  
453 nutrition (Smith 1935). In this case, contrary to nudibranchs, a long larval duration cannot in  
454 itself explain our observations as it seems extremely unlikely that the larvae would have drifted  
455 all the way from the north Pacific, especially in such high numbers. While the molecular  
456 identification of *C. iwatai* was based on a single specimen sequence archived in BOLD, the  
457 source of that specimen is reliable (Chernyshev 2013) and consistent with the tree-based  
458 identification. Two species of the *Cephalothrix* genus, *C. rufifrons* and *C. linearis*, are common  
459 in the North Atlantic (GBIF), including the Barents Sea for *C. linearis* (Buzhinskaja 2011).  
460 Neither closely matches our larval sequences despite being well-represented in BOLD. Our larval  
461 specimens therefore either belong to a yet non-barcoded species closely-related to *C. iwatai* or  
462 truly belong to *C. iwatai* which would therefore be presumed to have a much wider distribution  
463 range than is currently recognized.

464  
465 The bivalve *Serripes laperousii* was also found in all three areas of interest, appearing in a total  
466 of 8 samples. It is considered a Pacific species, but it also has some recorded occurrences in the  
467 Canadian Arctic (GBIF Secretariat 2021). The larvae's DNA-based identification was again  
468 based on a match with only two sequenced adults, but these sequences appear reliable (Layton et  
469 al. 2014). Another *Serripes* species, *S. groenlandicus*, is well known from the Barents Sea, but its  
470 DNA was not a direct match to our larval samples. A drift from the Canadian Arctic to the  
471 Barents Sea seems improbable, so we postulate that *S. laperousii* has a more widespread  
472 distribution than current records show.

473  
474 Human activity can also facilitate dispersal of species and may have contributed to some of our  
475 observations here. Transport in ballast waters, for example, has the potential to introduce new  
476 taxa to the Barents Sea despite mitigation protocols such as mid-ocean exchange (Rosenhaim et  
477 al. 2019). While mid-ocean exchange might reduce the transport of organisms from port to port,  
478 it may in fact contribute to transport to the Barents Sea if the exchange was to take place there. In  
479 the case of *C. iwatai* and *S. laperousii*, however, both taxa were found in several samples (52 and  
480 8, respectively) spanning all three areas of interest, so it is unlikely that recent transport in ship  
481 ballast waters alone could account for their presence in the Barents Sea. Plastic debris floating in  
482 the oceans can also serve as a vector for dispersal, sometimes acting on intergenerational  
483 timescales so as to enable the dispersal over greater distances than would be possible within the  
484 lifespan of a single larva (Haram et al. 2021). In fact, there is evidence that plastic debris has  
485 contributed to the dispersal of the blue mussel back to Svalbard after 1000-years absence  
486 (Kotwicki et al., 2021). Similar to ship ballast waters, this mechanism is unlikely to explain our



487 very frequent observations of *C. iwatai* in larval samples of the Barents Sea and Svalbard but  
488 could certainly have contributed to some of the dispersal.

489

#### 490 4.5 Outlook

491 This study highlights the potential for larval stages of benthic invertebrates to drift into the  
492 Barents Sea and around Svalbard from further south despite the bulk of the larval community  
493 being of local or regional origin. Continued surveys of both benthic adults and pelagic early life  
494 stages in the region, but also along the Norwegian coast, will be critical to track the northward  
495 progress of boreal taxa. Most of these non-local larvae, however, likely represent a ‘dead end’ as  
496 the conditions in the Arctic seas are, for now, presumably inhospitable (in terms of temperature,  
497 food availability, etc.) to the growth of a viable population. Indeed, while larval supply is the  
498 critical first step in establishment of a viable population, the larvae must settle successfully, grow  
499 to reproductive maturity and produce enough young to compensate for mortality. Rising  
500 temperatures associated with climate change and Atlantification of the Barents Sea (Renaud et al.  
501 2019, Polyakov et al. 2020) will likely allow some of these taxa to settle and reproduce  
502 successfully in the future (Renaud et al. 2015) as has been the case for the blue mussel (Berge et  
503 al. 2005) and other species. While this warming will likely be sufficient to enable some taxa to  
504 settle and reproduce, other factors such as bottom substrate, availability of specific prey items,  
505 etc., may limit the expansion of others. Those taxa that specialize in rocky shallow water  
506 environments for example would be unlikely to colonize the deeper soft-bottom Barents Sea shelf  
507 but could potentially settle on the coast of Svalbard. Additionally, new taxa may not be able to  
508 colonize the Barents Sea as long as local taxa occupy the same niche (so-called priority effects,  
509 Fraser et al. 2015). It should also be noted that increased temperatures are expected to speed up  
510 larval development (O’Connor et al. 2007) in a way that may reduce dispersal distance,

511 potentially counteracting, at least in part, the rate of expansion. The effects of climate change on  
512 the benthic communities of the Arctic are therefore complex to predict, but we show that some  
513 boreal taxa with long-duration larvae have the means of reaching the Arctic during their  
514 planktonic phase.

515

516

## 517 5. ACKNOWLEDGEMENTS

518 We would like to thank the chief scientists, crew and fellow scientists onboard the RV Helmer  
519 Hanssen, RV Kronprins Haakon and RRS James Clark Ross for their support during our  
520 sampling efforts. Thank you to Melissa Brandner, UiT, for her help with lab work. This research  
521 has been jointly funded by UiT the Arctic University of Norway and the Tromsø Research  
522 Foundation under the project “Arctic Seasonal Ice Zone Ecology”, project number 01vm/h1 as  
523 well as by the Fram Centre Flagship “Climate Change in Fjord and Coast” grant number 272019  
524 and the Fonds de Recherche Nature et Technologies du Québec (file number 270604). The  
525 ArcticPRIZE project (NE/P006302/1 – United Kingdom Natural Environment Research  
526 Council), and the Nansen Legacy project (Norwegian Research Council project 276730)  
527 contributed ship time for sampling.

528

529

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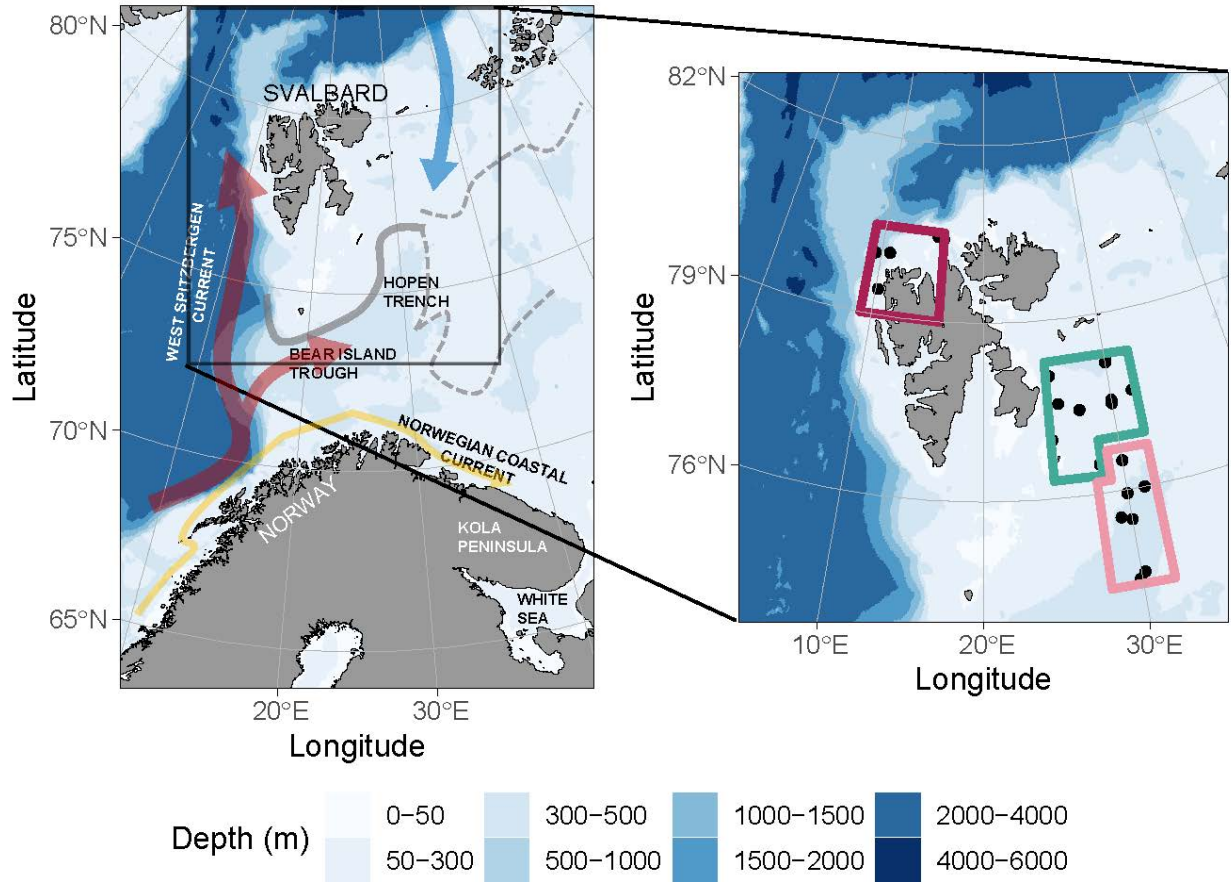
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Origin of Barents Sea Larvae

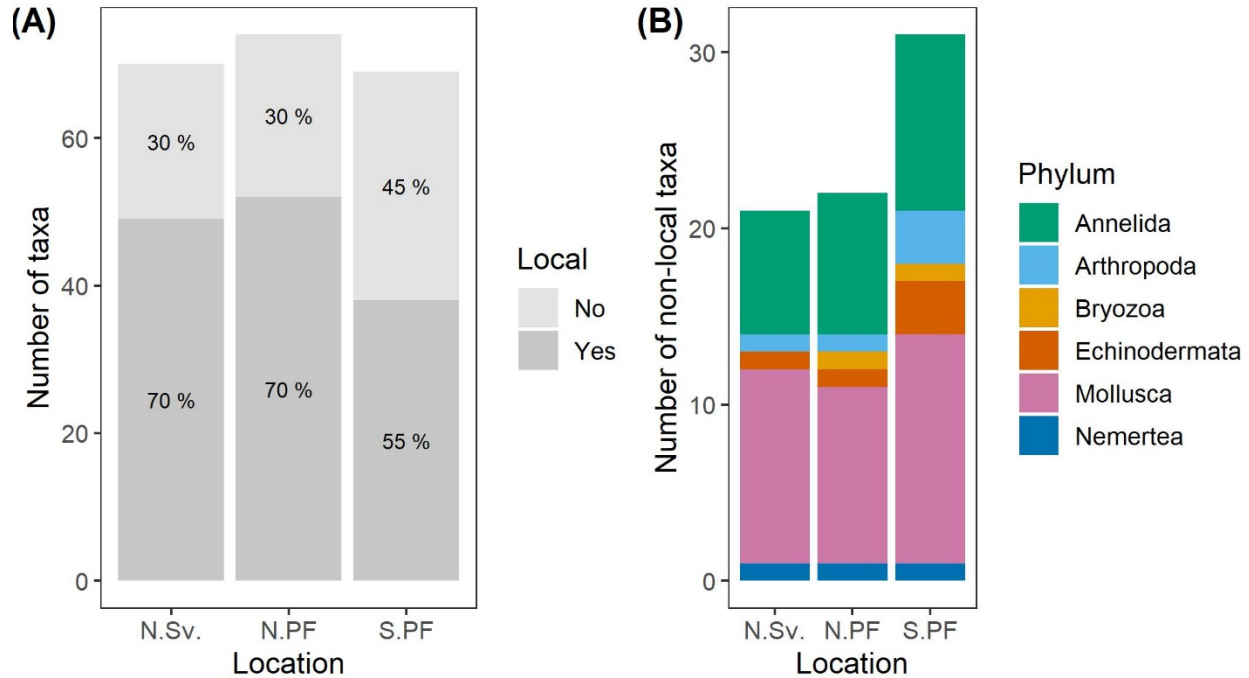
785 Table 1: List of non-local larval species for the three areas of interest, including frequency of occurrence (number of zooplankton samples in which  
 786 a taxon was detected within each area). Larval taxa whose closest known adults occur further than 1000 km away are highlighted in purple. n  
 787 indicates total number of samples per region.

Phylum	Class	North Svalbard (n = 11)		North Polar Front (n = 28)		South Polar Front (n = 22)	
Annelida	Polychaeta	<i>Dodecaceria concharum</i>	1	<i>Eunice pennata</i>	3	<i>Eunice pennata</i>	1
		<i>Eunice pennata</i>	2	<i>Eunoe oerstedii</i>	1	<i>Eunoe nodosa</i>	4
		<i>Laonice blakei</i>	2	<i>Harmothoe fragilis</i>	10	<i>Gattyana cirrhosa</i>	9
		<i>Paramphinome jeffreysii</i>	4	<i>Laonice blakei</i>	1	<i>Harmothoe fragilis</i>	9
		<i>Praxillella praetermissa</i>	1	<i>Nereis pelagica</i>	3	<i>Laonice blakei</i>	2
		<i>Proclea malmgreni</i>	3	<i>Paranaitis wahlbergi</i>	1	<i>Nereimyra woodsholea</i>	2
		<i>Terebellides gracilis</i>	2	<i>Polycirrus arcticus</i>	3	<i>Nereis zonata</i>	1
				<i>Polyphysia crassa</i>	7	<i>Nothria conchylega</i>	4
						<i>Proclea malmgreni</i>	3
				<i>Thelepus cincinnatus</i>	6		
Arthropoda	Malacostraca	<i>Bopyroides hippolytes</i>	2	<i>Bopyroides hippolytes</i>	5	<i>Bopyroides hippolytes</i>	1
						<i>Eualus gaimardii</i>	2
	Thecostraca					<i>Balanus balanus</i>	21
Bryozoa	Gymnolaemata			<i>Alcyonidium mamillatum</i>	13	<i>Alcyonidium mamillatum</i>	12
Echinodermata	Echinoidea					<i>Gracilechinus acutus</i>	1
	Holothuroidea					<i>Psolus phantapus</i>	3
	Ophiuroidea	<i>Ophiocten gracilis</i>	11	<i>Ophiocten gracilis</i>	28	<i>Ophiocten gracilis</i>	22
Mollusca	Bivalvia	<i>Serripes laperousii</i>	1	<i>Serripes laperousii</i>	5	<i>Serripes laperousii</i>	2
	Gastropoda	<i>Coryphella gracilis</i>	6	<i>Coryphella gracilis</i>	5	<i>Coryphella gracilis</i>	6
		<i>Cuthonella concinna</i>	1	<i>Cuthonella concinna</i>	3	<i>Cuthonella concinna</i>	1
		<i>Dendronotus elegans</i>	3	<i>Dendronotus elegans</i>	11	<i>Dendronotus elegans</i>	8
		<i>Dendronotus yrjargul</i>	7	<i>Dendronotus patricki</i>	7	<i>Dendronotus frondosus</i>	4
		<i>Doto coronata</i>	6	<i>Dendronotus yrjargul</i>	14	<i>Dendronotus patricki</i>	9
		<i>Doto maculata</i>	4	<i>Diaphana hiemalis</i>	10	<i>Dendronotus robustus</i>	3
		<i>Eubranthus rupium</i>	2	<i>Doto coronata</i>	1	<i>Dendronotus yrjargul</i>	12
		<i>Onchidoris muricata</i>	4	<i>Eubranthus rupium</i>	8	<i>Diaphana hiemalis</i>	5
		<i>Placida dendritica</i>	4	<i>Placida dendritica</i>	2	<i>Doto coronata</i>	9
		<i>Scaphander punctostriatus</i>	2			<i>Eubranthus rupium</i>	3
				<i>Onchidoris muricata</i>	1		
				<i>Placida dendritica</i>	1		
Nemertea	Paleonemertea	<i>Cephalothrix iwatai</i>	10	<i>Cephalothrix iwatai</i>	23	<i>Cephalothrix iwatai</i>	19

788

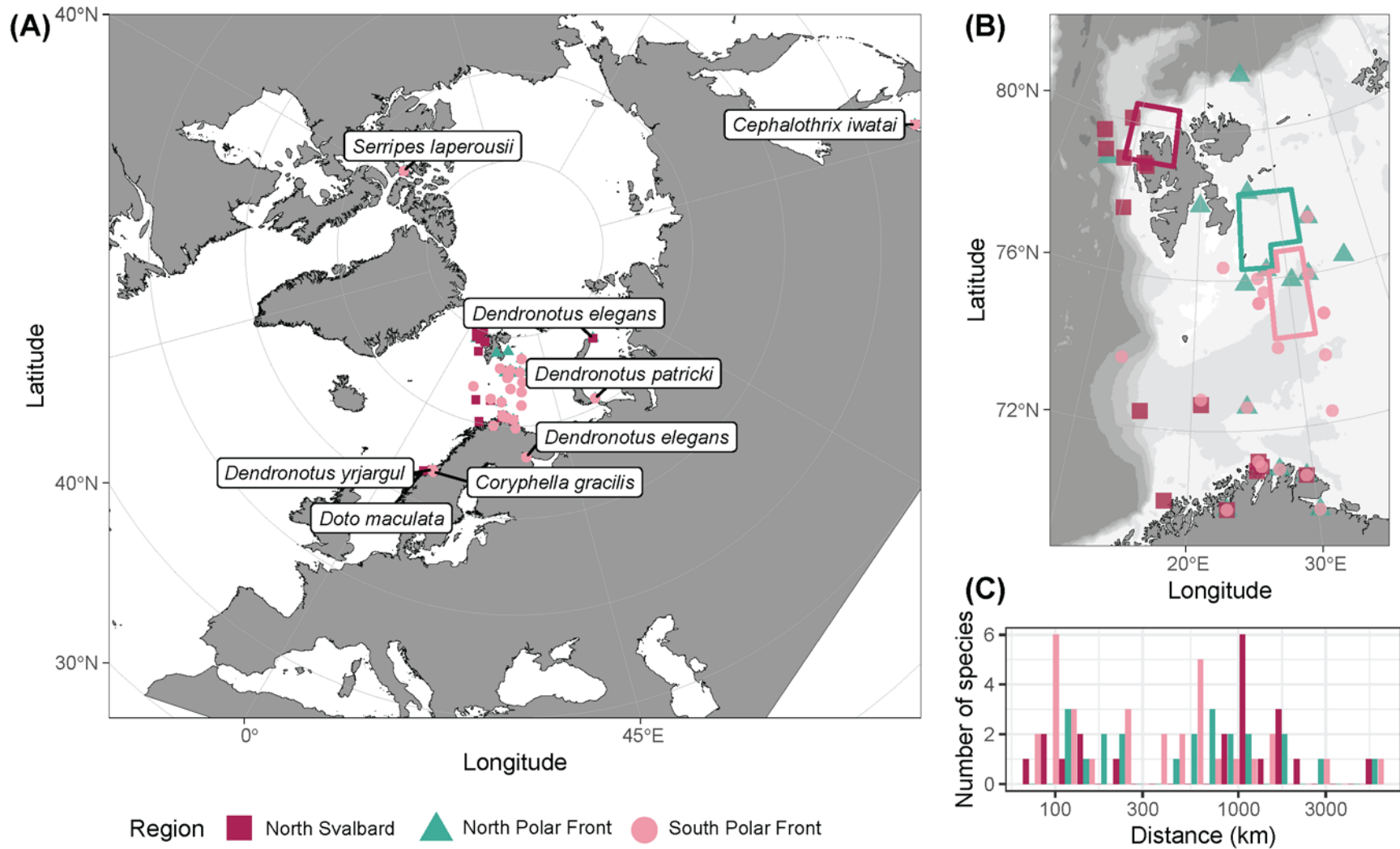


789  
 790 Fig. 1: Map of the study area with Atlantic (red arrows) and Arctic (blue arrow) water inflows, Norwegian  
 791 Coastal Current (yellow arrow) and Polar Front (grey lines). The inset shows the locations of zooplankton  
 792 sampling (black dots) and the three areas of adult community analysis and particle tracking target  
 793 (polygons): north of Svalbard (red), north of the Polar Front (green) and south of the Polar Front (light  
 794 pink). All maps presented here were produced with package ggOceanMaps (Vihtakari 2022) in R.  
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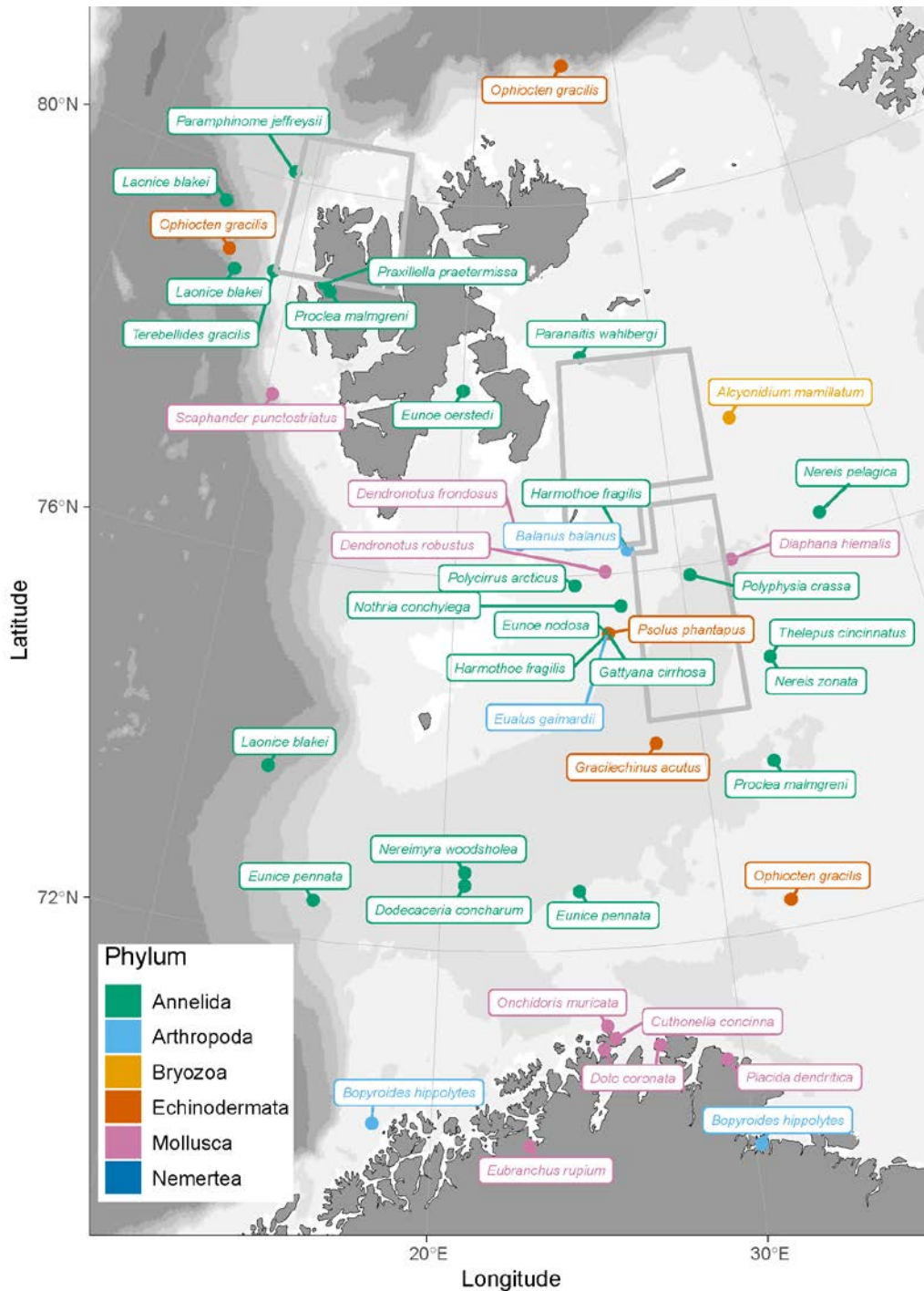


796  
 797 Fig. 2: (A) Number of benthic invertebrate taxa found in the larval pool of the Barents Sea/Svalbard for  
 798 which the adult inhabits the same area (local, dark gray) or for which the adult is not known in the area  
 799 (non-local, light gray). The percentages of local and non-local taxa are shown inside each bar. (B) The  
 800 number of non-local taxa belonging to each phylum for each of the three areas. Here, N.Sv, N.PF and  
 801 S.PF represent north of Svalbard, north of the Polar Front and south of the Polar Front, respectively.

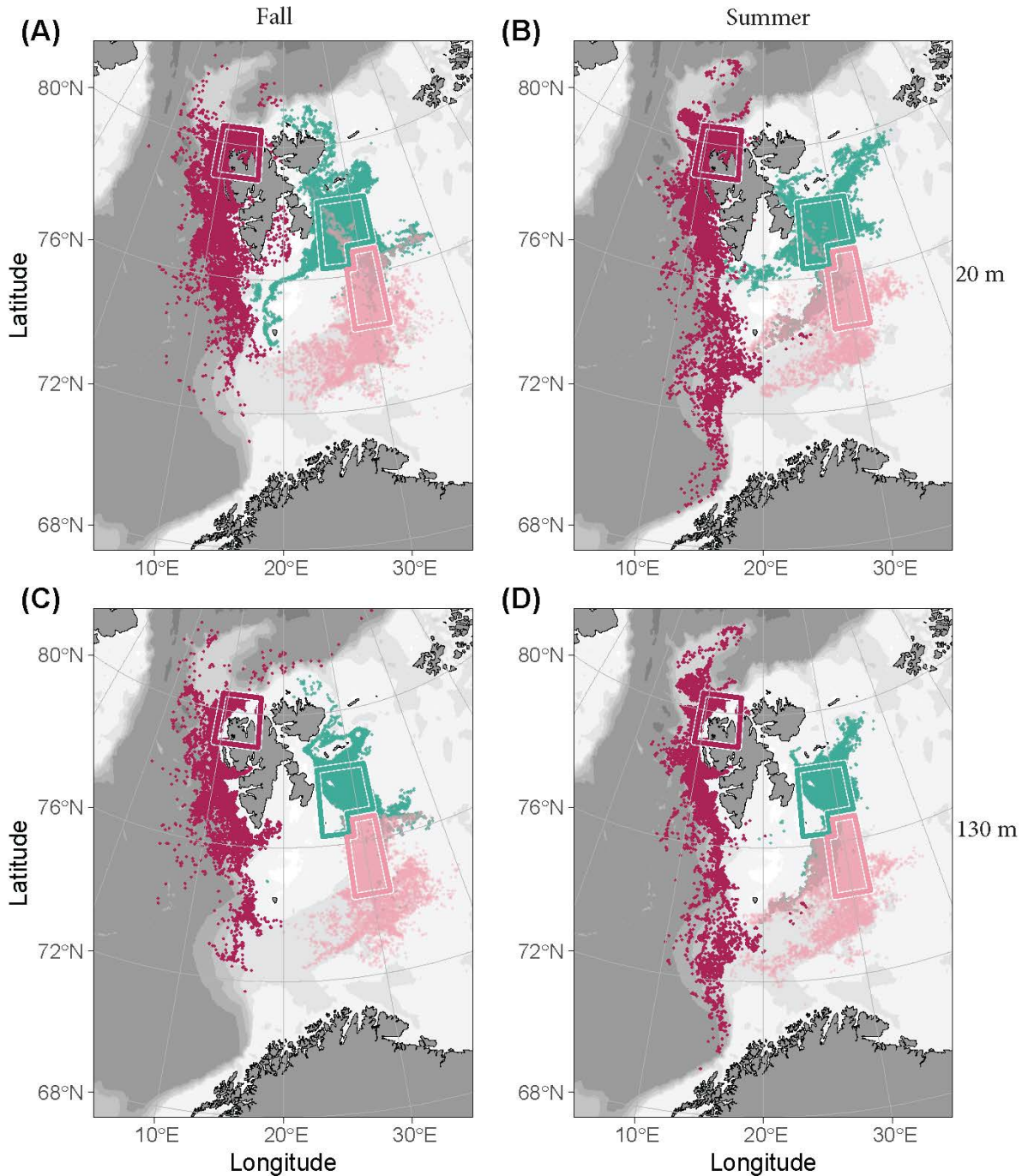




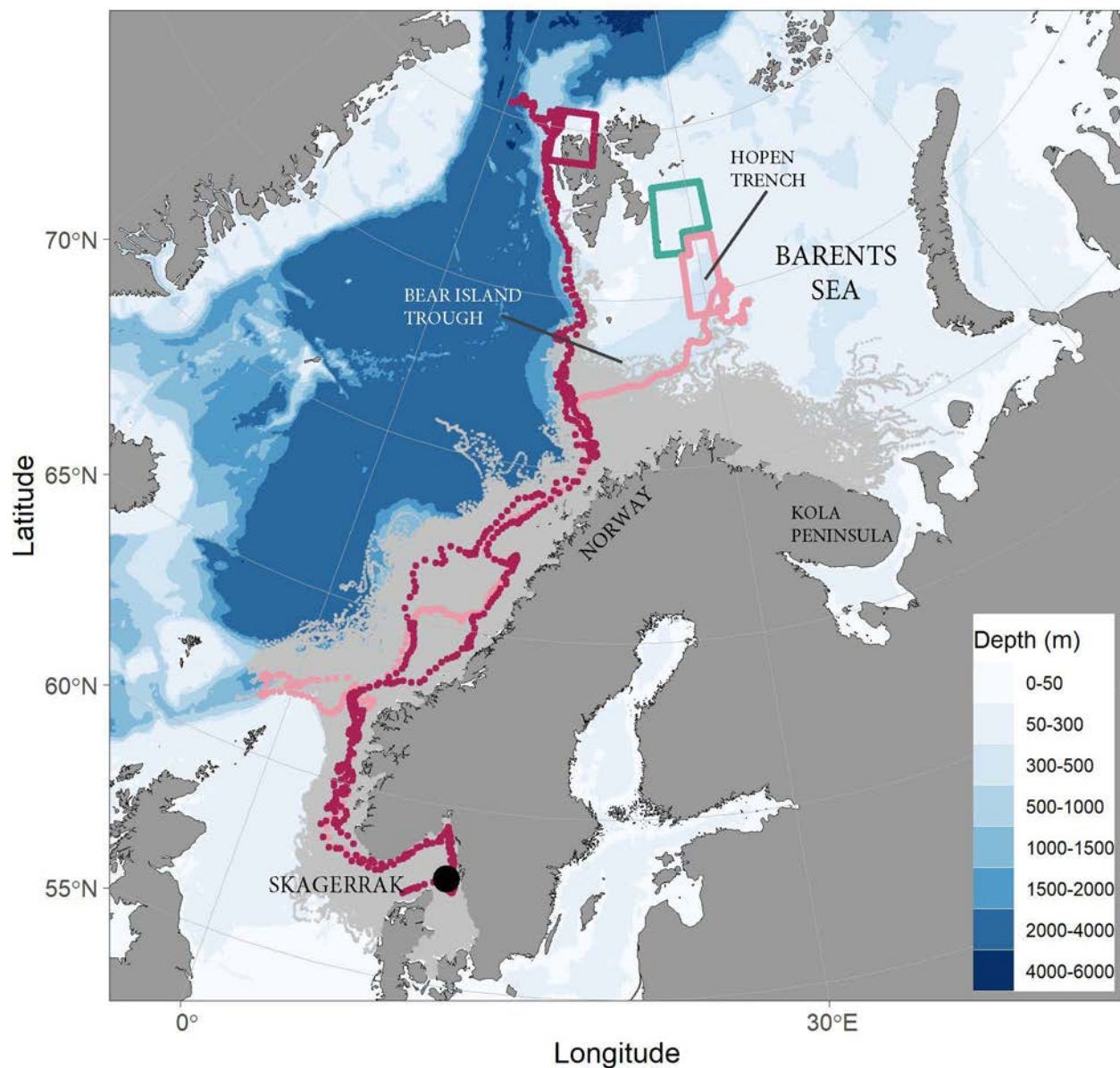
802  
803 Fig. 3: Nearest adult record for each larval taxon that was not found as adults in each of the three areas of interest in (A) a broad ocean view  
804 including labels for the taxa that are found particularly far away and in (B) a close-up of the Barents Sea, Svalbard and northern Norwegian coast.  
805 Histogram (C) showing the distribution of distances (shortest distance between two points on an ellipsoid) from the center of each area of interest  
806 to the nearest known adults (20 bins) with a log<sub>10</sub> x-axis to improve readability of lower distance values. Positions on the map and the histogram  
807 are color-coded according to the three areas of interest.



808  
 809 Fig. 4: Nearest known adults to the larval taxa that were found to be non-local to one of the three areas of  
 810 interest (grey polygons). On this map, taxa are color-coded by phylum. This figure omits the taxa for  
 811 which the nearest known adult was located in more distant locations which are presented in Fig. 3A. Note  
 812 that the presence of a taxon on this map does not necessarily mean that it is non-local to all three areas of  
 813 interest. Refer to Table 1 for a list of non-local taxa for each area of interest.



814  
 815 Fig. 5: Possible origins of particles (colored dots) drifting into one of three areas of interest (colored  
 816 polygons): north of Svalbard (red), north of Polar Front (green) and south of Polar Front (light pink),  
 817 anytime during a 3-month period starting on 23.08.2017 (fall, left) and 10.05.2018 (summer, right) at 20  
 818 m (top) and 130 m depth (bottom).



819  
 820 Fig. 6: Drift trajectories of simulated particles released in Skagerrak in January 2018 at 20 m depth and  
 821 allowed to drift for one year. The black dot represents the location where all particles were released while  
 822 the colored polygons represent the three areas of interest: north of Svalbard (red), north of the Polar Front  
 823 (green) and south of the Polar Front (light pink). All drift trajectories are shown in light gray, but the  
 824 trajectories of the two particles that eventually drifted into the area north of Svalbard are highlighted in red  
 825 and that of the single particle that reached the area south of the Polar Front in pink.  
 826

Table S1: Stations for which zooplankton samples were metabarcoded where Lat and Long represent latitude and longitude in decimal degrees, respectively, Depth is given in metres, Mesh size in  $\mu\text{m}$

Location	Cruise	Station ID	Lat	Long	Date	Depth	Net	Mesh	Fraction of sample metabarcoded
North Polar Front	Ecosystem survey	461	76.985	25.113	2017-09-18	40-0	WP2	180	0.5
		475	77.583	25.817	2017-09-21	168-0	WP2	180	0.5
	ArcticSIZE	B3	78.062	25.305	2017-11-22	50-0	WP2	64	0.5
			78.062	25.305	2017-11-22	100-50	WP2	64	0.5
			78.062	25.305	2017-11-22	115-100	WP2	64	0.5
		B4	77.441	27.411	2017-11-23	100-50	WP2	64	0.49
			77.441	27.411	2017-11-23	160-100	WP2	64	0.47
		ArcticPRIZE January	B34	77.472	29.968	2018-01-10	80-0	WP2	64
	77.472			29.968	2018-01-10	130-80	WP2	64	0.22
	77.472			29.968	2018-01-10	191-130	WP2	64	0.42
	B51		78.216	30.084	2018-01-10	70-0	WP2	64	0.5
			78.216	30.084	2018-01-10	225-70	WP2	64	0.5
			78.216	30.084	2018-01-10	313-225	WP2	64	0.5
	ArcticPRIZE April	HH70	76.491	28.366	2018-04-29	0-40	Multinet	180	0.48
			76.491	28.366	2018-04-29	80-40	Multinet	180	0.48
			76.491	28.366	2018-04-29	80-40	WP2	64	0.5
			76.491	28.366	2018-04-29	120-80	Multinet	180	0.44
			76.491	28.366	2018-04-29	140-120	Multinet	180	0.45
	ArcticPRIZE June	H51	78.166	29.999	2018-06-26	40-0	WP2	64	0.5
			78.166	29.999	2018-06-26	145-36	WP2	64	0.5
			78.166	29.999	2018-06-26	330-145	WP2	64	0.5
		B34	77.531	29.979	2018-06-27	15-0	WP2	180	0.23
			77.531	29.979	2018-06-27	130-15	WP2	64	0.19
			77.531	29.979	2018-06-27	195-130	WP2	64	0.38
	Nansen Legacy	R1	77.634	31.689	2018-08-11	30-0	Multinet	64	0.37
			77.634	31.689	2018-08-11	110-30	Multinet	64	0.48
			77.634	31.689	2018-08-11	140-110	Multinet	64	0.46
			77.634	31.689	2018-08-11	145-140	Multinet	64	0.43
North Svalbard	SI Arctic	119	80.389	15.964	2017-09-02	30-0	WP2	180	0.5
			80.389	15.964	2017-09-02	330-0	WP2	180	0.5
		125	80.029	9.395	2017-09-04	50-0	WP2	180	0.5
			80.029	9.395	2017-09-04	485-0	WP2	180	0.5
	ArcticSIZE	W1	79.429	10.212	2017-11-18	10-0	WP2	64	0.5
			79.429	10.212	2017-11-18	85-0	WP2	64	0.5
			79.429	10.212	2017-11-18	100-85	WP2	64	0.5
		NS1	80.427	15.418	2017-11-19	85-0	WP2	64	0.5
	80.427		15.418	2017-11-19	145-85	WP2	64	0.5	
	ArcticPRIZE June	JR78c	80.064	10.793	2018-06-13	20-0	WP2	180	0.5
80.064			10.793	2018-06-13	390-0	WP2	180	0.5	
South Polar Front	ArcticPRIZE January	B14	76.499	30.001	2018-01-08	273-180	WP2	64	0.5
		HH57	75.558	29.282	2018-01-12	200-0	WP2	64	0.46
			75.558	29.282	2018-01-12	250-200	WP2	64	0.43
			75.558	29.282	2018-01-12	321-250	WP2	64	0.41

	ArcticPRIZE April	B13	74.603	30.230	2018-04-26	10-0	Multinet	180	0.5
			74.603	30.230	2018-04-26	25-10	Multinet	180	0.5
			74.603	30.230	2018-04-26	50-25	Multinet	180	0.5
			74.603	30.230	2018-04-26	100-50	Multinet	180	0.5
		B14	76.498	30.013	2018-04-28	10-0	Multinet	180	0.5
			76.498	30.013	2018-04-28	40-10	Multinet	180	0.5
			76.498	30.013	2018-04-28	280-40	Multinet	180	0.5
		HH71	75.940	29.966	2018-04-30	40-0	Multinet	180	0.39
			75.940	29.966	2018-04-30	305-200	Multinet	180	0.45
			75.940	29.966	2018-04-30	310-0	WP2	64	0.5
	ArcticPRIZE June	B14	76.500	30.000	2018-06-29	50-0	WP2	64	0.5
			76.500	30.000	2018-06-29	275-50	WP2	64	0.5
		B13	74.493	29.862	2018-07-01	45-0	WP2	64	0.5
			74.493	29.862	2018-07-01	200-45	WP2	64	0.5
		B35	75.500	30.000	2018-07-02	45-0	WP2	64	0.47
			75.500	30.000	2018-07-02	345-45	WP2	64	0.45
	Nansen Legacy	P1	76.000	31.223	2018-08-09	30-0	Multinet	64	0.49
			76.000	31.223	2018-08-09	310-30	Multinet	64	0.49

# Paper III

Ershova EA, Descôteaux R, Wangensteen OS, Iken K, Hopcroft RR, Smoot C, Grebmeier JM, Bluhm BA (2019) Diversity and distribution of meroplanktonic larvae in the Pacific Arctic and connectivity with adult benthic invertebrate communities. *Front Mar Sci* 6:490, <https://doi.org/10.3389/fmars.2019.00490>









# Diversity and Distribution of Meroplanktonic Larvae in the Pacific Arctic and Connectivity With Adult Benthic Invertebrate Communities

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## OPEN ACCESS

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### Specialty section:

This article was submitted to  
Marine Ecosystem Ecology,  
a section of the journal  
Frontiers in Marine Science

**Received:** 24 May 2019

**Accepted:** 22 July 2019

**Published:** 13 August 2019

### Citation:

Ershova EA, Descoteaux R,  
Wangensteen OS, Iken K,  
Hopcroft RR, Smoot C,  
Grebmeier JM and Bluhm BA (2019)  
Diversity and Distribution  
of Meroplanktonic Larvae  
in the Pacific Arctic and Connectivity  
With Adult Benthic Invertebrate  
Communities. *Front. Mar. Sci.* 6:490.  
doi: 10.3389/fmars.2019.00490

Pelagic larval stages (meroplankton) of benthic invertebrates seasonally make up a significant proportion of planktonic communities, as well as determine the distribution of their benthic adult stages, yet are frequently overlooked by both plankton and benthic studies. Within the Arctic, the role of meroplanktonic larvae may be particularly important in regions of inflow from sub-Arctic regions, where they can serve as vectors of advection of temperate species into the Arctic. In this study, we describe the links between the distribution of larvae and adult benthic communities of bivalves, echinoderms, select decapods and cnidarians on the Pacific-influenced Chukchi Sea shelf during August–September in the time period 2004–2015 using traditional morphological and molecular tools to resolve taxonomic diversity. For most taxa, we observed little regional overlap between the distribution of larvae and adults of the same taxon; however, larvae of some organisms (e.g., the burrowing anemone *Cerianthus* sp., the sand dollar *Echinarachnius parma*) were only observed near populations of adult organisms. Larval stages of species not commonly observed in the Chukchi Sea benthos were also observed in the plankton; overall, shelf meroplanktonic communities were numerically dominated by larvae of coastal hard-bottom taxa, rather than local soft-bottom shelf species. Our results suggest that most larvae that we observe on the shelf are advected from other areas rather than produced locally, and most likely will not successfully settle to the benthos. Seasonality and distribution of water masses were the most important parameters shaping meroplankton communities. We discuss the implications of changing oceanographic and climatic conditions on the potential of range extensions by temperate species into the Arctic Ocean.

**Keywords:** meroplankton, zooplankton, Chukchi Sea, Pacific Arctic, DNA barcoding, benthic ecology, planktonic larvae

## INTRODUCTION

The Chukchi Sea is one of two inflow regions of the Arctic, providing the only connection between the temperate Pacific region and high Arctic Ocean (**Figure 1**). Every year, 0.8–1.2 Sv of Pacific water enter the Arctic through the Bering Strait (Woodgate, 2018), bringing in large quantities of heat, freshwater, nutrients, as well as rich pelagic communities of phyto- and zooplankton along

with their predators. Unlike the Fram Strait opening in the Atlantic, the Bering Strait is narrow and shallow. The broad and shallow (<50 m) Chukchi Sea shelf serves as an impediment to Pacific organisms being advected into the Arctic Ocean, because most of this advected biomass and local production will not travel past the shelf break (Kosobokova et al., 2011; Grebmeier and Maslowski, 2014).

Most of the flow entering the Chukchi Sea from the Pacific is dominated by a mixture of two currents: the Anadyr Current, originating on the Bering Sea slope and carrying cold, nutrient-rich oceanic water, and the Bering Shelf Current, which originates on the Bering Sea shelf and is warmer and lower in nutrients (Coachman and Aagaard, 1975). These two currents merge as they travel through the Bering Strait, forming Bering Sea-Anadyr Water, then split into three branches: one exiting north through Herald Canyon; one east of Hanna Shoal and through Barrow Canyon; and one through the Central Channel, which separates Herald Valley and Hanna Shoal (Weingartner et al., 2005; **Figure 1**). Due to uneven mixing of the two original currents, the western branch through Herald Valley is much richer in nutrients than the others, indicative of a higher contribution by Anadyr Water. Additionally, flowing adjacent to the Alaska coast is the highly seasonal, buoyancy-driven Alaska Coastal Current, which originates in the Gulf of Alaska, fed by river- and glacial discharge along its course, and carrying with it heat, freshwater and comparatively warmer-water organisms as far as the Beaufort Sea (Smoot and Hopcroft, 2017). Occasionally, winds carry this flow away from the coast, and during some years its signature has been observed covering the entire southern Chukchi Shelf (Pisareva et al., 2015b). Most of the water transport through the Bering Strait takes place in the summer months: during winter, the Chukchi and northern Bering Sea are completely covered by sea ice, the northward flow slows down or reverses (Woodgate et al., 2015), and the system “reverts” from an advection-dominated system to one resembling interior Arctic shelf seas (Williams and Carmack, 2015). Remnants of the cold, saline water mass that forms during ice formation remains near the sea floor on the northern Chukchi Shelf during summer as Winter Water (WW) (Weingartner et al., 2005), and is home to “resident” Chukchi Sea pelagic communities of organisms (Ershova et al., 2015a). A fourth water mass type, Siberian Coastal Water, is sometimes present in the western Chukchi Sea when the cold, buoyancy driven East-Siberian Coastal Current enters through Long Strait (Weingartner et al., 1999).

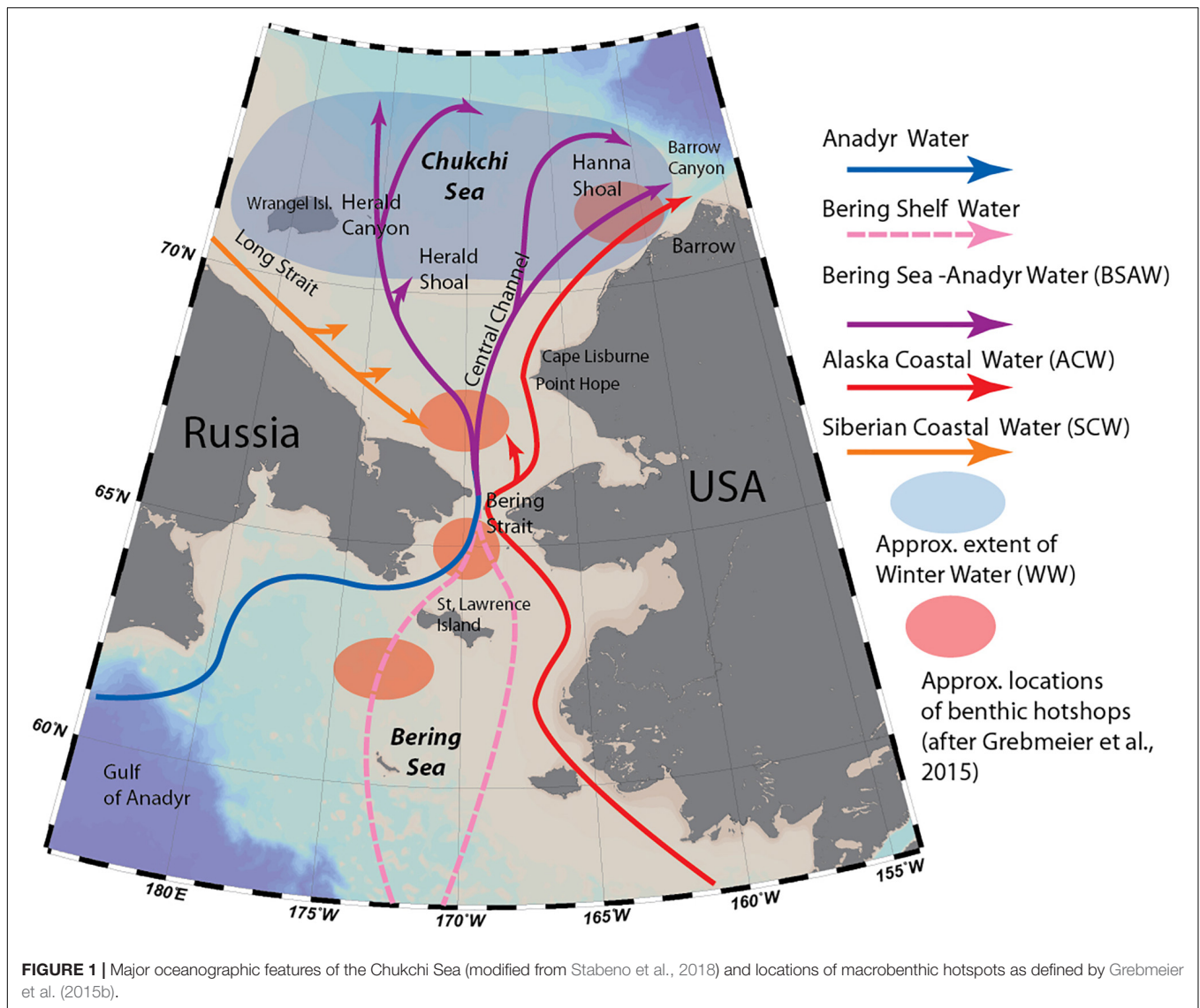
The persistent, high levels of advected and local pelagic primary production during the ice-free season on the Chukchi Shelf cannot be fully grazed by pelagic consumers, resulting in strong benthic-pelagic coupling, and very high benthic biomass in the region. In particular, the northern Bering Sea/Chukchi region is home to four benthic biomass “hotspots,” located in the pathway of the nutrient rich Anadyr water (**Figure 1**), characterized by a stable, exceptionally high (>20 g C m<sup>-2</sup>) biomass of macrobenthic organisms (Grebmeier et al., 2015b), which in turn provide a rich food source to a variety of higher trophic levels, such as walrus and diving sea

birds. The area is also home to diverse and biomass-rich epibenthic communities (Bluhm et al., 2009; Blanchard et al., 2013).

Most benthic organisms reproduce by means of a pelagic larval stage (meroplankton), which may live in the plankton for hours to months, and allows them to disperse across wide areas. The ecological significance of planktonic larvae is two-fold: they are a dispersal stage for benthic organisms (Shanks, 2009), determining the potential of benthic species to colonize adjacent habitats, but they can also constitute a major portion of zooplankton communities in high latitude waters (Gluchowska et al., 2016), potentially competing for resources with holoplanktonic species, and serving as food source for planktonic predators (e.g., Allen, 2008; Short et al., 2013). An important biophysical process in benthic ecosystems is larval transport, which in advection-dominated systems can cause spatial decoupling between the production of local communities and settlement of juveniles. In Arctic regions highly influenced by northward flow, such as the Chukchi Sea, meroplanktonic larvae can serve as vectors of advection of sub-Arctic species into the Arctic. For sessile or slow-moving benthic species, larval transport is one of the only apparent mechanisms by which this expansion can occur (Renaud et al., 2015), which makes studies of larval communities within key inflow regions of utmost importance.

Despite being an important life stage of many key benthic species, and a seasonally significant contributor to zooplankton, meroplankton has been historically overlooked by benthic community ecologists, while plankton studies, at best, have grouped them into broad taxonomic categories. Studies on meroplankton are hampered by their temporally patchy occurrence in the plankton, as well as lack of morphological features (and/or taxonomic expertise) to assign them beyond phylum or class level. In large part due to extensive DNA barcoding efforts, such as done through the Census of Marine Life (McIntyre, 2010) and a growing richness of reference libraries (i.e., Barcode of Life Data Systems, BOLD<sup>1</sup>), it is becoming increasingly feasible to use barcoding instead of, or in addition to, morphology to describe biodiversity in marine ecosystems. Yet to date, there are only a few studies focusing on meroplankton at the species level, and even fewer from the high latitudes (i.e., Stanwell-Smith et al., 1999; Sewell, 2005; Fetzer and Arntz, 2008; Bowden et al., 2009; Heimeier et al., 2010; Sewell and Jury, 2011; Gallego et al., 2015; Silberberger et al., 2016; Brandner et al., 2017). The Pacific Arctic region is no exception; although both benthic (Grebmeier et al., 2006; Bluhm et al., 2009; Grebmeier, 2012; Iken et al., 2018) and pelagic (Hopcroft et al., 2010; Eisner et al., 2013; Ershova et al., 2015a; Pinchuk and Eisner, 2017) summer communities of the Chukchi Sea, particularly on the United States side, have been studied extensively and described in great detail during the last decades (Day et al., 2013; Sigler et al., 2017), meroplankton has been largely overlooked, with

<sup>1</sup><http://v3.boldsystems.org/>



the exception of some studies focusing on specific groups (i.e., Landeira et al., 2017).

Presumably, the meroplanktonic communities in the Chukchi Sea consist of larvae both produced by the local benthic organisms, as well as advected with currents from other regions. In this study we examined the patterns in summer distribution of planktonic larvae in the Chukchi Sea region with reference to the distribution of their adult populations. To achieve previously intractable taxonomic resolution, one of the objectives of this study was to develop a time and cost-effective DNA barcoding protocol that would allow us to routinely resolve taxonomic diversity of larvae within the zooplankton and examine select taxonomic groups at or near the species level. We use these data to describe the diversity and distribution patterns of planktonic larvae on the Chukchi shelf during summer for five study years each with distinct oceanographic and thermal regimes, and with special focus on potential range expanders from the sub-Arctic. We aimed to identify the main driving factors that

shape the meroplanktonic communities on the Chukchi sea shelf, anticipating that both local production and advection play important roles in the distribution and the fate of the larvae that we observe in this region.

## MATERIALS AND METHODS

### Zooplankton and Benthos Data

Data on meroplankton distribution were obtained from published (Hopcroft et al., 2010; Eisner et al., 2013; Ershova et al., 2015a) and unpublished zooplankton surveys, conducted during summer-fall of 2004, 2007, 2009, 2012, and 2015 (Table 1) in the Chukchi Sea. All studies used a 150  $\mu\text{m}$  Bongo net (ring diameter 60 cm), which was hauled vertically from a few meters off the bottom to the surface with a wire speed of 0.5 m/s and General Oceanics Flowmeters attached at the mouth opening. For all surveys, meroplankton was

**TABLE 1** | List of data sources used in the study.

Cruise	Dates	Year	Area	Type of data included
AMBN	8 August–10 September	2015	SE/NE Chukchi Sea	Meroplankton, Epifauna, Oceanography
RUSALCA	8 August–24 August	2004	S/W Chukchi Sea	Meroplankton, Epifauna, Infauna, Oceanography
	1 September–30 September	2009	S/W Chukchi Sea	Meroplankton, Epifauna, Infauna, Oceanography
	1 September–15 September	2012	S/W Chukchi Sea	Meroplankton, Epifauna, Infauna, Oceanography
BASIS	4 September–17 September	2007	SE Chukchi Sea	Meroplankton, Epifauna, Oceanography

counted quantitatively from formalin-preserved samples and identified to macrotaxa level (i.e., Bivalvia, Echinodermata, Polychaeta, etc.), with the exception of crab megalopa in 2012 and 2015 that were identified to species. Since crab larvae are good swimmers and can avoid nets (Porter et al., 2008), for this group we included data from selected stations collected with a semi-oblique 505  $\mu\text{m}$  Bongo net, which is much better at capturing larger and faster swimming planktonic organisms. For the purposes of our study, we converted all zooplankton abundance values to  $\text{ind m}^{-2}$ . For more details on zooplankton sample processing and enumeration, see Ershova et al. (2015a).

Macrofaunal and megafaunal abundance and biomass estimates were obtained from benthic van Veen grab and beam trawl catches, respectively, taken concurrent to zooplankton collection during each expedition (Bluhm et al., 2009; Grebmeier et al., 2015a; Iken et al., 2018). Macrofauna samples were washed over 1 mm screen and bivalves were identified to species or family level, enumerated and weighed, with typically four replicates per station. Crabs and echinoderms were sorted from beam trawl catches (7 mm mesh, 4 mm in cod end), enumerated and weighed after identification in the field; taxonomic identification was later confirmed by taxonomic experts based on voucher material where needed. Densities of the burrowing anemone *Cerianthus* sp. were taken from a photographic survey (Sirenko and Gagaev, 2007), since this taxon rarely is captured with trawls or grabs. For each station, the abundances of benthic invertebrates in a given taxon were combined to match the taxonomic resolution of the meroplankton identification and averaged for each station over all sampling years. While some interannual/interdecadal trends in benthic biomass are recognized (Grebmeier et al., 2018), large-scale benthic communities biomass patterns in the Chukchi Sea have remained relatively stable on an inter-annual basis (Grebmeier et al., 2015a).

## Water Mass Distribution and Thermal Characteristics

Bottom depth, and depth-stratified temperature and salinity measurements were obtained for each station (collected by a Seabird 911 + CTD, with all physical data binned into 1-m intervals during post-processing). For each station, we calculated surface (top 10 m) and bottom (10 m above the seafloor to bottom) temperature and salinity values.

The distribution and properties of the water masses, as well as the overall thermal characteristics and patterns in zooplankton communities in the Chukchi Sea during the expeditions are described in detail elsewhere for all years except 2015 (Pickart

et al., 2010; Ershova et al., 2015a,b; Pisareva et al., 2015a,b). Surface- and bottom- water at each station was assigned to a water mass type based on temperature and salinity characteristics in accordance with methodology for previous years (Ershova et al., 2015b). Three major water masses dominated the region (**Figure 2**): Alaska Coastal Water (ACW), Bering-Sea Anadyr Water (BSAW), and WW, although the boundary between the first two was not always well defined and an intermediate water mass (ACW/BSAW) was assigned to stations with mixed properties. ACW was mostly found in the eastern Chukchi, although its signature was also observed away from the coast, especially in 2009, when this water mass was spread across the entire shelf as far north as Herald Canyon (**Figure 2**). BSAW was widespread across the shelf during all years, although it was pushed westward in 2009. WW was observed in the northern Chukchi during all years except 2007, when the more northern regions above 70°N were not sampled. The summers of 2004 and 2007 were the warmest of the study years, with an average surface temperature (SST) of 6–8°C over the sampled stations, and with surface waters as warm as 10–12°C present along the Alaska Coast and at the entrance of the Herald Canyon region. The years 2009 and 2012 were markedly colder, with the coldest SST observed in September 2012, averaging only 3.4°C, despite the all-time low ice-minimum extent observed during that year in most parts of the Arctic. In 2013–2014 the system shifted to warmer conditions again (Wood et al., 2015), and 2015 was also characterized by warmer than average conditions.

## DNA Barcoding

The diversity of bivalve and echinoderm larvae was investigated using DNA barcoding. These two groups were chosen because they were among the best represented both in the meroplankton and the benthic communities, as well as had the most complete reference libraries and were challenging to identify morphologically. While barnacle and polychaete larvae were also very common in the meroplankton, the former were presumably composed of only one or two species, limited in their adult distribution to rocky coastal regions, and the reference libraries for the latter are still among the poorest. The majority of the analyzed zooplankton samples had a second replicate preserved in 97% ethanol. For a total of 26 stations, we randomly selected 20–30 individuals of each bivalve and echinoderm larva from these ethanol-preserved samples. The stations were chosen based on overall abundance of the larvae, spatial coverage, and sample quality/availability. Each individual larva was soaked for ~10 min in MilliQ water, then transferred using sterile tools into individual wells on a 96-well plate containing 25  $\mu\text{l}$

Alkaline Lysis Buffer (ALB) on ice. Bivalves were crushed with the flat blade of a sterilized micro-scalpel prior to transfer. DNA extractions were conducted using the HotShot method (Truett et al., 2000); the plates containing larvae in ALB were incubated at 95°C for 30 min, then the plates were transferred onto ice and 25  $\mu$ l of Tris-HCl was added to each well. This extraction method is very quick (30 min), inexpensive, and requires only two pipetting steps with no transfer of DNA, which ensures minimal loss of material. A total of 1030 larvae were processed in this way. Massive parallel barcoding of all extracts was achieved using a high-throughput sequencing strategy. A  $\sim$ 313 base pair (bp) fragment from the 5' region of the COI gene was amplified using a single-PCR protocol using tagged versions of the highly degenerated primer set Leray-XT (Wangensteen et al., 2018), containing forward primer mCOIintF-XT 5'-GGWACWRGWTGRACWITITAYCCYCC-3' and reverse primer jgHCO2198 5'-TAIACYTCIGGRTGICCRAARAAYCA-3' (Geller et al., 2013). Sample tags of 8 bp were attached to both ends of the primers. Amplification was conducted using AmpliTaq Gold DNA polymerase, with 1  $\mu$ l of each 5  $\mu$ M forward and reverse tagged primers, 3  $\mu$ g of bovine serum albumin and 2  $\mu$ l of extracted DNA in a total volume of 20  $\mu$ l per sample. The PCR protocol consisted of a denaturing step of 10 min at 95°C, 35 cycles of: 94°C for 1 min, 45°C for 1 min and 72°C for 1 min, and a final extension of 5 min at 72°C. To test the DNA quality, 80 samples, selected randomly, were sequenced using Sanger Sequencing. Sequencing reactions were performed on amplicons purified with ExoSap, using the BigDye Terminator v3.1 kit (Applied Biosystems). The sequencing products were analyzed on the ABI 3130  $\times$  1 genetic analyzer (Applied Biosystems). The resulting sequences were assembled and aligned in BioEdit, the ends trimmed, and searched across both BLAST and BOLD (Barcode of Life) databases for identification. The remaining samples were purified using Minelute PCR purification columns<sup>2</sup> and pooled (96 samples per library). Ten Illumina libraries were built from the DNA pools using the NextFlex PCR-free library preparation kit (Perkin-Elmer). This protocol incorporates Illumina adapters using a ligation procedure without any further PCR step, thus minimizing biases. The resulting libraries were equalized using qPCR then sequenced on an Illumina MiSeq using a V3 2  $\times$  250 bp kit.

## Bioinformatics

Metabarcoding sequences were analyzed following a similar pipeline as in Siegenthaler et al. (2019). Initial steps and quality control were based on OBITools (Boyer et al., 2016). Paired-end reads were aligned using illumina-paired-end, and reads with alignment score >40 were retained. Demultiplexing and primer removal were achieved using ngsfilter with the default options. Sequences with length outside the 300–320 bp range and containing ambiguous bases were filtered out. Obiuniq was then used to dereplicate the reads and chimeric sequences were removed using the uchime\_denovo algorithm implemented in VSearch (Rognes et al., 2016). We used Swarm 2.0 (Mahé et al., 2015) with a distance value of  $d = 13$

<sup>2</sup>www.qiagen.com

(Wangensteen and Turon, 2017) to cluster unique sequences into OTUs. After removal of singletons, taxonomic pre-assignment of the representative sequences of every OTU was performed using ecotag (Boyer et al., 2016). Finally, taxonomic assignment of the most abundant OTU in each sample was checked by querying the sequences against the NCBI nucleotide database using BLAST and against the BOLD database (Ratnasingham and Hebert, 2007). All taxon names were standardized to the World Register of Marine Species (Costello et al., 2013).

## Data Analysis

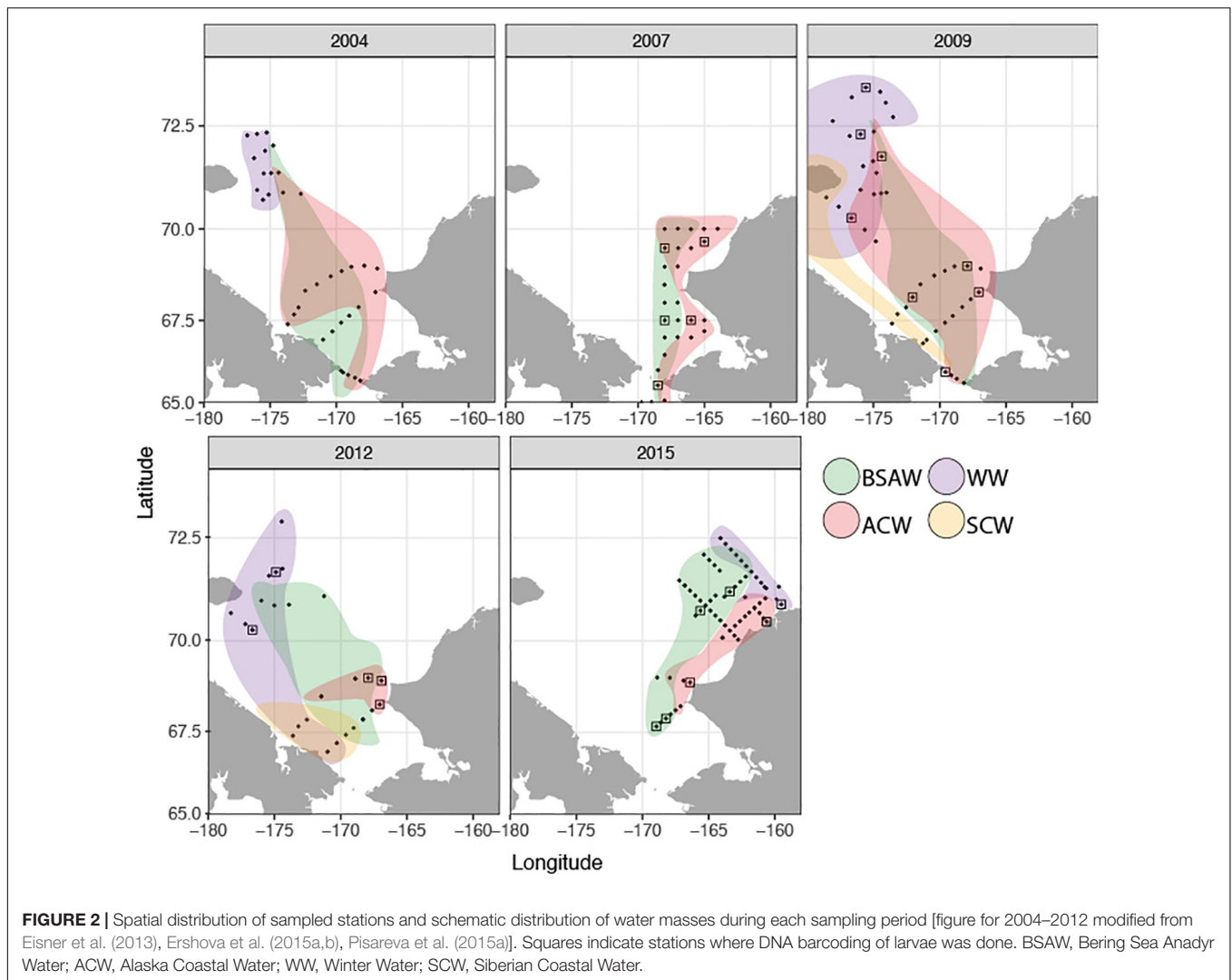
All mapping and analyses were performed in R, using the package vegan (Oksanen, 2013). Patterns in community structure were examined using non-metric multidimensional scaling (nMDS) of Bray-Curtis similarities between stations. Prior to analysis, abundance matrices of examined taxa (separate matrices for macrotaxa and barcoded bivalves and echinoderms) were log-transformed, and then standardized using a Wisconsin standardization. Only species/groups that contributed at least 5% of the abundances at any station were included. The resulting ordination was correlated to taxa abundances and physical characteristics using the *envfit* function to determine which species or groups, as well as oceanographic, spatial and temporal characteristics (surface and bottom temperature and salinity, bottom depth, latitude and longitude, day of year sampled) drove the separation of the communities. The significance ( $p$ ) and goodness of fit ( $R^2$ ) of the correlated variables were determined via 999 permutations of the variables.

Non-parametric permutational multivariate analysis of variance (PERMANOVA) was applied to test community differences between pre-assigned water mass types (surface and bottom) and sampling years (function *adonis*). The Bray-Curtis similarity index was calculated for log-transformed abundances, similar to the previous analysis. Dispersion within groups was tested using the function *betadisper*; non-significant results of the test were assumed to meet the PERMANOVA assumption of equal dispersion. Pseudo- $F$ ,  $p$  and  $R^2$  values were calculated based on 999 permutations of the residuals. Significant differences between specific pairs (years and/or water masses) were determined using a permutational multivariate pairwise  $T$ -test with a Holm adjustment for the resulting  $p$ -values.

## RESULTS

### Overall Meroplankton Distribution

Meroplankton was observed at every sampled station and in highly variable numbers, with abundances ranging from <100 ind  $m^{-2}$  to over 500,000 ind  $m^{-2}$  (Figure 3), and composing 1–90% of total zooplankton abundance on some stations (12% on average). The highest numbers of planktonic larvae were observed in August 2004, when the average across all stations was  $105,000 \pm 330,000$  (mean and SD) ind  $m^{-2}$  and the mean contribution to overall zooplankton abundance was >30%. The lowest abundances were observed in September 2012 (mean  $5800 \pm 11300$  ind  $m^{-2}$ , 5% of overall zooplankton). During most years, the highest numbers of larvae were observed at



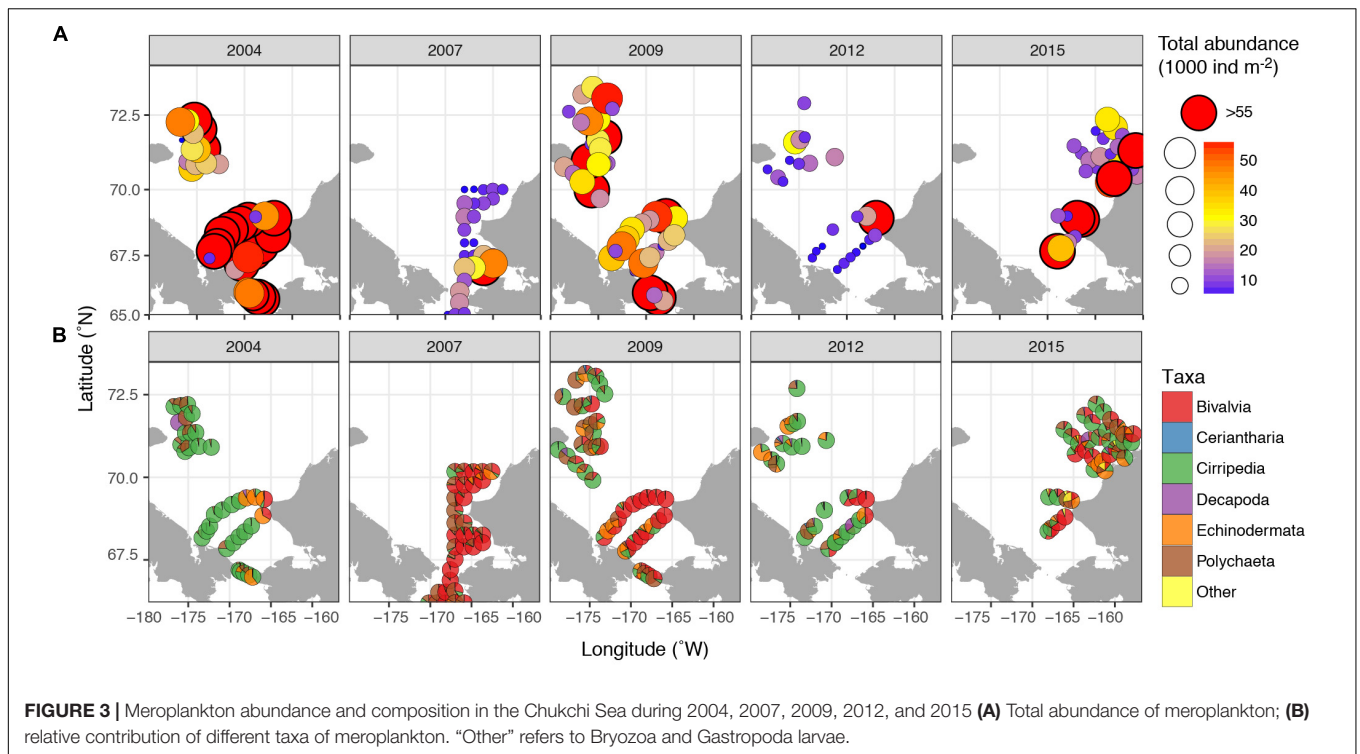
stations near the Alaskan Coast, or if away from the coast, in water influenced by the Alaska Coastal Current (2004 and 2009) (Figures 2, 3).

The most abundant meroplankton taxa across all years and stations were barnacle (Cirripedia) and bivalve larvae (Figure 3 and Table 2). Barnacle larvae were particularly abundant in the central and northwestern Chukchi, and were most numerous during August 2004, when on average they composed almost  $60,000 \text{ ind m}^{-2}$ , in distinct contrast to 2007, when they were practically absent. Bivalves were numerous during all years, especially 2007 and 2009, and were particularly dominant in the eastern Chukchi Sea (or in waters influenced by the Alaska Coastal Current, as in 2009). Larvae of echinoderms also composed a substantial percentage of the meroplankton in some areas: particularly near the Alaska coast in 2004 and 2015, as well as the Wrangel Island/Herald Canyon region during 2009 and 2012. Abundance of polychaete larvae was highest at stations in the northeast and northwest Chukchi, as well as south of Cape Lisburne in 2007, where at several stations they dominated absolute

zooplankton abundance. Decapods (shrimp zoea and juveniles, crab and hermit crab zoea, and crab megalopa) were rare in the  $150\text{-}\mu\text{m}$  vertical samples, but their abundance may have been underestimated due to net avoidance, as suggested by data from the  $505\text{-}\mu\text{m}$  nets (see section on “Decapods” below). Larvae from other taxa (Cnidaria, Gastropoda, Bryozoa, and Nemertea) were also recorded, but generally occurred in extremely low abundances.

## Results of DNA Barcoding

Of 1005 DNA extracts, usable sequences were obtained for 932, or about 93%. The remaining samples either failed to produce a corresponding sequence match in the library (e.g., were identified only at the level of Eukaryota), or matched to organisms unrelated to those sampled (e.g., *Homo sapiens*, or Copepoda), presumably due to contamination or degradation of the target DNA, although we cannot exclude the possibility that some species are not amplified due to primer bias. The rate of success was about equal for bivalves and echinoderms. A list of 35 OTU's was produced (Table 3); however, the



number of "species" was lower, both due to more than one OTU often being assigned to the same species (different bins), as well as due to the frequent absence of reference species in the library, and consequent assignment to a higher ranking (family or order). A total of 7 OTU's were produced for Echinodermata, and 28 for Bivalvia; 10 of the Bivalvia OTU's were only found once.

## Species-Specific Distribution of Larvae and Adults

### Bivalves

A hotspot of adult bivalve biomass ( $>20 \text{ g C m}^{-2}$ ) was located in the southwestern Chukchi Sea, and was dominated both in biomass and abundance/density by *Macoma calcarea*

(Figure 4). In other areas, bivalve biomass was substantially lower (generally  $< 10 \text{ g C m}^{-2}$ ), and abundance was dominated by a variety of species from the families Nuculanidae, Thyasiridae, Yoldiidae and the superfamily Galeommatoidea (which includes the families Lasaeidae and Montacutinae) (Table 4). In contrast, the hotspot for larval abundance was consistently observed near the Alaska coast and in waters influenced by the Alaska Coastal Current (in 2009) (Figure 5). Within the northwest Chukchi, bivalve larvae were present in 2009, but were entirely absent in 2004 and 2012. Similarly, they were found at very few stations and in very low numbers in the northeast Chukchi in 2015.

A total of 28 larval OTUs were identified, of which three were classified biogeographically as boreal-Pacific, 14 as boreal-Arctic, and the rest of unknown affinity due to lack of

**TABLE 2 |** Mean abundances (ind  $\text{m}^{-2}$ ) and percent contribution of each taxon to the total meroplankton abundance.

Taxon	2004		2007		2009		2012		2015	
	Mean abund.	%	Mean abund.	%	Mean abund.	%	Mean abund.	%	Mean abund.	%
Bivalvia	6344	6	8287	81	10697	46	2418	41	5316	24
Cirripedia	59748	56	363	4	5859	25	2055	35	5349	24
Decapoda	44	0	0	0	38	0	44	1	115	1
Echinodermata	35551	34	108	1	2414	10	1123	19	4853	22
Bryozoa	+	+	+	+	+	+	+	+	1253	6
Polychaeta	4249	4	1405	14	4042	17	209	4	4145	19
Cnidaria	0	0	0	0	59	0	21	0	0	0
Gastropoda	0	0	23	0	270	1	19	0	1020	5
TOTAL	105936		10186		23379		5889		22051	

taxonomic resolution. The majority of the sequenced larvae (90%) belonged to only 10 OTUs, of them two species that are presumed to be of North Pacific origin (*Mytilus trossulus* and *Mactromeris polynyma*). The taxonomic composition of the larvae was not at all reflective of the co-occurring adult bivalve communities, with only 2 of the 23 most common bivalve species represented in the larval communities. The most common taxa among the larvae was the coastal species *Hiatella arctica*, which was found at every station, and dominated most stations south of Cape Lisburne during all years. Adults of

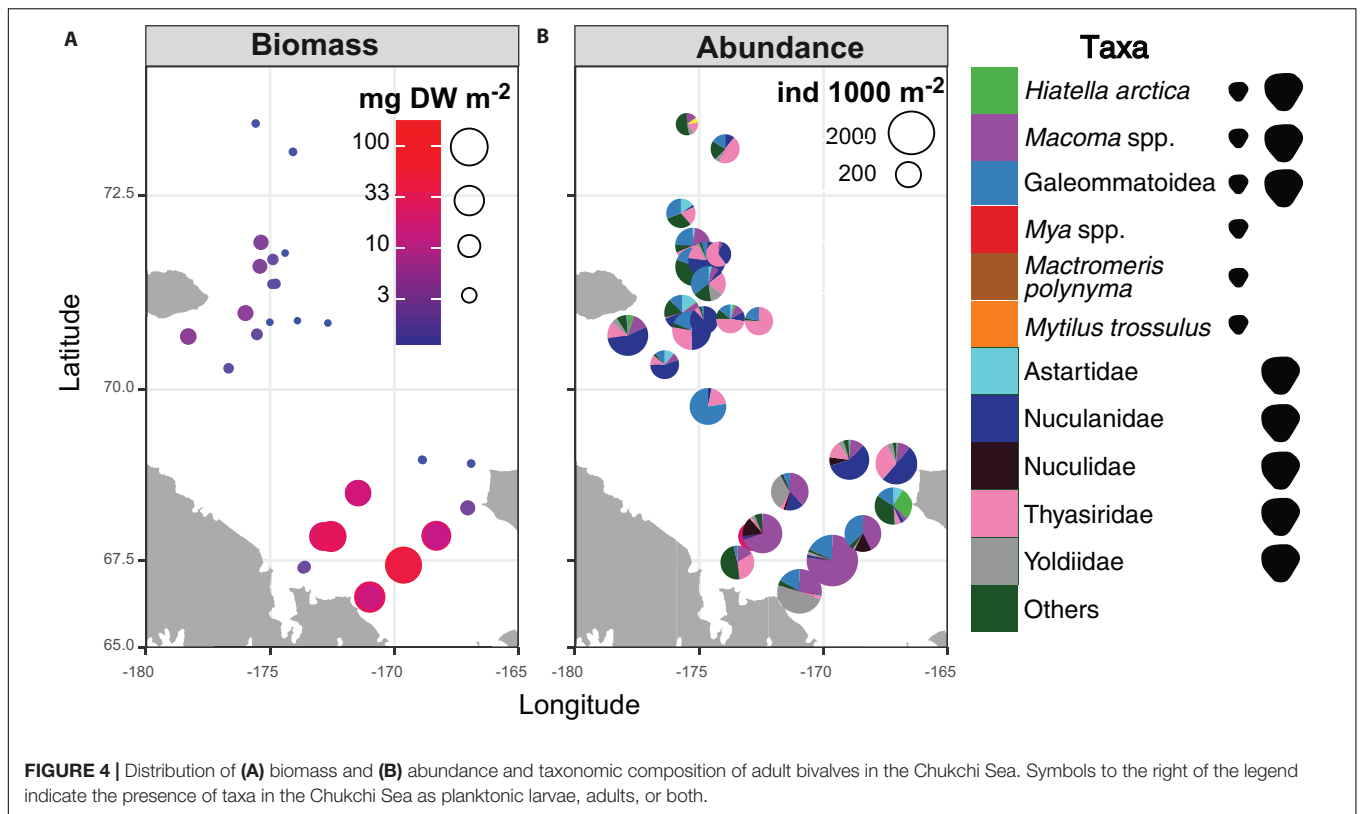
this species were numerous only at one coastal station near Alaska and, to a lesser extent, at one station near Wrangel Island. The 4 OTUs identified as *Mya* spp. (of them, 2 bins of *Mya truncata*) were the next most dominant group, also observed at nearly every station, and especially dominant around the Cape Lisburne area in 2007, 2012, and 2015. Larvae of the dominant bivalve species in the benthos, *M. calcarea*, were found in relatively large numbers in the Bering Strait region and in Herald Canyon in 2009, and in low numbers in ACW in 2012, but were notably absent in other areas and

**TABLE 3** | List of OTU's identified using molecular barcoding.

Phylum	OTU (closest match)	Biogeography	Source	% similarity	# of specimens	
Bivalvia	Cardiidae sp. (1)	–		94	1	
	Cardiidae sp. (2)	–		95	1	
	Cardiidae sp. (3)	–		94	2	
	<i>Chlamys behringiana</i> (Pectinidae)	Widespread Pacific Boreal-Arctic	Sirenko, 2009	100	1	
	<i>Ciliatocardium ciliatum</i> (Cardiidae)	Widespread circumpolar Boreal-Arctic	Sirenko, 2009	100	1	
	Galeommatoidea sp.	–		84	65	
	<i>Hiatella arctica</i> (1) (Hiatellidae)	Coastal, Subtropical-Arctic	Sirenko, 2009	100	234	
	<i>Hiatella arctica</i> (2) (Hiatellidae)	Coastal, Subtropical-Arctic	Sirenko, 2009	100	36	
	Hiatellidae sp. (1)	–		87	1	
	Hiatellidae sp. (2)	–		90	1	
	<i>Limecola balthica</i> (Tellinidae)	Widespread Atlantic boreal-Arctic	Väinölä, 2003	100	1	
	<i>Macoma calcarea</i> (Tellinidae)	Widespread Boreal-Arctic	Kędra et al., 2010	100	57	
	<i>Macoma</i> sp. (1) (Tellinidae)	Boreal-Arctic*		95	1	
	<i>Macoma</i> sp. (2) (Tellinidae)	Boreal-Arctic*		90	4	
	<i>Mya</i> sp. (1) (Myidae)	Boreal-Arctic*		93	22	
	<i>Mya</i> sp. (2) (Myidae)	Boreal-Arctic*		94	21	
	<i>Mya truncata</i> (1) (Myidae)	Coastal, widespread boreal-Arctic	Sirenko, 2009	100	16	
	<i>Mya truncata</i> (2) (Myidae)	Coastal, widespread boreal-Arctic	Sirenko, 2009	100	13	
	<i>Mya uzenensis</i> (Myidae)	Boreal-Pacific		100	1	
	Myoida sp.	–		77	4	
	<i>Mytilus trossulus</i> (Mytilidae)	Widespread Boreal-Pacific	Sirenko, 2009	100	9	
	Pholadidae sp.	–		89	2	
	<i>Serripes laperousii</i> (Cardiidae)	Widespread Boreal-Pacific	Sirenko, 2009	100	4	
	<i>Spisula</i> sp., presumed <i>Mactromeris polynyma</i> (Mactridae)	Widespread Boreal-Pacific	Sirenko and Vassilenko, 2009	99	29	
	Tellinidae sp.	–		90	3	
	Venerida sp.	–		87	4	
	<i>Zirfaea pilsbryi</i> (Pholadidae)	Widespread Boreal-Pacific	Sirenko and Vassilenko, 2009	100	1	
	Echinodermata	Amphiuridae sp. (Ophiuroidea) (presumed <i>Amphiodia craterodmeta</i> )	Widespread Boreal-Pacific	Smirnov, 1994	84	102
		<i>Echinarachnius parma</i> (Echinoidea)	Widespread Pacific West Atlantic Boreal	Smirnov, 1994	100	82
		<i>Lethasterias nanimensis</i> (Asteroidea)	Widespread Boreal-Pacific	Smirnov, 1994	100	11
<i>Ophiocten sericeum</i> (Ophiuroidea)		Atlantic high boreal-Arctic circumpolar	Smirnov, 1994	100	48	
<i>Ophiopholis aculeata</i> (Ophiuroidea)		Amphiboreal	Smirnov, 1994	100	7	
<i>Ophiura sarsii</i> (Ophiuroidea)		Widespread Arctic circumpolar	Smirnov, 1994	100	94	
Ophiuridae (nearest match <i>Ophiura robusta</i> , 90% similarity, presumed <i>Ophiura maculata</i> )		Widespread Boreal-Pacific	Smirnov, 1994	90	43	
Failed sequences		No match		NA	41	
		Wrong taxa		NA	32	
		TOTAL			1005	

Similarity is the identity percent of the representative sequence of the OTU with the reference sequence. \*Indicates taxa that comprise more than one species, so cannot confirm the biogeographic distribution.

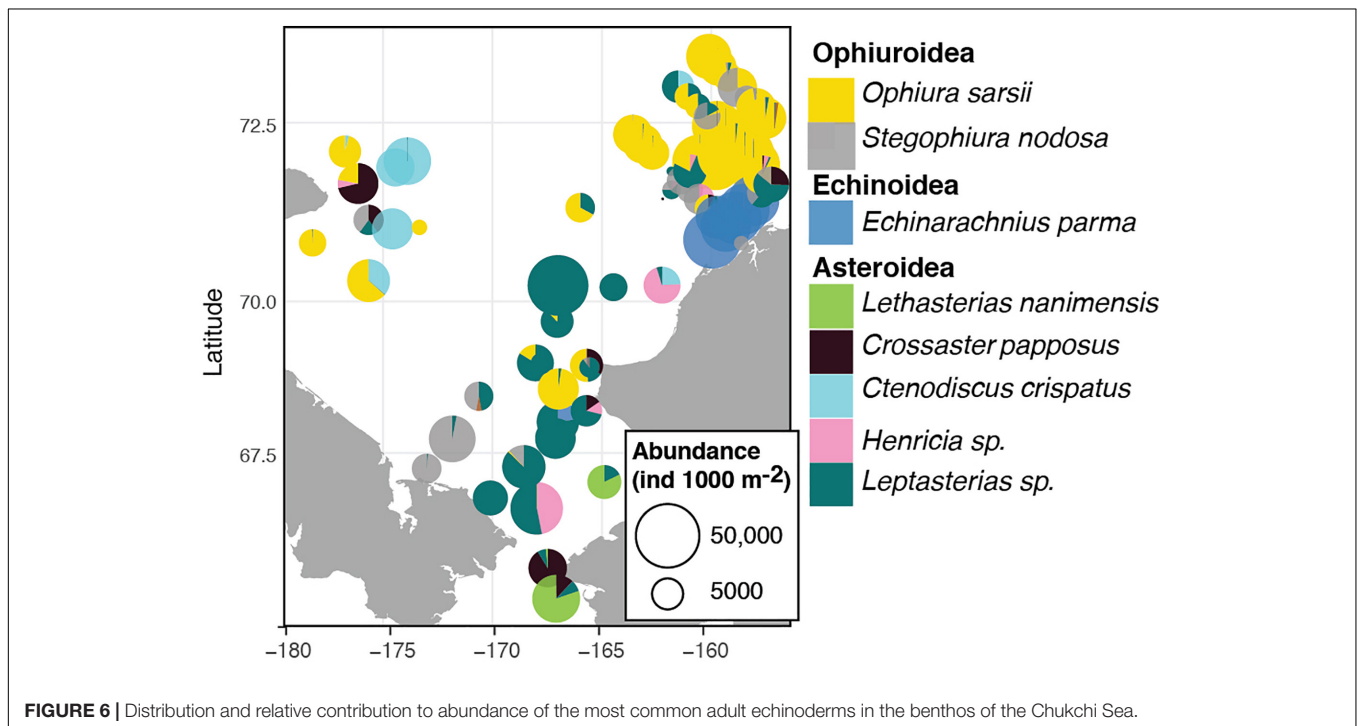
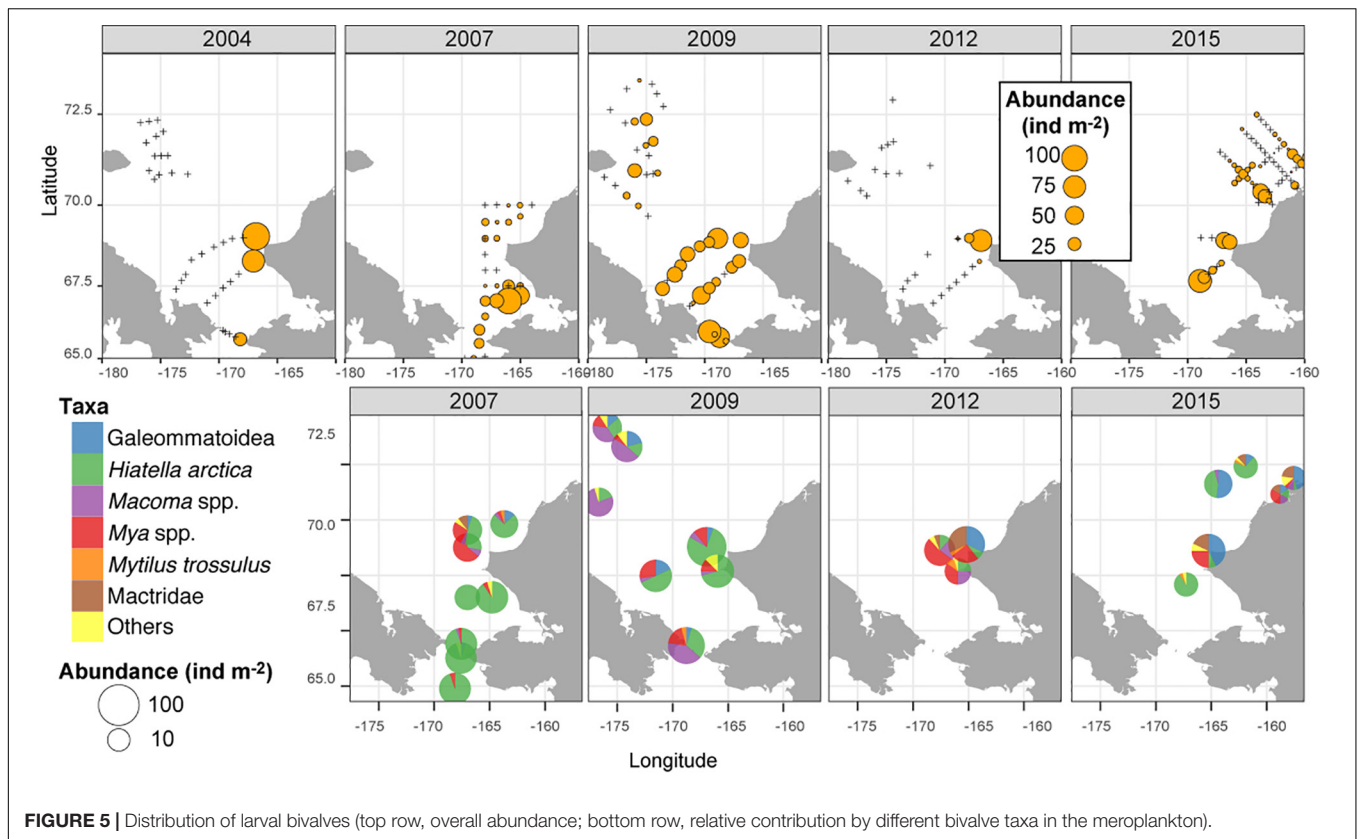




**TABLE 4 |** Most abundant adult bivalve species within the infauna of the Chukchi Sea.

Bivalve family	Common species	Average abundance (ind 1000 m <sup>-2</sup> )	Max. abund.	Barcode available	Larvae observed
Astartidae	<i>Astarte montagui</i>	3.3	52.5	Yes	No
	Astartidae	3.9	37.5	–	No
suprfrm. Galeommatoidea	Montacutinae	30.6	197.5	–	*
	<i>Kurtiella tumida</i> (Lasaeidae)	3.3	40.0	No	*
	<i>Kurtiella bidentata</i> (Lasaeidae)	20.0	301.3	Yes	*
	<i>Mysella</i> sp. (Lasaeidae)	9.2	240.0	Yes	*
Hiatellidae	<i>Hiattella arctica</i>	5.2	135.0	Yes	Yes
Tellinidae	<i>Macoma calcarea</i>	121.5	1994.2	Yes	Yes
	<i>Macoma moesta</i>	14.4	136.7	Yes	No
	<i>Macoma</i> sp.	10.4	52.5	Yes	*
Nuculanidae*	<i>Nuculana radiata</i>	37.9	380.0	Yes	No
	<i>Nuculana pernula</i>	70.8	680.0	Yes	No
	<i>Nuculana</i> sp.	3.0	75.0	–	No
Nuculidae*	<i>Nucula nucleus</i>	3.8	87.5	Yes	No
Thyasiridae	<i>Thyasira flexuosa</i>	7.0	182.5	Yes	No
	<i>Axinopsida</i> sp.	2.4	60.0	Yes	No
	Thyasiridae	39.0	132.5	–	No
Yoldiidae*	<i>Yoldia hyperborea</i>	37.7	642.5	Yes	No
Others	<i>Cyclocardia crebricostata</i> (Carditidae)	1.0	25.0	No	No
	<i>Pododesmus macrochisma</i> (Anomiidae)	1.9	50.0	No	No
	<i>Musculus glacialis</i> (Mytilidae)	2.1	55.0	No	No
	<i>Musculus discors</i> (Mytilidae)	0.9	22.5	Yes	No
	<i>Diplodonta</i> sp. (Ungulinidae)	3.8	100.0	No	No
	<i>Nutricola tantilla</i> (Veneridae)	0.8	20.0	Yes	*

\*, indicates unknown larval presence due to absence of reference barcodes.



during all other years. Other fairly common larvae included an OTU belonging to the superfamily Galeommatoidea, an OTU identified as belonging to the family Mactridae, and

*M. trossulus*. The rest of the taxa (18 OTUs) together composed no more than 10% of the larval bivalve abundance at any of the stations.

## Echinoderms

The dominant echinoderms in the epibenthos across the southern Chukchi Sea were the sea stars *Leptasterias* spp. and *Henricia* sp. as well as the ophiuroid *Stegophiura nodosa* (Figure 6 and Table 5). The northwestern Chukchi communities (Herald Canyon area), in contrast, were dominated by the sea star *Ctenodiscus crispatus* and to a lesser extent the ophiuroid *Ophiura sarsii*, while the northeastern Chukchi Sea was heavily dominated by *O. sarsii* and, near the coast, by the sand dollar *E. parma*. Other species, such as the sea star *Crossaster papposus* and *Lethasterias nanimensis*, were locally dominant at some stations (Figure 6 and Table 5).

The spatial distribution of the echinoderm larvae was variable among years, ranging from 0 to >100,000 ind. m<sup>-2</sup> in different locations and during different years (Figure 7A). Similar to the bivalves, the highest abundances were also observed near the Alaska coast during all years, and especially in 2004 and 2015. Larvae were observed in the southwestern Chukchi in 2009, when this area was strongly influenced by ACW, but were absent in 2004 and 2012. Similarly, echinoderm larvae were common in the Herald Canyon region in September 2009 and 2012, but were entirely absent in 2004. It is noteworthy that with the exception of a narrow band along the coast, larvae were rare or absent in the northeastern Chukchi region in 2015.

The vast majority of echinoderm larvae were ophioplutei (76%), which were matched to 5 different species of ophiuroids: *O. sarsii*, *Ophiocten sericeum*, and *Ophiopholis aculeata*, as well as two species from the families Amphiuroidae and Ophiuridae that were absent in the reference databases. Of the remaining individuals, 21% were echinoplutei, all of which were identified as *E. parma*, and 3% were bipinnaria, all identified as *L. nanimensis* (Figure 7B). No sea cucumber larvae were observed. Larvae of

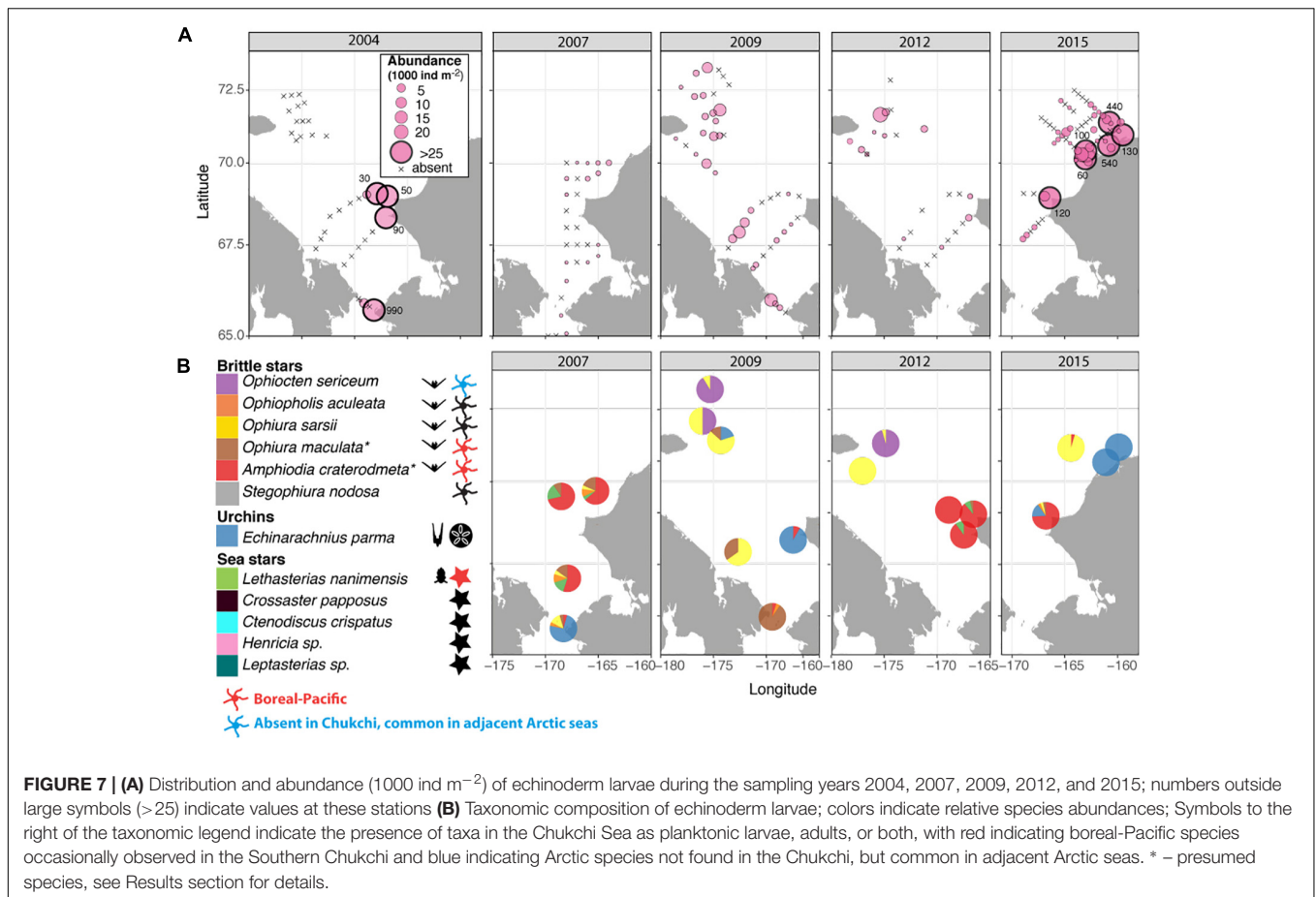
the most widely distributed and abundant adult ophiuroid species within the benthos in this area, *O. sarsii*, were abundant in the western and northwestern Chukchi stations in 2009 and 2012, but were notably rare or absent in the eastern and northeastern Chukchi during all study years, despite this area being a major hotspot for adult abundance. Surprisingly, the western Herald Canyon region in 2009 and 2012 was dominated by larvae of *O. sericeum*, another common Arctic ophiuroid, although adults of this species were absent on the Chukchi shelf.

The larval echinoderm communities at southeastern stations during 2007, 2012, and 2015 were heavily dominated by an ophiuroid species identified as an Amphiuroidae. The only member of this family commonly found in the Chukchi epibenthos is the Pacific species *Amphiodia craterodmeta*, which notably lacked a reference barcode in the BOLD database (Ratnasingham and Hebert, 2007). We assumed these larvae to most likely belong to this species; however, its adult distribution was limited to the southwestern Chukchi and did not overlap with the distribution of the larvae. One other ophiuroid larva (listed as Ophiuridae sp., brown color on Figure 7B) did not produce a species match, but was most similar (90%) to *O. robusta*. We deduced this species to be *O. maculata*, an uncommon sibling Pacific species (that also lacks a barcode in BOLD) that is the only other member of the genus *Ophiura* ever observed in the region. Larvae of this species were present at most stations in the southern Chukchi Sea during all years, and were the dominant meroplankton species in the Bering Strait in 2009 (abundance approaching 14,000 ind/m<sup>2</sup>), with declining numbers toward the north. *O. aculeata* was the least common of the ophiuroid larvae, although found in low quantities at most stations near the Bering Strait and near the Point Hope/Cape Lisburne area. Adults of this species were also relatively uncommon and restricted

**TABLE 5** | Most abundant echinoderm species within the benthos of the Chukchi Sea.

Common species	Average abundance (ind 1000 m <sup>-2</sup> )	Max. abundance	Barcode available	Larvae observed
<b>Asteroidea</b>				
<i>Asterias amurensis</i>	278.0	2523.0	Yes	No
<i>Crossaster papposus</i>	241.4	4284.6	Yes	No
<i>Ctenodiscus crispatus</i>	1353.0	11882.2	Yes	No
<i>Henricia</i> sp.	530.0	9475.2	Yes	No
<i>Leptasterias</i> sp.	3718.6	55060.2	Yes	No
<i>Lethasterias nanimensis</i>	200.4	4647.7	Yes	Yes
<i>Pteraster</i> sp.	55.4	1085.3	Yes	No
<b>Ophiuroidea</b>				
<i>Amphiodia craterodmeta</i>	49.4	856.8	No	Yes*
<i>Gorgonocephalus</i> sp.	116.7	950.0	Yes	No
<i>Ophiacantha bidentata</i>	323.3	4174.3	Yes	No
<i>Ophiocten sericeum</i>	639.7	10601.8	Yes	Yes
<i>Ophiura sarsii</i>	14062.5	911659.5	Yes	Yes
<i>Stegophiura nodosa</i>	870.9	13847.2	Yes	No
<b>Echinoidea</b>				
<i>Echinarachnius parma</i>	4967.5	59780.2	Yes	Yes
<i>Strongylocentrotus pallidus</i>	99.6	445.7	Yes	No

\*, presumed, see section "Results."



to just a few locations characterized by the presence of harder substrate preferred by this species as occur near Point Hope/Cape Lisburne, in the northeast Chukchi, and in Herald Canyon. Of the common ophiuroid species found in the benthos, we did not observe larvae of three: *S. nodosa*, *Ophiacantha bidentata*, and *Gorgonocephalus* sp. Of these, the latter two ophiuroids are believed to reproduce via external brooding or benthic/demersal larvae, which would not have been captured by our sampling (Carmack and Wassmann, 2006; Shanks, 2001).

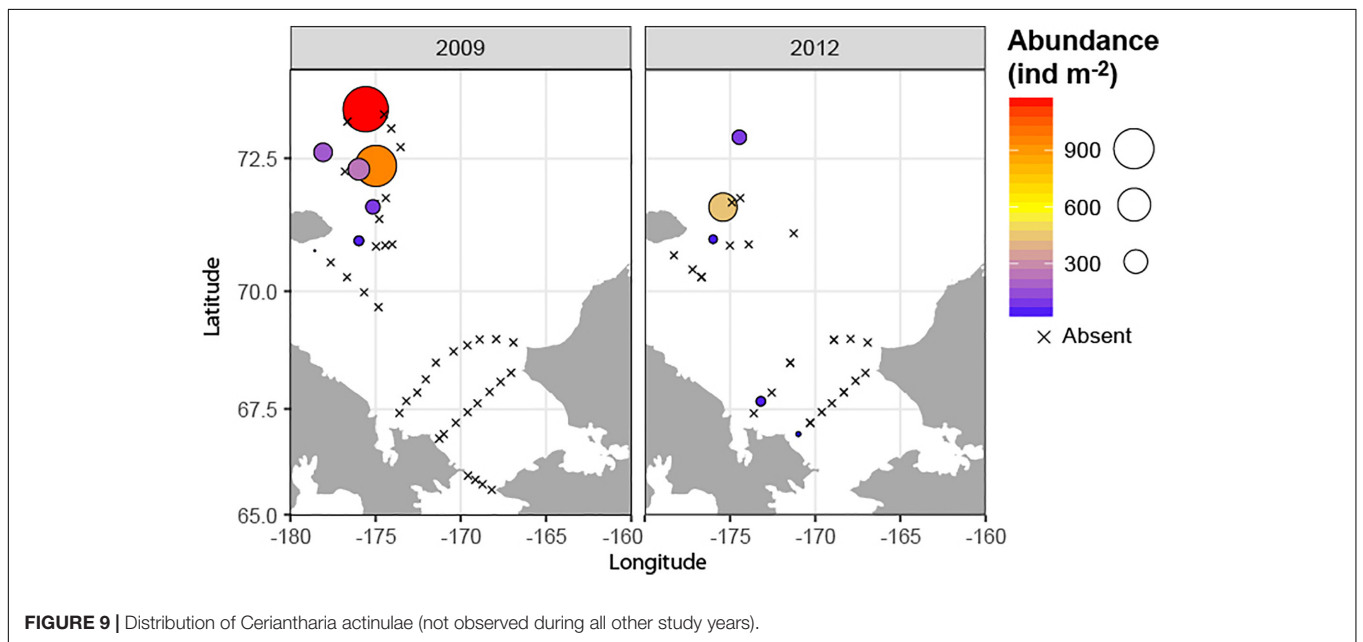
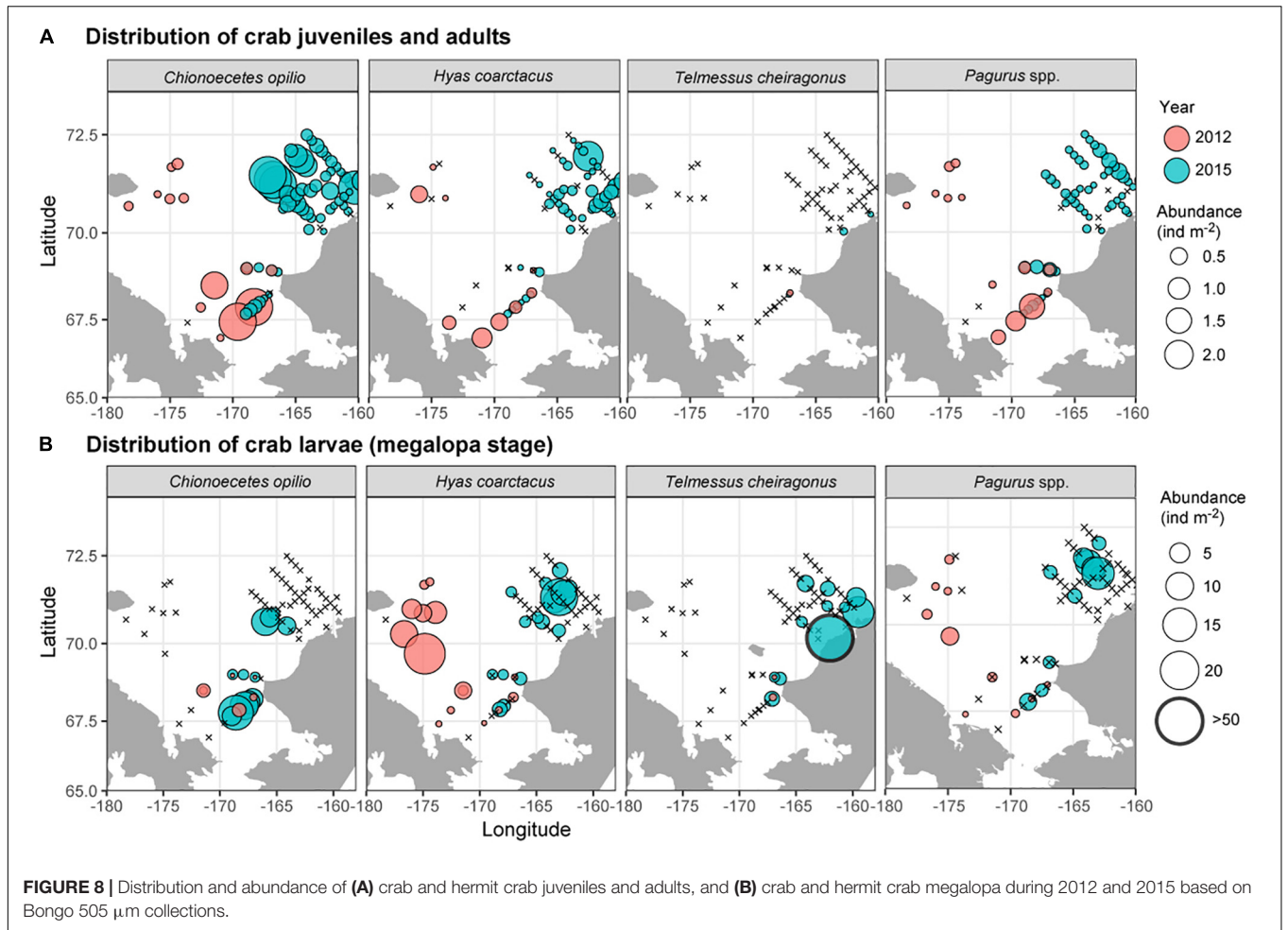
Notably, the larva of only one sea star (*L. nanimensis*, a boreal-Pacific species) was observed within the plankton, despite many species of sea stars being commonly present in the benthic communities. In the meroplankton, *L. nanimensis* larvae were common just south of the Bering Strait and found in low numbers at all stations around and just south of the Cape Lisburne area; notably, the adults of this species were highly abundant just south of that cape. The stations with extremely high abundances of echinoplutei, all belonging to *E. parma*, were located near the northeast Alaska coast, coincident with the location of high adult abundance. Both larvae and adults of this species were also found in lower numbers elsewhere, generally overlapping in their distributions. It is noteworthy that we observed no larvae of the other sea urchin present in the Chukchi Sea, *Strongylocentrotus pallidus*, although not entirely surprising due to the timing of our sampling (Falk-Petersen and Lonning, 1983).

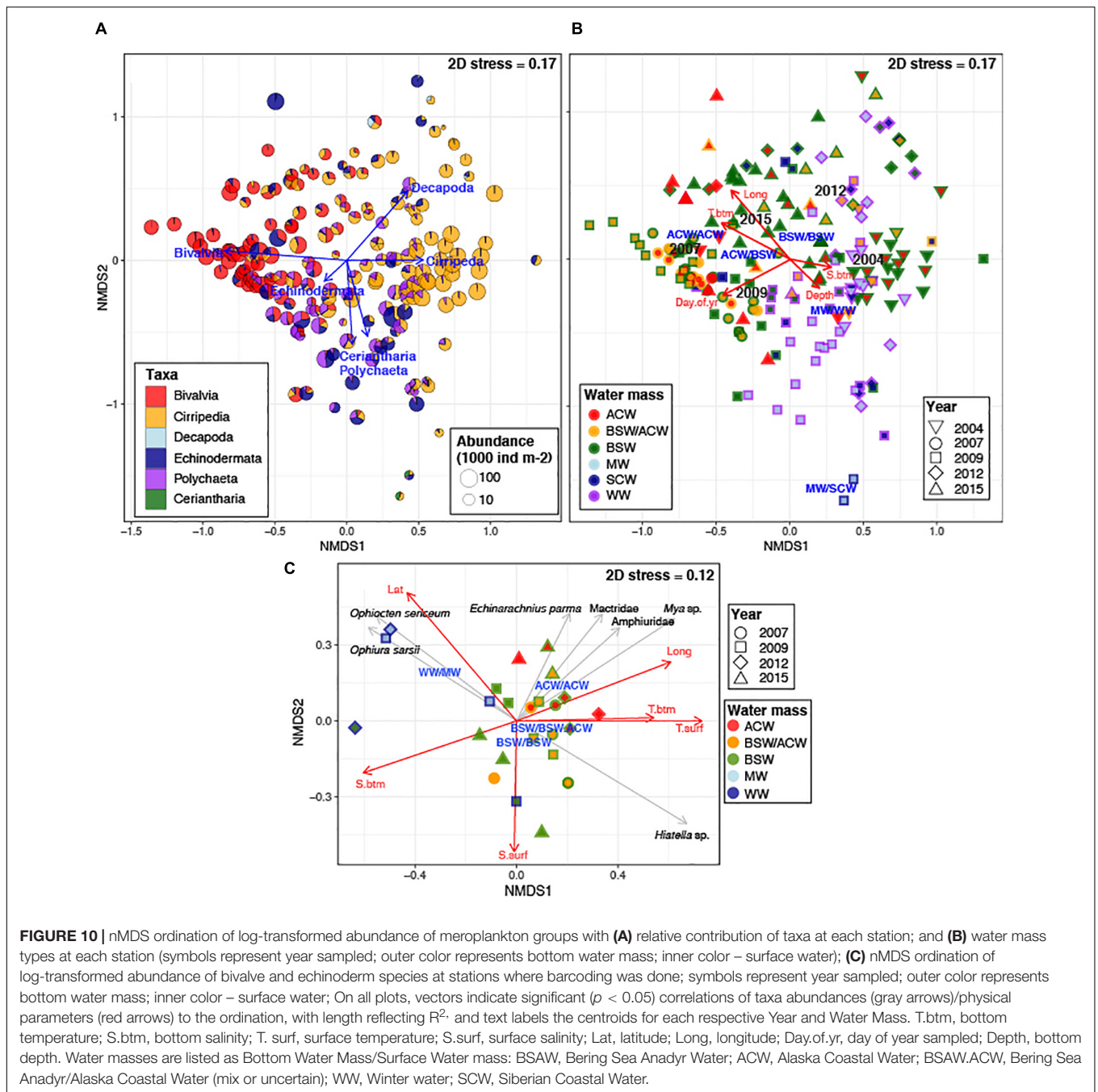
## Decapoda

Observed decapod larvae included shrimp zoea stages of the families Hippolytidae and Pandalidae (not shown), zoea and megalopa larvae of the anomuran crab family Paguridae and zoea and megalopa stages of three brachyuran crab species: the snow crab, *Chionoecetes opilio*, the Arctic lyre crab, *Hyas coarctatus* and the helmet crab, *Telmessus cheiragonus* (Figure 8). Among the adult crabs, *C. opilio* was the most common, with adults present at nearly every sampled location. The megalopae of this species, on the other hand, were only observed in the eastern Chukchi Sea and at stations influenced by the Alaska Coastal Current. Both adults and megalopae of *H. coarctatus* were found throughout the Chukchi Sea, although maximum densities of larvae were observed in the area where adults were relatively scarce (northwestern Chukchi region). *T. cheiragonus* adults were only observed on two stations near the Alaska Coast in extremely low numbers; larvae, on the other hand, were common at stations along the coast, with abundances occasionally exceeding 50 ind m<sup>-2</sup>.

## Cnidaria

Actinulae of burrowing anemones (Ceriantharia) were observed exclusively in the western Herald Canyon region in 2009 and 2012 in abundances up to 1000 ind m<sup>-2</sup>, as well as, in lower numbers near the Siberian coast at stations containing Chukchi





WW (Figure 9). We did not observe these larvae in any other area during any of the years. Estimates of adult abundances are difficult to obtain for this organism, but video surveys of the area only observed adults at the same, or nearby, stations as we observed the larvae, in aggregations with densities of up to  $3\text{--}4$  specimens  $\text{m}^{-2}$  (Sirenko and Gagaev, 2007).

### Assemblages and Environmental Drivers

Non-metric multidimensional scaling of log-transformed abundance of meroplankton groups (macrotaxon level) showed a moderate separation of meroplankton communities (2D

stress = 0.17), mainly driven by bivalve, barnacle, and polychaete larvae, with bivalves and barnacles driving separation along the first axis, and polychaetes and decapods driving the separation along the second axis (Figure 10A). The ordination was moderately correlated to spatial (Longitude,  $R^2 = 0.37$ ) and temporal (Sampling Day,  $R^2 = 0.26$ ) gradients, as well as sampling year ( $R^2 = 0.39$ ) and water mass characteristics (bottom temperature and combined water mass type) ( $R^2 = 0.27$  and  $0.21$ , respectively), although significant overlap between categories was observed. The centroids for ACW coincided with the maximum bivalve abundances, with a decreasing contribution of bivalve

larvae in BSW water, while the centroids for WW and SCW were located near stations containing maximum contribution of polychaete, echinoderm and Ceriantharia larvae. PERMANOVA showed significant differences in meroplankton community abundance between all sampling years and most water mass types (**Supplementary Table 1a**), as well as the interaction between them; together they accounted for 57% variability. The most pronounced differences of larval assemblages between water masses were between BSW and WW, BSW and ACW, and WW and ACW (**Supplementary Table 1b**).

Non-metric multidimensional scaling analysis of bivalve and echinoderm assemblages at stations where molecular identification was done (26 stations) produced a much clearer separation (2D stress = 0.12), as well as much stronger correlations to geography (Lat, Long;  $R^2 = 0.44$  and  $0.42$ ) and oceanographic parameters [surface and bottom temperature ( $R^2 = 0.53$  and  $0.29$ ), surface and bottom salinity ( $R^2 = 0.26$  and  $0.41$ ), as well as surface water mass type ( $R^2 = 0.49$ )] (**Figure 10B**). There was no separation by sampling years or by date sampled. The ACW influenced stations were characterized by significantly higher contribution of the bivalves *Mya* sp. and family Macluridae as well as echinoderms Amphiruridae and *E. parma* (**Figure 10C**). The three western Herald Canyon stations (2009 and 2012) were highly dissimilar to the rest, driven mainly by the presence of *O. sericeum* and *O. sarsii*. PERMANOVA showed significant differences in community structure between water mass types, but not sampling years, with surface and bottom water mass type together accounting for 47% of observed variability, most of it explained by surface water mass type (**Supplementary Table 2a**). Pairwise-comparisons showed differences in larval communities to be significant between surface ACW and BSW, ACW and MW, and BSW/ACW and MW, as well as bottom BSW and WW (**Supplementary Table 2b**).

## DISCUSSION

### Advances and Challenges of Studying Meroplankton Using Molecular Methods

Benthic species within the same phylum, or even within a family or genus, can differ significantly in their ecology and distribution patterns, both at the larval and adult stages. Our study highlights the importance of approaching meroplankton at the species level, since both spatial and temporal patterns are obscured when larvae are grouped into broad categories. Species-specific morphological features are limited in early life stages, and morphological identification to higher taxonomic ranks in our study was only possible for crabs. Our study is among the first for Arctic meroplankton to supplement the morphological identification of meroplankton with molecular barcoding to better resolve taxonomic diversity of larvae. Our lab protocol resulted in a much higher success rate (>90%) than in previous attempts, where it did not exceed 20–50% (e.g., Webb et al., 2006; Heimeier et al., 2010; Brandner et al., 2017), and we hope that our effective, simple identification protocol will pave the way for future biodiversity studies of meroplankton.

Yet, limitations of our approach remain, such as the requirement to process larvae individually, restricting the scope of any even broader-scale ecological study. A metabarcoding assay of bulk DNA extracted from whole plankton samples could circumvent this limitation, but uncertainties in the quantitative relation between sequencing read abundances and biomass per species have to be clarified before the results of such approach can be compared to previous morphological surveys. Furthermore, a current constraint of molecular identification is the gaps in the reference libraries, making a match to species level in many cases impossible. Despite significant contributions by the Census of Marine Life, Polar Barcode of Life and other efforts (Hardy et al., 2011), of the approximately 112 echinoderm and bivalve species found in the region (Sirenko and Vassilenko, 2009), barcodes are currently only available for 40 (or 35%). The numbers are even smaller for polychaetes and other less studied groups, with gaps including many abundant and ecologically significant species. We strongly encourage the scientific community to continue adding additional species to the Barcode of Life Database so as to improve the identification success of larvae (and other samples of interest) in future studies.

### Diversity and Distribution Patterns of Meroplankton

The summer-fall meroplanktonic communities within the Chukchi Sea were characterized by overall low diversity, yet comparable to other high latitude regions in the northern hemisphere (e.g., Fetzer and Arntz, 2008; Silberberger et al., 2016; Michelsen et al., 2017), although undoubtedly the species list would grow substantially if other groups (e.g., polychaetes) were also barcoded. Additionally, our estimates of both abundance and diversity might be underestimated due to the mesh size used and many larvae being smaller than 150  $\mu\text{m}$  and would be better sampled with finer-mesh nets. Furthermore, we may underestimate larval diversity due to many larval types occurring just above the bottom, as our samples were collected 3–5 m off the seafloor. The low diversity observed in our study and other parts of the Arctic (30–50 taxa) is in contrast with Antarctic regions, where the number of distinct taxa among the larvae is often estimated to be in the hundreds (Stanwell-Smith et al., 1999; Bowden et al., 2009). The difference in species richness between our study and the Antarctic studies mentioned above could represent a real natural pattern but could also be due to a more complete seasonal coverage in those studies and/or smaller mesh sizes capturing a wider size range of larvae.

Meroplankton abundance within summer-fall zooplankton communities in the Chukchi Sea ranged over four orders of magnitude (from  $<100 \text{ ind m}^{-2}$  to over  $500,000 \text{ ind m}^{-2}$ ), and was characterized by extremely large spatial and inter-annual variability, although we recognize the challenge of comparing inter-annual data with such different spatial coverage. The high variability between locations and sampling years is a consequence of the inherently patchy distribution of larvae in time and space, due to the limited larval duration and episodic reproduction events in many benthic organisms (Shanks, 2009). For example,

the exceptionally high abundance of barnacle larvae observed in 2004 was likely due to the earlier timing of the sampling by 3–4 weeks that year compared to other years, as in other Arctic regions, peak abundances of barnacle larvae have been recorded early in the summer (Brandner et al., 2017; Michelsen et al., 2017). A study on decapod larvae in the Chukchi Sea spanning two consecutive years similarly found abundances to differ over several orders of magnitude, which the authors explain by the slightly different timing of the cruises (Landeira et al., 2017). Although significant differences in larval assemblages were observed between years at the macrotaxa level, we did not observe any temporal trends over the examined time span of changes in abundances or composition, with each year being distinct in larval composition from the others. Yet, even with the high levels of variability there were persistent trends in larval distribution, mostly associated with water mass distribution, and especially when communities were examined at the species level. ACW was the “hotspot” for larval abundance and diversity during most years, characterized by a community composed mainly of bivalve and echinoderm larvae of coastal and North Pacific origin. In contrast, Bering Sea Anadyr Water was dominated by barnacle larvae, and generally carried lower quantities of meroplankton (with the exception of 2004). Similarly, distinct meroplankton groups, containing a higher contribution of decapod and polychaete larvae, were similarly observed in the northeast (Herald Canyon) and northwest Chukchi at stations containing WW. Future climate related changes in the relative transport and characteristics of these water masses into the Chukchi will also affect the composition of the larval communities carried within them.

It is noteworthy how few of the dominant benthic species, many of which presumably reproduce via pelagic larvae, we observed in the meroplankton. One possible explanation is that the reproductive window for these species falls outside of our sampling periods, all of which were during late summer-fall. Long-term sediment trap data from the northeastern Chukchi Sea, however, showed meroplankton abundance (mostly represented by polychaetes and barnacles) to peak around September, coinciding with our sampling periods (Lelande et al., in review). However, in other regions of the Arctic, the main meroplankton peaks often occur during or shortly after the spring bloom (Kuklinski et al., 2013; Stübner et al., 2016). A recent seasonal study in a Svalbard fjord observed two major abundance peaks for most meroplankton groups, with the first one occurring early in the spring, and the second in the summer-fall (Stübner et al., 2016), which could indicate different timing of reproduction for different species within the same phylum. The spawning of the sea urchin *S. pallidus*, for example, in the northeast Atlantic occurs in early spring (Falk-Petersen and Lønning, 1983), which likely explains the absence of the larvae of this species in our samples. Another explanation is that the planktonic larvae are too short-lived or do not rise up sufficiently from the seafloor to even be captured with nets, or do not reproduce via a planktonic larva altogether. Thorson’s Rule (Thorson, 1950; Mileikovsky, 1971) predicted a decline in the incidence of pelagic development with latitude, although these studies did not take into account organisms

with planktonic lecithotrophy, and this rule has been partially disputed by newer evidence (Stanwell-Smith et al., 1999; Marshall et al., 2012; Landeira et al., 2017). However, as an example in support of the absence of pelagic larvae, recent live observations have suggested that *S. nodosa* may have brooding development (Lauren Sutton, University of Alaska Fairbanks, pers. obs.), rather than a typical pluteus larva, and the fact that we never observed larvae of this very common ophiuroid in any of our samples supports this observation. Within the bivalves, three of the most common families found in the Chukchi (Nuculanidae, Nuculidae, and Yoldiidae) belong to the subclass Protobranchia, which reproduce via a lecithotrophic pericalymma larva, rather than the typical bivalve veliger. These larvae are short-lived (hours to days) and stay near the bottom, which likely explains why we did not observe them in our net samples. The species that we did consistently observe in the meroplankton across the study area during most study years are likely characterized by an extended period of reproduction, or multiple overlapping spawning periods, as well as long larval duration. Larvae of *H. arctica*, *M. calcarea*, and *Mya* spp., as well as *Cerianthus* sp., were observed over periods of 5–8 months during the course of a year in other high latitude regions (Von Oertzen, 1972; Couwelaar, 2003; Brandner et al., 2017).

Inversely, for some species that we observed in the meroplankton no data were available on the distribution of adult forms. For example, the deep-dwelling clam families Myidae and Mactridae, both of which were among the most numerous bivalves in the meroplankton, cannot be adequately sampled by van Veen grab (Jay et al., 2014), so no estimates of distribution and abundance of these groups exist for the Chukchi Sea other than from the stomach contents of marine mammals. In the Bering Sea, *Mya* spp. and *Mactromeris* (= *Spisula*) *polynyma* (the only member of the family Mactridae in the Pacific sub-Arctic) are among the dominant prey items for walrus (Sheffield and Grebmeier, 2009; Jay et al., 2014) and bearded seals (Frost and Lowry, 1980), and *M. polynyma* is harvested by native communities in northwest Alaska (Magdanz et al., 2007). Walrus within the Chukchi Sea, however, mostly feed on other organisms (Sheffield and Grebmeier, 2009), suggesting that these clams are less common as adults in the benthos in this area. Similar to marine mammal diet analysis, the distribution of larvae of these groups may indirectly provide important insight on the distributions of adults within the benthos, although possible advection of larvae needs to be accounted for.

## Comparison of Patterns of Larval and Adult Invertebrates

One striking, if not entirely surprising, result of our study was the distinct spatial mismatch between most adult benthic populations and their larval stages. Notable exceptions to this were organisms such as *Cerianthus* sp. and the sand dollar *E. parma*, whose larvae were only found close to the adult populations, suggesting either recent spawning from this local population as a source or larval retention by means of local oceanographic features and/or larval behavior. Larval behavior (e.g., vertical migration, response to turbulence, or chemical signals) coupled with local



hydrodynamics can sometimes result in much shorter dispersal distances than predicted by larval duration solely, favoring retention of propagules closer to their spawning grounds (Shanks, 2009; Gaylord et al., 2013). Consistent with our observations of high densities of larvae near adult populations, it has been documented that *E. parma* larvae react to chemical cues produced by adults, which contributes to the dense aggregations of this species in the benthos (Pearce and Scheibling, 1990). It is also noteworthy that no significant sand dollar populations are present upstream from these locations.

However, most other larval types that we observed were presumed to be far from their points of origin. Most significantly, the meroplanktonic communities were heavily dominated by coastal forms (together Cirripedia and *H. arctica* composed on average >75% of meroplankton abundance). Therefore, the absence of these taxa on the Chukchi Sea shelf is most likely due to a lack of suitable substrate rather than larval supply. This is supported by the observations of dense patches of newly settled recruits of *H. arctica* covering moorings – an artificial hard substrate – in the south-central Chukchi Sea (K. Iken, pers. obs.). The high density of larvae of these hard-bottom coastal organisms that we observed across the studied area during all years reflects their life strategy: high fecundity, extended periods of reproduction, and long larval durations (Grantham et al., 2015), which is likely what makes them so common and successful in nearshore environments across all Arctic- and sub-Arctic seas.

Some of the other more common larvae presumably belonged to Pacific expatriates, such as the ophiuroids *A. craterodmeta* and *O. maculata*, and the crab *T. cheiragonus*, adults of which are rarely observed outside of the southern Chukchi Sea (Smirnov, 1994; Sirenko and Gagaev, 2007), although a large density of this species has been observed in kelp habitats in Peard Bay (Iken et al., personal observation). On the contrary, the Arctic ophiuroid *O. sericeum* is practically absent on the Chukchi shelf, but very numerous in the adjacent Beaufort and East-Siberian seas. The numerous larvae of this species that we observed in Herald Canyon is likely a consequence of local circulation patterns, which occasionally carry water from the East Siberian Sea into this region (Pisareva et al., 2015b). Therefore, its scarcity in the benthos in that region is not a consequence of lack of larval supply, but may be related to competition with *O. sarsii* or other dominant epibenthic species (Ravelo et al., 2017). While larval forms of some of the most abundant benthic species were numerous in the plankton (i.e., *M. calcarea* or *O. sarsii*), they were generally observed outside of the areas with the highest adult abundances.

A mismatch between adult and larval distribution is not surprising given the dispersive nature of the pelagic larval stage. Larval dispersal distance can be highly variable across taxa, dictated in large part by the time a larva spends in the water column prior to settling, ranging from a few minutes to several months (Shanks, 2009). Data on size distributions and developmental stages of larval populations could predict how recently these larvae were produced. Unfortunately, pelagic larval duration and dispersal distance are known only for

a handful of species, and closely related species often show disparate dispersal strategies (Levin and Bridges, 1995), so it is difficult to predict duration without empirical evidence. Temperature also affects pelagic larval duration so that larvae in cold waters spend more time in the water column prior to settling and consequently can disperse much greater distances (O'Connor et al., 2007). This has major implications for the Chukchi and other Arctic seas where larvae could, in theory, disperse over longer distances than their boreal compatriots. Conversely, the recent warming seawater temperatures may result in larvae spending less time in the warming surface water and settling more quickly to benthos, and Arctic species settling farther to the south than dictated by their life history strategy.

## Fate of Meroplankton in the Chukchi Sea

The Chukchi Sea is a special environment within the Arctic in that at least during summer, it represents a direct extension of the Bering Sea and North Pacific. This Pacific connection is unique compared with the rest of the Arctic shelf seas that are either influenced by Atlantic inflow (i.e., Barents/Kara Seas), and/or are governed by local processes (i.e., interior shelves) (Williams and Carmack, 2015). As a result, the Chukchi Sea is characterized by unique pelagic communities, heavily dominated by Pacific expatriates. Even the species that are shared with adjacent Arctic seas may represent distinct populations of Pacific origin within the Chukchi Sea (Nelson et al., 2009; Ershova et al., 2015b). For holozooplankton, Pacific zooplankton species are occasionally observed in the surface waters of the deep Arctic Basin, but they never compose a significant part of the communities in contrast to Atlantic expatriates, such as *C. finmarchicus* (Kosobokova et al., 2011). Thus, the Chukchi Sea serves as a chokepoint for these species invading the Arctic. As with holoplanktonic expatriates, some meroplanktonic larvae get transported from the Chukchi Sea to the basins (Smoot and Hopcroft, 2017), but only eurybathic species would be able to settle at depths beyond the shelf break. Among the meroplankton taxa identified to species level, we did not detect deep-water species from the North Pacific. The long residence time on the Bering and Chukchi shelves likely inhibits the propagation of larvae of deep-water benthic species from the North Pacific to the Arctic Ocean, while larvae of most deep-water species likely never reach surface waters necessary to carry them northward in the first place.

In contrast to holozooplankton that permanently associate with hydrography (Pisareva et al., 2015a), meroplankton recruit to the seafloor and need to encounter conditions matching their habitat preference. We document the arrival of high densities of larvae of hard bottom species such the bivalve *H. arctica* and barnacles, but the predominantly fine sediment type (mud and silt), characteristic of the Chukchi Shelf (Feder et al., 1994; Grebmeier et al., 2015a), seems to largely preclude the settlement of such species. Since most of the sampling within our study has been conducted far off shore, we are limited in the knowledge of the distribution of the adults and larvae of these species in coastal domain of the study region. However, offshore the potential for successful range expansions through the Bering

Strait is essentially limited to soft-bottom shelf species, such as many crabs, polychaetes, ophiuroids or bivalves. Larvae of species with potential of range expansion that we have already observed in our study include the North Pacific echinoderms *L. nanimensis*, *O. maculata*, and *A. craterodmeta*, the bivalves *S. polynyma* and *M. trossulus* and the crab *T. cheraigonus*. In support of this trend, adults of *M. trossulus* have been observed in the Northeastern Chukchi Sea in the late 1990s, whereas historically they had been absent in this area (Feder et al., 2003). The remainder of the advected larvae that fail to eventually settle to the benthos, play the same ecological role of advected holoplanktonic expatriates that cannot complete their life cycle in the Arctic, and instead become prey for planktonic predators or a sink to the benthos as detritus (Carmack and Wassmann, 2006).

## CONCLUSION

The distinct mismatch between larval and adult benthic communities within the Chukchi Sea suggests that advection is the main factor driving larval distribution in this region. Our results suggest the vast majority of the larvae in terms of their abundance that we observe on the Chukchi Sea shelf during summer months are advected “visitors” from neighboring regions: from the North Pacific through the Bering Strait, from adjacent Arctic seas, and most significantly, from hard bottom coastal areas. The absence or rarity of adult forms of these advected species on the Chukchi Shelf is an indication that the vast majority of their larvae will not settle successfully and will become a food source for pelagic predators or a carbon sink to the benthos.

## DATA AVAILABILITY

The datasets generated and compiled for this study can be found in the Mendeley Data Repository at <http://dx.doi.org/10.17632/rhd9z8x86h.1>.

## AUTHOR CONTRIBUTIONS

EE, RD, and BB conceived the study. EE, RH, and CS collected and analyzed the zooplankton data. BB, JG, and KI collected and analyzed the benthic data. EE, RD, and OW completed

the molecular work. OW completed the bioinformatics. EE performed the data analysis and wrote the manuscript with contributions from OW, RD, BB, KI, RH, CS, and JG.

## FUNDING

This research has been jointly funded by the UiT – The Arctic University of Norway and the Tromsø Research Foundation under the project “Arctic Seasonal Ice Zone Ecology,” project number 01vm/h15. The collection and processing of 2015 samples by KI, RH, and CS was done within the framework of the AMBON program funded through a National Ocean Partnership Program (NOPP Grant NA14NOS0120158) by the National Oceanic and Atmospheric Administration (NOAA), the Bureau of Ocean Energy Management, and the Shell Exploration & Production, under management of the Integrated Ocean Observing System (IOOS). The 2004, 2009, and 2012 samples were collected within the framework of the RUSALCA program with funds from the NOAA under cooperative agreements NA17RJ1224, NA13OAR4320056, and NA08OAR4320870 with the University of Alaska. The publication charges for this article were funded by the publication fund of the UiT – The Arctic University of Norway.

## ACKNOWLEDGMENTS

We thank Kim Præbel for providing lab facilities and advising with the development of the molecular protocol, Kyle Dilliplace and Cheryl Hopcroft for providing lab support, Seth Danielsen for providing CTD data for the 2015 AMBON expedition. We also thank Sarah Hardy for providing ethanol samples and Lisa Eisner for providing the zooplankton data from the BASIS expedition. We acknowledge the support in the field during all expeditions, which contributed data to this manuscript. We also thank the two reviewers for their helpful comments on improving the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00490/full#supplementary-material>

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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