In the North Atlantic and Arctic Oceans, the zooplankton biomass is dominated by copepods of the genus *Calanus* which are key species in linking algae and bigger animals in the food web. Up to four different *Calanus* species overlap in distribution here and each species have distinct environmental preferences, making them useful as climate indicators. However, these *Calanus* species are morphologically similar and during the work with this thesis the importance of correct identification has been highlighted. In this thesis, ecological and molecular methods have been combined to ensure correct species identification when documenting the distribution range of the different *Calanus* species, gaining new insights into the biology of the poorly known *Calanus* males and comparing the adaptations of the Arctic *C. glacialis* and Atlantic *C. finmarchicus* in the high Arctic. During this thesis, the species distributions were found to be more extensive and overlapping than previously described for all four species and were partly redrawn. New insights into the biology of males show that *C. glacialis* males are fatter and more active than females in January when they mate. It was revealed that the short life span of males could not be explained by depletion of the lipid resources and further research on the role of e.g. aging is needed. In a high Arctic fjord (Isfjorden), co-occurring *C. glacialis* and *C. finmarchicus* showed similar patterns in seasonal migration and changes in enzyme activity, except for few specific differences. Presence of adults, reproduction and seasonal descent were found to be earlier in *C. glacialis* than *C. finmarchicus*. The earlier wake-up and reproduction in *C. glacialis* is most likely an adaption to the highly unpredictable shelf seas with short algae blooms. With larger sizes and thus more fat, *C. glacialis* females mature and lay eggs prior to the bloom, allowing their offspring to utilize the productive season more optimally while *C. finmarchicus* who need food to mature and produce eggs is better adapted to a longer productive season. From this study, it appears that as long as the productive season remain similarly short and the temperatures do not exceed what *C. glacialis* thrives in, *C. glacialis* will most likely remain the best adapted and most numerous species in high Arctic shelf seas.

PhD in Aquatic Biosciences // No. 33 - 2019 Maja Karoline Viddal Hatlebakk

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ISBN: 978-82-93165-32-3
Trykk: Trykkeriet, Nord universitet
www.nord.no

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A thesis for the degree of
Philosophiae Doctor (PhD)

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ISBN: 978-82-93165-32-3

Print: Trykkeriet NORD

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Preface

This thesis is submitted in fulfilment of the requirements for the degree of Philosophiae Doctor (PhD) at the Faculty of Biosciences and Aquaculture (FBA), Nord University, Bodø, Norway. The original research presented in this these through four research papers was carried out from 01.01.15 to 18.09.19. This research has been internally financed by the University Centre in Svalbard (UNIS) and was part of the following projects funded by the Norwegian Research Council: IMOS (246747) and COPPY (227139)

The project team consisted of the following members:

Maja Hatlebakk, MSc: PhD student

Galice Hoarau, Professor, FBA, Nord University, Bodø, Norway: Primary supervisor

Janne E. Søreide, Associate Professor, Department of Arctic Biology, the University Centre in Svalbard, Longyearbyen, Norway, co-supervisor

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The thesis work was conducted within the ARCTOS PhD School

Maja Hatlebakk,

Longyearbyen, September 2019

Nothing in life is to be feared, it is only to be understood.

Now is the time to understand more, so that we may fear less.

- Marie Curie

Acknowledgment

First and foremost I want to thank my supervisor at UNIS, Janne Søreide, for the opportunity to do this PhD. Thank you for supporting and guiding me through this project. I have learnt so much from you and the adventures you have sent me on, from a multi-disciplinary field campaign in the Arctic Ocean to the narrowest copepod conference in California. Thank you to Galice Hoarau, my supervisor at Nord University, for introducing me to the wonders of molecular biology and for your encouragement and support throughout this project.

To my co-supervisors at Alfred Wegener Institute: Martin Graeve and Barbara Niehoff. Thank you for advising me in your respective fields of expertise and not least for letting me work with you at AWI for part of this project.

Thank you to the Norwegian Polar Institute and the captain and crew of RV Lance for the opportunity to join the N-ICE campaign, to the university in Tromsø and the captain and crew of RV Helmer Hanssen for the polar night cruises, Murmansk Marine Biology institute and captain and crew of RV Dalnie Zelentsy for welcoming me onboard and to UNIS logistics for all the day trips in all kinds of weather out in Isfjorden. Also thank you to everyone else who joined me out in the field. This project would not be possible without you.

Thank you to my fellow PhD students and colleagues at UNIS for creating a social and interesting work environment. Thank you to my fellow PhD students at Nord University for always including me whenever I spent time in Bodø. Thank you to friends and family outside the science bubble for all your support and encouragement.

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Published in Biology letters **13**:20170588

Paper II Daase M, Kosobokova K, Last K, Cohen J, Choquet M, **Hatlebakk M**, Søreide JE.
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Published in Marine Ecology Progress Series ***607****:53-69*

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Paper IV **Hatlebakk M**, Niehoff B, Eide H, Daase M, Choquet M, Hoarau G, Søreide JE.
Seasonal changes in population dynamics and enzyme activity of *C. glacialis* and *C. finmarchicus* in the high Arctic
Manuscript

Abstract

In the North Atlantic and Arctic Oceans, the mesozooplankton biomass is dominated by Calanoid copepods of the genus *Calanus* which play a central role in the lipid driven pelagic food of high-latitude seas. Up to four different *Calanus* species overlap in distribution here, but as each species show distinct environmental preferences, *Calanus* spp. are frequently used as climate indicators. One major challenge, however, is that these *Calanus* species are morphologically similar which shed doubt on previously drawn conclusions on species distributions and species-specific life histories.

The main aim of this thesis was therefore to get new insights into 1) the distribution of *Calanus* in the North Atlantic and Arctic Ocean, using molecular identification tools, 2) the biology and physiology of the poorly known *Calanus* males and 3) the life histories and physiology in a high Arctic fjord of *Calanus glacialis* (cold Arctic indicator species) and *C. finmarchicus* (warm Arctic indicator species).

The species distributions were found to be more extensive and overlapping between species than previously described for all four species, and the *Calanus* distributions in the North Atlantic and Arctic Ocean were partly redrawn. *Calanus glacialis* was found to be resident in several fjords along the coast of Norway and not strictly Arctic, while the boreal *C. helgolandicus* had a much more northerly distribution than suspected. These findings demonstrate the challenges to correctly identify the *Calanus* species in areas where they co-occur and the importance of implementing molecular tools in ecological studies.

New insights into the biology of males show that *C. glacialis* males are more lipid-rich and more active than females in January at the time of mating. Life span and male body condition were determined by laboratory incubations combined with image analyses and revealed the short (max 76 days) life span of males could not be explained by depletion of the lipid resources. Further research on the role of essential fatty acids and aging are therefore needed.

In a high arctic fjord (Isfjorden), co-occuring *C. glacialis* and *C. finmarchicus* showed similar patterns in seasonal migration and enzyme activity regulations, except for few ontogenetic specific differences. Molting to adults, reproduction and seasonal descent were found to be earlier in *C. glacialis* than *C. finmarchicus*. The earlier wake-up and reproduction in *C. glacialis* is most likely an adaption to the highly unpredictable shelf seas with short pulsed phytoplankton blooms. With larger sizes and thus more lipids, *C. glacialis* females mature and spawn prior to the bloom (capital breeding), allowing their offspring to utilize the productive season more optimally while *C. finmarchicus* which is a primary income breeder has a reproductive strategy that is better adapted to a longer, rather high phytoplankton summer production.

From this study, it appears that as long as the productive season remain similarly short and the temperatures do not exceed what *C. glacialis* thrives in, *C. glacialis* will most likely remain the best adapted and most numerous species in high Arctic shelf seas.

1

Introduction

Copepods are small aquatic crustaceans of high diversity and abundance. They have successfully colonized a wide range of habitats (Huys and Boxshall, 1991), from marine trenches of 10 000 meters depth (Belyaev, 1989) to over 5000 meters in the Himalayan Mountains, sub-zero polar oceans to hot springs (Grainger, 1965, Reid, 1994, Sommaruga, 2010).

Currently more than 17 000 species of copepods are described, of which more than 13 000 are marine species (Walter and Boxshall, 2019). Their total abundance makes them highly important in the marine food webs and they are even considered the most important primary consumers (Huys and Boxshall, 1991). Filter feeding copepods are the first link between the minute algae and higher trophic levels, thus forming the foundation for virtually all pelagic food webs (Huys and Boxshall, 1991, Falk-Petersen et al., 2007). In the Arctic and Sub-arctic seas copepods of the genus *Calanus* dominates the mesozooplankton community, in terms of biomass, and are key species in the Arctic marine ecosystem (Dahl et al., 2003, Wassmann et al., 2006).

The Arctic is characterized by strong seasonality in light climate, from complete absence of sunlight in winter to constant sunlight in the summer. As light returns in spring and nutrient rich water are stratified by melt waters and increased surface temperatures, a short and intense period of high primary production is initiated. In ice covered areas the returning light first triggers a bloom of ice algae followed by a short but intense pelagic spring bloom which fades into a smaller summer and fall

production (Leu et al., 2015). However, the Arctic has experienced substantial warming since the 1950's. Sea surface temperatures and the temperature of Atlantic water in the Arctic Ocean and its marginal seas have increased and are affecting the Arctic cryosphere. The annual Arctic sea ice extent has declined by 3.5-4.1 % per decade (1979-2012) and areas previously experiencing seasonal ice cover are now ice free year round (Comiso and Hall, 2014, IPCC, 2014). Consequently, these areas have also lost the early ice algae bloom but still seem to retain the short and intense phytoplankton bloom (Leu et al., 2015). In such strongly seasonal environments where the main inflow of energy comes in one short intense pulse in spring, grazers must coordinate their reproduction and feeding according to the seasonally fluctuations in food availability and they need to rely on stored resources for up to 10-11 months of the year (Falk-Petersen et al., 2009, Hirche, 2013).

Calanus spp. are filter feeders, primarily feeding on diatoms, though they can consume alternative prey such as eggs, fungi and heterotrophic microorganisms (Cleary et al., 2017, Frank-Gopolos et al., 2017). From their diet, they build up large lipid reserves which can make up the majority of their body mass (Sargent and Falk-Petersen, 1988, Lee et al., 2006). Lipids have the highest energy density of the macro nutrients and are therefore the most efficient for long term energy storage. In *Calanus* spp. there are mainly two types of storage lipids: triacylglycerols and wax esters, where triacylglycerols are utilized most rapidly and wax esters serve as long term energy storage (Lee et al., 2006, Kattner and Hagen, 2009). Triacylglycerols are composed of a glycerol backbone esterified with three fatty acids and wax esters are esters of one fatty alcohol and one fatty acid. *Calanus* generally incorporate fatty acids unmodified into their lipid reserves, but fatty alcohols are usually absent in marine phytoplankton and are formed either by reduction of fatty acids or from *de novo* synthesis from protein and carbohydrate (Sargent and Henderson, 1986, Graeve et al., 2005). Essential polyunsaturated fatty acids (PUFA), fatty acids the copepods can't synthesize, are preferentially retained from the diet, which is

reflected in relatively high amounts of 20:5(n-3) (eicosapentaenoic acid, EPA) and 22:6(n-3) (docosahexaenoic acid, DHA) in *Calanus* (Graeve et al., 2005). These omega-3 (n-3) fatty acids are exclusively produced by marine algae, but are essential for reproduction and growth of all marine organisms (Ackman, 1989), as well as for human health (Riediger et al., 2009). Thus the efficient accumulation of lipids, including essential fatty acids, is not only important for their own survival, but also for the transfer of energy from the primary producers to higher trophic levels (Falk-Petersen et al., 1990, Dahl et al., 2003,), making *Calanus*, a key part of the Arctic marine ecosystem.

Another important adaptation to the seasonal food availability is energy conservation during periods of low food availability (e.g. Hirche, 2013). *Calanus* spp. achieves this by descending to deeper waters and entering a state of dormancy known as diapause. During diapause they go through several phases of physiological changes to cope with a long period of food scarcity (Hirche, 1983, Hirche, 1996, Ingvarsdóttir et al., 1999). The five stages of diapause were first described in insects by (Mansingh, 1971), but the same phases were later described in copepods (Elgmork and Nilssen, 1978, Hirche, 1996): (1) Preparatory phase: development and growth are arrested and the organisms accumulate energy stores. (2) Induction phase: metabolic activity is lowered and the organisms stop feeding. (3) Refractory phase: metabolic activity is at its lowest during this phase and the organisms are torpid. (4) Activation phase: the organisms regain the ability to develop and processes like gonadogenesis start. (5) Termination phase: organisms regain the full potential of metabolic activity, growth and development.

What cues that triggers onset and termination of diapause is still not well understood, but connections have been observed between changes in metabolic activity and different external and endogenous factors. Freese et al. (2017) suggested that metabolism could be reduced as a response to starvation after

descent, and Daase et al. (2013) found that in areas with earlier bloom, copepods ascended earlier as well. Temperature also appeared to have some effect in that high surface temperatures can trigger descent (Kosobokova, 1999, Niehoff and Hirche, 2005). Of potential endogenous cues, it has been suggested that a minimum requirement for lipid stores is necessary to initiate and maintain diapause (Maps et al., 2010, Rey-Rassat et al., 2002) and that lipid depletion can initiate termination (Maps et al., 2010, Miller et al., 1991). Furthermore, in a recent study, Häfker et al. (2018) found indications of circannual clock creating an endogenous rhythm. Most likely the drivers behind diapause in *Calanus* spp. are a combination of external and endogenous cues (Johnson et al., 2007, Miller et al., 1991) and more studies are required (Baumgartner and Tarrant, 2017, Häfker et al., 2018).

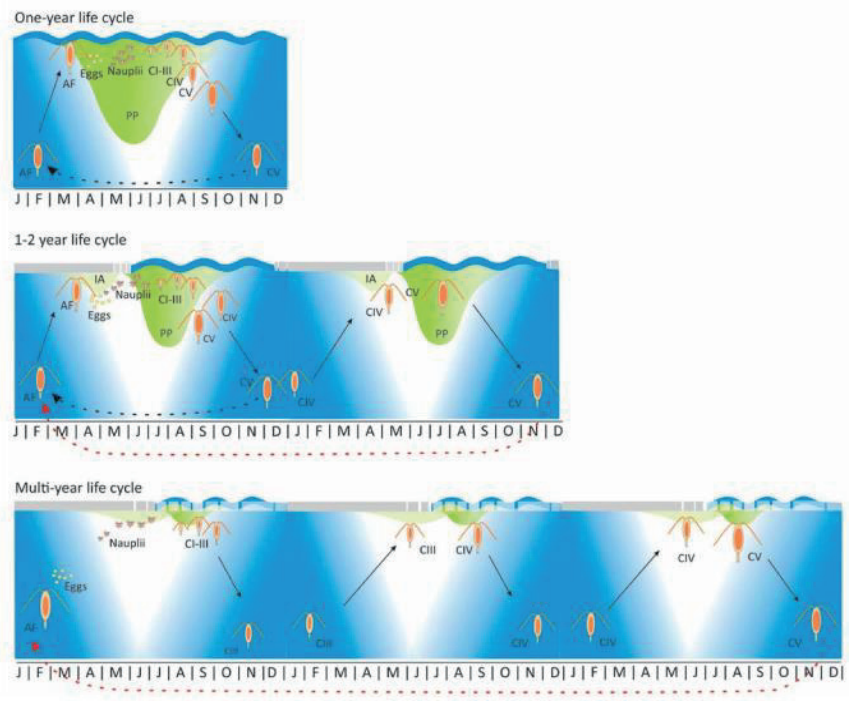


Figure 1: Life cycles of *Calanus* spp. One year life cycle (top) is the most common for *Calanus finmarchicus*. One to two year life cycle (middle) is common for *Calanus glacialis*. Multi-year life cycle (bottom) is the most common for *Calanus*

Three species of the genus *Calanus* co-occur in the European part of the Arctic: the two “Arctic species” *Calanus hyperboreus* and *C. glacialis*, and the “North Atlantic” *C. finmarchicus* (Conover, 1988, Heath et al., 2000, Hirche and Kosobokova, 2007, Melle and Skjoldal, 1998). They all perform seasonal vertical migration and go through diapause as they accumulate lipids in the surface waters during the productive season before they descend to deeper waters to overwinter in a dormant state (Conover, 1988, Falk-Petersen et al., 2009). All three species follow the same development: from eggs they develop through six naupliar stages (NI-NVI) followed by five copepodite stages (CI-CV) before they molt to adults (CVI). The Arctic deep water species, *C. hyperboreus*, can overwinter from stage CIII and have a life cycle of 2-4 years (Figure 1c). It has its core distribution in the Greenland Sea (Conover, 1988), and can be distinguished from the others by its bigger size and by an acute spine on the last thoracic segment appearing from copepodite stage IV. *C. glacialis* has a pan-arctic distribution with core area connected to the shallower shelf seas (Figure 2) (Jaschnov, 1970, Conover, 1988). The core distribution of *C. finmarchicus* is in the Northern Norwegian Sea and Labrador Sea (Beaugrand et al., 2002, Falk-Petersen et al., 2009, Jaschnov, 1970). However, it is widely distributed (Figure 2), closely following the path of the North Atlantic current into the Fram Strait and the Barents Sea and all the way into the Arctic Ocean (Gluchowska et al., 2017, Hirche and Kosobokova, 2007). Because the main study location for this project is Isfjorden, Svalbard where *C. hyperboreus* are only found in low numbers (Arnkværn et al., 2005, Scott et al., 2000) the focus has been on the much more numerous *C. glacialis* and *C. finmarchicus*.

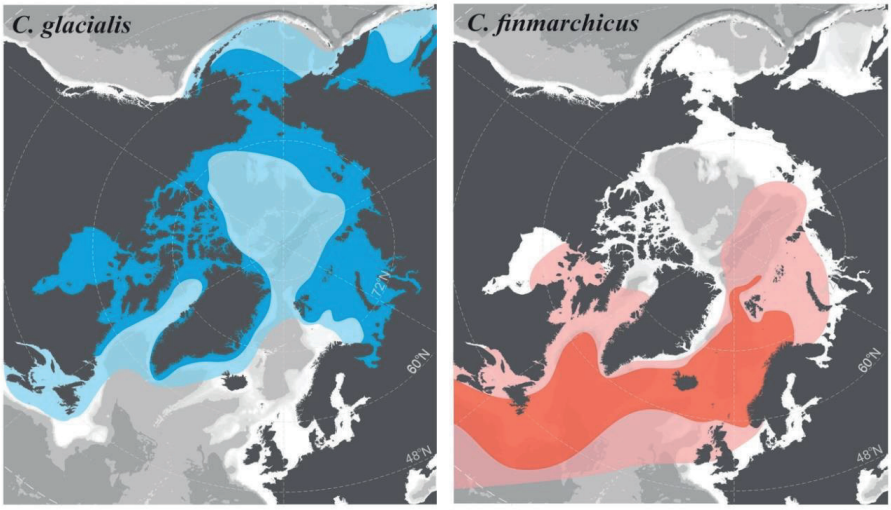


Figure 2: Distributional range of *Calanus glacialis* and *Calanus finmarchicus* based on morphological identification from previous studies (Sources in electronic supplementary material, S8 of Paper I).

C. glacialis is considered endemic to the Arctic. However local populations along the Norwegian coast have been observed (e.g. Bucklin et al., 2000, Niehoff and Hirche, 2005). *C. glacialis* is capable of both capital and income breeding (Kjellerup et al., 2012, Melle and Skjoldal, 1998). Capital breeding means reproduction fueled by stored energy reserves while income breeding is reproduction fueled by external food resources (Varpe et al., 2009). Early in the season, prior to the bloom, *C. glacialis* mature and produce eggs based on their lipid reserves. When the bloom starts they utilize the extra resources to boost the egg production (Kjellerup et al., 2012, Madsen et al., 2001). Over the summer, they grow to their overwintering stages, copepodite stage CIV or CV, and accumulate lipids for surviving the winter before they descend to the deep and enter diapause (Conover and Huntley, 1991, Falk-Petersen et al., 2009). Their life cycle is 1-2 years (Figure 1B), depending on which copepodite stage they reach by winter. Those overwintering as CIV leave diapause in spring and molt to CV before descending for a second overwintering,

while the ones descending as CV molt during winter to adult males and females and mate mid-winter (Kosobokova, 1999).

Even though *C. finmarchicus* is present in Arctic waters, there is no evidence that they successfully recruit new generations there and thus they are considered a boreal expatriate (Hirche and Kosobokova, 2007, Wassmann et al., 2015). Hirche and Kosobokova (2007) hypothesized that the poor recruitment is due to the late onset of the phytoplankton bloom rather than low temperatures. Unlike *C. glacialis*, *C. finmarchicus* is mainly an income breeder, meaning they need food for both maturation and egg production (Conover, 1988, Varpe et al., 2009). Further south, *C. finmarchicus* can have multiple generations in one year, but in its northernmost distribution area it takes one year to complete its life cycle (Figure 1 A), and the reproduction is timed with the spring bloom (Eiane and Tande, 2009, Møller et al., 2016).

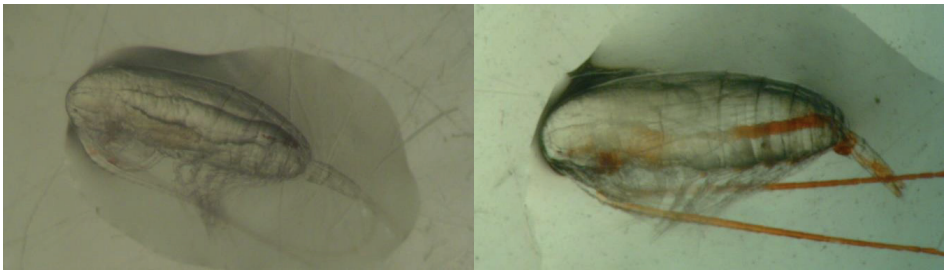


Figure 3: *Calanus finmarchicus* female (left) with pale antennules and genital somite and *Calanus glacialis* female (right) with red antennules and genital somite.

Calanus glacialis and *C. finmarchicus* are morphologically very similar and can be hard to distinguish from each other (Choquet et al., 2018). *C. glacialis* is generally slightly bigger than *C. finmarchicus*, but their size distribution is overlapping (Choquet et al., 2018, Gabrielsen et al., 2012). This can lead to misidentification, especially of smaller *C. glacialis* as *C. finmarchicus*. At higher latitudes *C. glacialis* tends to have more red pigmentation than *C. finmarchicus* (Figure 3) and this in combination with prosome length be used to separate the two species with reasonable confidence, although molecular ID is the only 100 % accurate method (Choquet et al., 2018).

With the ongoing global warming, an extensive borealization of the Arctic zooplankton community is predicted (Hays et al., 2005). In the area surrounding the path of the Norwegian Atlantic current into the Fram Strait and Barents Sea, a significant increase in the boreal *C. finmarchicus* has already been observed (Aarflot et al., 2017, Gluchowska et al., 2017, Hop et al., 2019). Because of the affiliation of *C. glacialis* and *C. finmarchicus* to Arctic and Atlantic water masses, respectively, they are regarded important beacons of climate change in the Svalbard-Barents Sea region as changes in their distribution can indicate changes in Atlantic water circulation (Hays et al., 2005, Wassmann et al., 2015). A high proportion of *C. finmarchicus* to *C. glacialis* reflects a relatively warm Arctic climate while the opposite reflects a colder Arctic climate. A shift in their distribution, where *C. finmarchicus* becomes dominant in areas where *C. glacialis* used to be, is likely to have consequences for the higher trophic levels (Falk-Petersen et al., 2007, Kitaysky and Golubova, 2000, Vihtakari et al., 2018). A shift in size can impact predators that actively select larger individuals (Martens et al., 2015), such as the little auk (*Alle alle*) (Kwasniewski et al., 2012, Møller et al., 2018, Vogedes et al., 2014). However, a recent study suggests that being fed on smaller zooplankton may not result in a decline of chicken growth and adult body-condition (Amélineau et al., 2016). Furthermore, Renaud et al. (2018) suggest that a shift to *Calanus* spp. of smaller

body size may be compensated by higher turnover rate in terms of total lipid per unit mass and thus such a shift may not be detrimental to the lipid based food-web contributions from *Calanus.*

Due to the key role of *Calanus* spp. in the Arctic food web and the uncertainties around its fate in a warming Arctic, *Calanus* have been the focus of many studies in the recent years (e.g. Gabrielsen et al., 2012, Mayor et al., 2015, Renaud et al., 2018). Nonetheless many knowledge gaps remain. Among them are uncertainties around correct species identification of *C. finmarchicus* and *C. glacialis*, and knowledge about the physiological mechanisms behind their different life strategy in arctic conditions. Particularly little is known particularly about the males. They are present only for a short period and mainly in winter with highest occurrence of males for *C. glacialis* reported between December and February (Madsen et al., 2001, Niehoff et al., 2002, Wold et al., 2011) and March and April for *C. finmarchicus* (Madsen et al., 2001). The timing, especially for *C. glacialis* coincides with darkness, low temperatures and potentially difficult ice conditions, making sampling logistically challenging. More knowledge about the biology of *Calanus* males is necessary to fully understand the reproductive strategy of these species.

2

Objectives

The overall goal of this thesis was to increase our knowledge on *Calanus glacialis* and *C. finmarchicus* life histories and to combine existing and new knowledge to better understand the fate of these two species, regarded as important beacons of climate change in a rapidly warming Arctic. This was accomplished through the following objectives:

1. Validate the distributions of the four *Calanus* species in the North Atlantic using molecular tools. (Paper I)
2. Study the behavior and life span of the poorly known *Calanus* males to better understand *Calanus* reproductive strategies (Paper II, III and IV)
3. Investigate the population dynamics and corresponding physiology of co-occurring *C. glacialis* and *C. finmarchicus* in high Arctic environments to identify potential advantages and disadvantages to the current climate. (Paper IV)

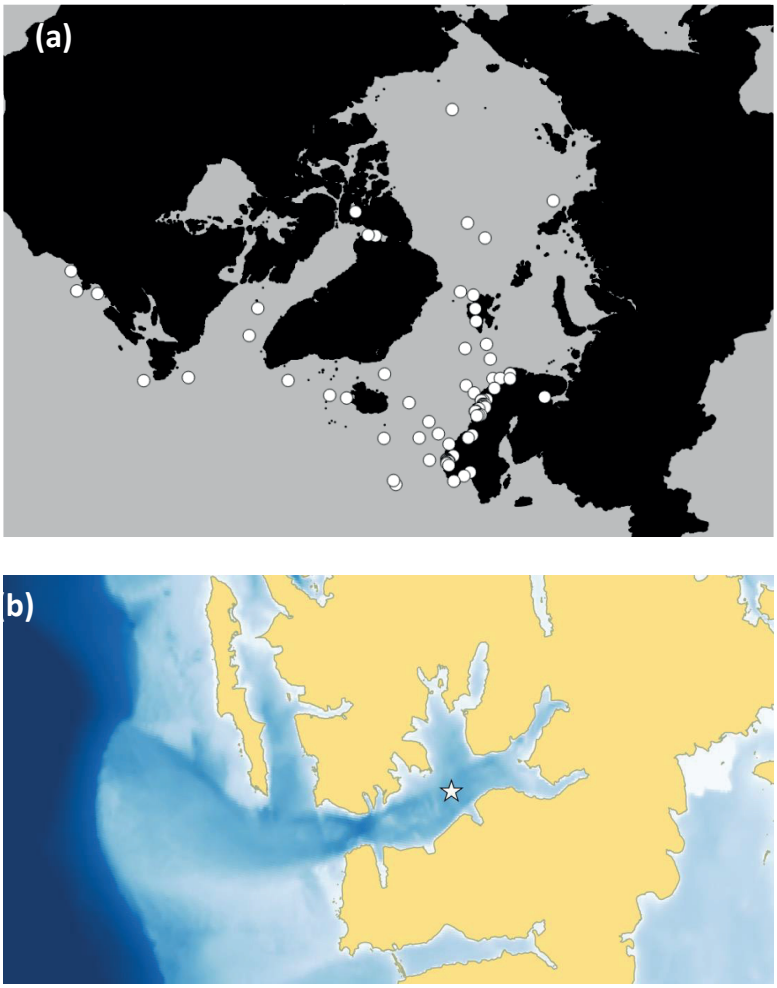


Figure 4: Maps indicating the sampling locations for the *Calanus* distribution study (a) and the main study area in Isfjorden, Svalbard (b) (Map data: NPI, GEBCO, GADM)

3

Approach

3.1 Study area

For re-assessment of the *Calanus* distribution, zooplankton samples were collected from 83 locations in the North Atlantic and Arctic Oceans (Figure 4a).

For the high-resolution year-round sampling we focused on Karlskronasdjupet (78°19'N; 015°10'E), a 274 m deep basin in the central part of the Isfjorden system on Spitsbergen, Svalbard (Figure 4b) in near vicinity to the University Centre in Svalbard. Spitsbergen is the largest island of the Svalbard Archipelago and is situated between 76° and 81° N. Off the west coast, two north flowing currents affects the coastal area: the Arctic East Spitsbergen Current (ESC) following the shelf and the Atlantic West Spitsbergen Current (WSC) following the shelf break (Nilsen et al., 2008). Several fjords are located on the west coast of Spitsbergen, and they are to varying degree influenced by these two currents as well as by local water production. The largest fjord is Isfjorden, an open fjord system with relatively strong influences from the Atlantic WSC since 2005. This has led to ice free conditions for most parts of Isfjorden, except the innermost shallow fjord arms and the fjord arms with a distinct, shallow sill, preventing warm Atlantic water to penetrate (Muckenhuber et al., 2016, Nilsen et al., 2008).

3.2 Methods

Various methods have been applied throughout this thesis. The central part of the thesis has been extensive field sampling to follow the *Calanus* population in

Isfjorden throughout a whole year. The seasonal changes in the population composition have been assessed (Paper IV) along with the seasonal variations in the relative activity of different enzymes (Paper IV). The behavior of males has been specifically targeted through several winter cruises (Paper II and Paper III). A grazing experiment was conducted with ^{13}C labeled algae to assess the feeding potential of males (Paper III) and new insight into their behavior was revealed through respiration and activity assessments (Paper II). Molecular markers for species identification have been applied throughout this study for verification of morphological identification (Paper IV), assessment of prosome length frequency distributions (Paper II) and last but not least the work with assessing the distributional range of *Calanus* (Paper I).

3.2.1 Morphological identification of *Calanus* spp.

Several characteristics have been used to distinguish between the different species of *Calanus.* The classical, though time consuming, method have been to examine certain morphological traits such as the structure of the fifth pair of swimming legs or the coxal endid of the mandibles (Beklemishev, 1959, Jaschnov, 1955), although recent work has challenged the validity of such characters (Choquet et al., 2018). A faster and more convenient method is to separate by prosome length (e.g. Arnkværn et al., 2005, Daase and Eiane, 2007), and recently pigmentation have been suggested as a trait for distinguishing *C. glacialis* from *C. finmarchicus* (Nielsen et al., 2014).

For the present work, the animals needed to be sorted quickly and in good shape, hence a combination of prosome length and pigmentation was used to distinguish between *C. glacialis* and *C. finmarchicus*. The reliability of the different traits varies between regions, but these two have been proved as good indicators in the Arctic (Choquet et al., 2018).

The animals were sorted by prosome length according to Daase and Eiane (2007) with adjustments for CV and adult females after Gabrielsen et al. (2012) as summarized in Paper II. From copepodite stage CIV, *C. hyperboreus* was also distinguished by the presence of an acute spine on the fifth thoracic segment.

Pigmentation was used as an additional species indicator, where *C. glacialis* tend to have more red pigmentation on the antennules and the female genital segment of the urosome compared to *C. finmarchicus* (Choquet et al., 2018, Nielsen et al., 2014,).

Table 1: Length classes used to distinguish between the three *Calanus* species at the different copepodite stages. *Distinguished by acute spine on the fifth thoracic segment.

	Prosome length (µm)		
Stage	*C. finmarchicus*	*C. glacialis*	*C. hyperboreus*
CI	< 810	810-900	> 900
CII	< 1170	1170-1350	> 1351
CIII	< 1470	1470-1950	> 1950
CIV	< 2010	2010-2910	*
CV	< 2900	> 2900	*
AF	< 2950	> 2950	*

3.2.2 Molecular identification of *Calanus* spp.

For molecular identification of *Calanus* spp. DNA was extracted from the antennule only following the hotshot DNA extraction (Montero-Pau et al., 2008). From the extracted DNA, six molecular markers characterized by length mutations caused by insertion or deletion, so called InDels, were amplified by Polymerase Chain Reaction (PCR) and the size of the amplicons determined. Nuclear InDel polymorphisms mainly ensue from a single mutation event and have low mutation rates, thus providing a fairly conserved phylogenetic signal (Liu and Cordes, 2004, Nagy et al., 2012). Within a species, the size of an InDel marker will be consistent, and thus

differences in size between species will be consistent. Species specific profile of the six markers used in this study was developed by Smolina et al. (2014) and can be used to identify and distinguish all *Calanus* species in the North Atlantic and Arctic oceans.

3.2.3 Population composition

Abundance was analyzed from samples preserved in formalin. The samples were drained, rinsed in filtered sea water and then soaked in filtered sea water to wash out the formalin. After washing the samples were transferred to a beaker and diluted to a known volume. Subsamples of a known volume were taken out and analyzed under a stereo microscope. All *Calanus* spp. copepodites in the subsample were enumerated and identified morphologically to species and development stage. Subsamples were taken until a minimum of 200 individuals was reached.

Weighted mean depth was calculated for total population of C*. glacialis* and *C. finmarchicus* as well as copepodite stages within the species according to equation 1.

$$\frac{\sum_{i=1}^{n}(a_i d_i)D_i}{\sum_{i=1}^{n} a_i d_i} \qquad \text{(Equation 1)}$$

Where a_i is the number of individuals per m^3 of species a in depth stratum i, d_i is the sampled distance in depth stratum i, D is average depth in depth stratum i and n is the number of depth strata at a station.

3.2.4 Feeding experiment

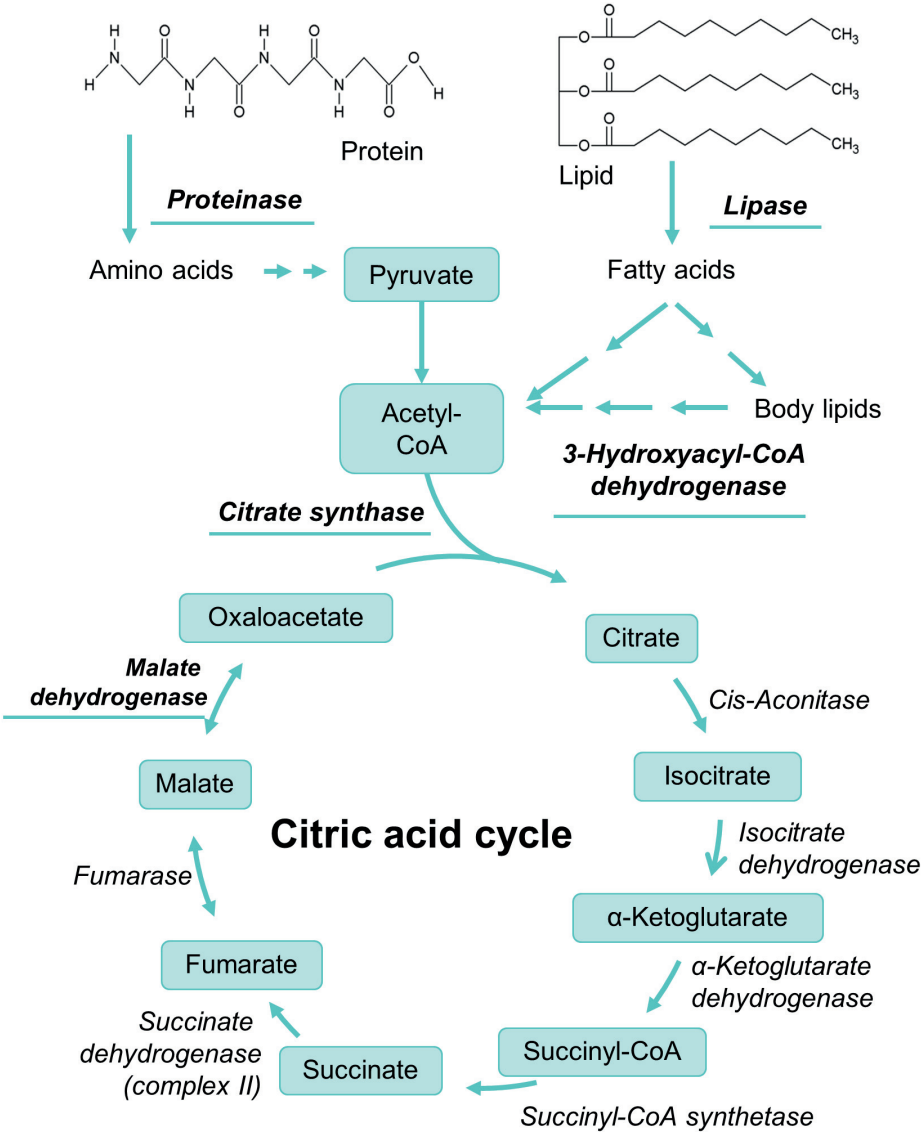
Males and females of *C. glacialis* were incubated in bottles and fed ^{13}C labelled algae (Paper III). The algae culture was enriched with ^{13}C sodium bicarbonate which makes it possible to trace fatty acids containing elevated levels of this isotope from the diet to the assimilated lipids of the copepods (Paper III). Through the incubation experiment the bottles, with the copepods were kept on a rotating plankton wheel to keep the microalgae in constant suspension. From the bottles we regularly counted the number of fecal pellets produced as a proxy for grazing activity and regular samples of copepods were collected for lipid composition and compound specific stable isotope analyses (CSIA) to evaluate the changes in lipid content and composition and to trace the assimilation of the ^{13}C stable isotope.

3.2.5 Enzyme analyses

Reduced metabolism and diapause can be indicated by the level of activity of enzymes participating in central metabolic pathways (Paper IV). To assess the physiological state of the copepods throughout the different seasons, enzyme activity of key enzymes linked to digestion, catabolism of body lipids and overall metabolic activity was measured.

The relation of the analyzed enzymes to the central metabolic pathway, the citric acid cycle, is illustrated in box 1.The advantage of enzyme measurements compare to incubation methods like respiration is the reduction of handling effect (Ohman et al., 1998).

Box 1: Overview of the role of the enzymes analysed in relation to the central metabolic pathway, the citric acid cycle. All substrates/products are noted in plain font, enzymes are noted in italics, and the enzymes analysed in this study har highlighted in bold and underlined.



In the present study we have used citrate synthase (CS) and malate dehydrogenase (MDH) which both catalyze different reactions in the citric acid cycle (Box 1) and thus can be used as indicators of the aerobic potential of an organism (Meyer et al., 2002, Teschke et al., 2007, Torres and Somero, 1988). CS catalyse the first step of the citric acid cycle, the condensation of acetyl-Coenzyme A (acetyl-CoA) and oxaloacetate to form citrate, while MDH reversibly catalyses the last step, the oxidation of malate to oxaloacetate.

For utilization of ingested resources, digestive enzymes break down the components of the ingested food so it can be assimilated for growth and reproduction. The major components of the food the copepods ingest are proteins and lipids, which are processed by proteinase and lipase/esterase, respectively (Box 1). They are both groups of several enzymes with variations in the specific mechanisms, but in general, proteinases break down proteins by hydrolyzing the peptide bonds between the amino acids (Mayzaud, 1986), and lipases/esterases break down lipids by cleaving the ester bonds of carboxylic acids (Luppa and Andrä, 1983).

For utilization of the stored lipid resources, 3-hydroxyacyl-CoA dehydrogenase (HOAD) is a good indicator of the catabolism of body lipids as it is an important enzyme for the β-oxidation of fatty acids to produce acetyl-CoA for use in the citric acid cycle (Auerswald and Gäde, 1999, Hassett, 2006) (Box 1).

Because of the uncertainty of distinguishing between *C. glacialis* and *C. finmarchicus* based on morphology, the remaining pellet of tissue after enzyme extraction was kept and genetically tested. Species composition of the tissue pellet was assessed by analysis of InDel markers as described in section 3.2.2. From these markers we got profiles of the tissue pellets from the enzyme extraction that either matched one of the species specific profiles from Smolina et al. (2014), indicating a pure sample, or contained mixed signals for both *C. glacialis* and *C. finmarchicus,* indicating a mixed sample.

4

Main findings and General Discussion

4.1 Distribution of *Calanus* spp. in the North Atlantic and Arctic

The use of molecular markers for species ID, revealed that the distribution of all four *Calanus* species in the North Atlantic and Arctic Oceans have wider distributional ranges than previously reported (Paper I). *Calanus helgolandicus* was found as far north as 70°N, 12° further north than its previously known (Barnard et al., 2004), and the Arctic *C. hyperboreus* was found as far south as 58°N (Paper I). However, it is not clear if these are sustainable populations or rather a result of advection from their respective core areas (Broms et al., 2009). *C. glacialis* was mostly found adjacent to the Arctic shelf seas, but local fjord populations were found along the Norwegian coast, co-existing with *C. finmarchicus*. The presence of *C. glacialis* populations has been recorded in Norway previously, e.g. Lurefjorden (Bucklin et al., 2000, Niehoff and Hirche, 2005), but not to such extent (Paper I). The high morphological similarities and the almost completely overlapping size distributions of *C. glacialis* and *C. finmarchicus* in these Norwegian fjords and large parts of their distribution area (Choquet et al., 2018) have resulted in failure of identifying *C. glacialis* in these fjords earlier and to underestimation of *C. glacialis* elsewhere (Gabrielsen et al., 2012, Paper I).

The range of *C. finmarchicus* extended as far North as 87°N and as far east as the Laptev Sea (78°N, 113 E). These areas are influenced by warm Atlantic inflow, and the *C. finmarchicus* found here are most likely transported from populations further

south (Wassmann et al., 2015), supporting the continued use of *C. finmarchicus* as a valid indicator species of Atlantic water (Paper I).

The main take home message from this large scale distribution study is that there are likely many erroneous species distribution and population data from the past and that we have to start using molecular tools to correctly identify species before conclusions can be drawn. We have to continue to revise the large-scale patterns in *Calanus* distribution by using molecular tools in order to validate our current use of *C. glacialis* and *C. finmarchicus* as climate indicator species.

4.2 Life span and behavior of *Calanus* males

Calanus have been studied extensively, but knowledge of *Calanus* males is surprisingly poor (Paper II). Males of *C. glacialis* appeared up to two months earlier than males of *C. finmarchicus* in Svalbard (Paper II, IV). Life span for males is known to be short, and males kept in the laboratory suggested an average life span of 43 days (maximum 73 days). Life span for *C. finmarchicus* males were not studied, but is likely to be even shorter since *C. finmarchicus* males was rarely encountered (Paper II, IV). Several factors indicated that *C. glacialis* males were actively mating in January. Males were particularly numerous in January and high female:male sex ratios were found (Paper II). Furthermore, almost none (<2.5%) of the remaining *C. glacialis* CV were found to be males (indicated by gonad investigations) and 10% of the females had spermatophores attached (Paper II). Other observations, such as high swimming activities and twice as high respiration rates in males versus females (Paper II, Paper IV) further strengthen that males were actively mating (Kiørboe, 2008). Males of *C. glacialis* are mainly present in winter when food is scarce (Paper II, Paper IV). Higher swimming activity results in higher risk of predator encounter, but also higher chance of encountering food particles. In our study we found that males are capable of feeding and assimilating lipids from the food. However, they were less efficient than females (Paper III). We found indications that *C. glacialis*

male is more omnivorous than females, and given that they are present mainly in winter when primary production is absent, it is possible that they are better adapted to non-algae food. A recent study has also shown that *Calanus* feed on alternative prey outside the productive season (Cleary et al., 2017) and even if the overall biomass in winter is low (Kubiszyn et al., 2017), organisms are present and could potentially be consumed by e.g. *C. glacialis* males to supplement their mate search and sperm production (Paper III).

One common hypothesis explaining the short life span in males is that males die due to energy shortage. Controlled laboratory incubations where males' lipid sac sizes were followed over time until their death rejected this hypothesis (Paper III).

The life span of male *Calanus* do not appear to be restricted due to lack of lipid reserves as parallel experiments of starved males and females showed males to die with substantial amounts of lipids left while females completely depleted their lipid sac. However, it is possible that certain vital components, e.g. essential fatty acids or other elements (e.g. Mayor et al., 2009) got depleted. Males can most likely only produce a limited amount of high quality spermatophores on internal lipid sources (Hopkins, 1978) and by actively searching for females they are exposing themselves to higher predation risk (van Duren and Videler, 1996). These are both factors that can limit the life expectancy. The males might benefit from investing all their resources into reproduction at the cost of maintaining their own somatic tissue because they do not gain any benefit from staying alive for longer.

In conclusion, at the time of mating in January, *C. glacialis* males are the most active in the search for a mate and possibly they can compensate for some of the mating costs by feeding. However, pure energy depletion do not appear to be the limiting factor of their short life span, and further studies are recommended on the role of essential fatty acids and aging.

4.3 Population dynamics and physiology

Reproduction

C. glacialis dominated the population and had a more successful recruitment of a new generation than *C. finmarchicus* during our study (Paper IV). This seems to be a result of the short productive season and how well that fit with the different reproduction strategy of the two species. Adult males and females of *C. glacialis* appeared in December, two months prior to *C. finmarchicus* in Isfjorden (Paper IV) and this separation in time was also found elsewhere in the Svalbard region (paper II). From mating it takes some time before females mature and produce eggs (Niehoff and Hirche, 1996). Females of *C. glacialis* mature their gonads and start egg production based on their lipid stores, so called capital breeding, before food is present. Since they are already mature when the spring bloom starts, they are immediately ready to utilize it to boost egg production (Paper IV). *C. finmarchicus* on the other hand is an income breeder, and need food to mature their gonads and produce eggs. This was evident from the egg production rates which pick up later for *C. finmarchicus* and lasted longer compared to *C. glacialis* (Paper IV). The difference in egg production carried over to the timing of young stages (CI-CIII). Like the egg production, *C. glacialis* CI-CIII were present for a shorter time earlier in the productive season than *C. finmarchicus* where CI was observed as late as August. By being mature and spawning already prior to the spring bloom, *C. glacialis* provides its offspring with a longer part of the productive season to grow and store lipids for over wintering, while the late start of *C. finmarchicus* means a lot of the offspring most likely is lost prior to overwintering because the productive season is too short (Paper IV).

Vertical migration and metabolic activity

C. finmarchicus and *C. glacialis* largely followed the same seasonal patterns of migration and seasonal regulation of their metabolic activity (Paper IV). By August,

when the population descended to depth, the population was dominated by the overwintering stages. *C finmarchicus* was dominated by CV and *C. glacialis* by CIV, reflecting their predominantly one and two year life cycle respectively (Paper IV). A substantial amount of *C. finmarchicus* CIV was also found in the overwintering population. These CIVs may be late-born *C. finmarchicus* which did not have time to develop beyond CIV (e.g. Arashkevich et al., 2004), but we cannot exclude that many of these CIVs may have been misidentified *C. glacialis* CIV (Choquet et al., 2018, Gabrielsen et al., 2012).

Vertical migration and metabolism was shown to be closely connected with a strong negative correlation, indicating that when the population is deeper in the water column, metabolic activity (CS and MDH) is lower (Paper IV), reflecting how *Calanus* overwinter by descending to depth and entering diapause. What exactly triggers and regulates metabolism, and particularly the onset and termination of diapause, is still poorly understood for *Calanus*, but most likely it is interactions between several external and internal factors (Johnson et al., 2007, Miller et al., 1991). In our study we found a strong correlation to day length for both CS and MDH. Photoperiod as a direct trigger to terminate diapause has been questioned (Hind et al., 2000; Johnson et al., 2007), however, Speirs et al., (2005) suggest it may be important for synchronizing the population near the end of diapause. The seasonal patterns in MDH was also not only similar between *C. finmarchicus* and *C. glacialis* in Isfjorden in our study, but also with *C. glacialis* from a similar study by Freese et al. (2017) in the colder Billefjorden and *C. finmarchicus* sampled off shelf north of Svalbard (Paper IV), suggesting similar seasonal physiological response independent of temperature and species (Paper IV). This consistent seasonal pattern in MDH could indicate a response explained at least in part by a circannual clock as suggested by Häfker et al. (2018).

In conclusion, *C. finmarchicus* and *C. glacialis* follow much the same patterns of seasonal migration and regulation of metabolic activity. It is still not clear what triggers changes in metabolic activity. A circannual clock seems to be part of it, as well as photoperiod, though the latter may not cue changes in diapause directly but rather work to synchronize the population towards the end of diapause.

4.4 Fate of *Calanus*

The key factor of the higher success of *C. glacialis* in the current environment is its capability to perform capital breeding which makes it very flexible in terms of being ready to reproduce when the spring bloom starts (Paper IV). Because capital breeding is based on lipid stores and lipid content in *Calanus* is positively correlated to body size (Renaud et al., 2018, Vogedes et al., 2010), the capacity for capital breeding becomes larger for larger specimen (Varpe et al., 2007, Varpe and Ejsmond, 2018). However, a bigger body size also means higher risk of predation (Varpe and Ejsmond, 2018). The optimal body size of *Calanus* in a given environment is thus a balance between bottom-up (e.g. food availability and accumulated lipid resources) and top-down processes (Varpe and Ejsmond, 2018). In the Arctic where the production season is short and predation pressure low, a longer life cycle and larger body size with more lipid stores is beneficial. Further south where productive season is longer and predation pressure higher, smaller body size and shorter life cycle appears to be a better strategy. The effect of these factors on *Calanus* body size is evident in the discovery of *C. glacialis* population in Norwegian fjords which have been overlooked because their size distribution completely overlaps with *C. finmarchicus* (Paper I).

Because *C. finmarchicus* is an income breeder, their life strategy is more restricted in the Arctic. Studies from further south have shown the ability to produce multiple

generations in a season where early in the season parts of the population descend and enter diapause while another part matures and produce a second generation (Häfker et al., 2018). In our study, we saw a prolonged period of young stages, which could indicate the same strategy as described in Häfker et al. (2018), however the short productive season most likely means that the late spawned off spring were lost (Paper IV).

One concern regarding climate change in the Arctic is the ability of Arctic species to handle the warmer temperatures. Studies have shown that *C. glacialis* lack heat protective responses compare to *C. finmarchicus* (Smolina et al., 2015). When exposed to increasingly higher temperatures, *C. finmarchicus* showed a strong response in upregulation of genes related to handling heat stress, while the response in *C. glacialis* was lacking (Smolina et al., 2015). In our study of HOAD, which indicates catabolism of body lipids for energy during the non-productive season, we found differences between our station and a similar study by (Freese et al., 2017) in the colder Billefjorden which indicates they are able to adjust enzyme activity according to temperature to some extent (Paper IV). However, the main strategy of *C. glacialis* appears to be avoiding warmer temperatures, as shown by (Niehoff and Hirche, 2005) in Lurefjorden, Norway, where *C. glacialis* descended to colder depths when the surface temperatures were at 5-6 °C.

As long as the productive season remain short and the temperatures do not exceed what *C. glacialis* thrives in, *C. glacialis* will most likely remain the best adapted and most numerous species in high-Arctic shelf seas. A longer productive season will improve the living conditions for *C. finmarchicus*, but still early reproduction favors *C. glacialis.* The reduction in sea ice and earlier onset of the spring bloom and thus slighter longer overall duration of the open water primary production has even led to a boost in the *C. glacialis* population in Svalbard, due to more females being present in the population when algal food appears (Paper IV).

5

Conclusions and Perspective

This thesis is one of the first studies comparing the population structure and the *in situ* physiological adaptations of co-occurring *C. glacialis* and *C. finmarchicus* in strongly seasonal, high-Arctic environments. Distribution ranges of *Calanus* species in the North Atlantic and Arctic Ocean were found to be wider than previously known, but our findings still support *C. glacialis* and *C. finmarchicus* as climate indicator species.

The new insight in physiological adaptations of co-occurring *C. glacialis* and *C. finmarchicus* from this study enable us to better understand why these two species have different success in terms of abundance in their respective habitat of preference. Although the two species largely follow similar patterns of seasonal migration and physiological adaptations, some distinct differences allow *C. glacialis* to be more successful in high-Arctic where the strong seasonality in light restricts the window of primary production. Indeed, the short productive season appears to be the main driver for the difference in success, as it is too short for the income breeder strategy of *C. finmarchicus* while *C. glacialis* have adapted to utilize this much more efficiently.

The present study also contributes to new knowledge on *Calanus* males, primarily *C. glacialis* since they were the only one present in January and males of *C. finmarchicus* was rarely found. Males were capable of feeding and lipid resource depletion was not found to be the main reason for their short lifespan. In future

studies, we recommend more research on the role of essential fatty acids and aging as determining factors for males' life span to resolve the biology and role of males in the population.

By implementing molecular species identification we can be confident that the physiological comparison of these two climate indicator species is truly valid. However, it has not been possible to address some aspects with the same certainty because of the potential of misidentification where only morphological species identification has been applied. For example, the interpretation of the substantial amount of *C. finmarchicus* CIV in the winter population (Paper IV) and the pre-bloom egg production by *C. finmarchicus* (Paper IV), must be treated with caution due to the possibility of misidentified *C. glacialis.* This highlights again the importance of including molecular tools in ecological studies to eliminate uncertainties and further untangle differences in species specific adaptations to better predict their fate in a warming Arctic.

From this study it appears that as long as the productive season remain short and the temperatures do not exceed a certain threshold, *C. glacialis* will most likely remain the best adapted and most numerous species in high Arctic shelf seas. A longer productive season could make conditions better for *C. finmarchicus*, but because of the strategy of *C. glacialis* to be ready earlier than *C. finmarchicus* it is doubtful that this will be a direct threat to the presence of *C. glacialis*.

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Paper I

Choquet M, Hatlebakk M, Dhanasiri AKS, Kosobokova K, Smolina I, Søreide JE, Svensen C, Melle W, Kwaśniewski S, Eiane K, Daase M, Tverberg V, Skreslet S, Bucklin A, Hoarau G.

Genetics redraws pelagic biogeography of *Calanus*

Biology letters **13**:20170588

BIOLOGY LETTERS

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Research

Cite this article: Choquet M *et al*. 2017 Genetics redraws pelagic biogeography of *Calanus*. *Biol. Lett.* **13**: 20170588.
http://dx.doi.org/10.1098/rsbl.2017.0588

Received: 21 September 2017
Accepted: 27 November 2017

Subject Areas:
ecology, molecular biology, taxonomy and systematics

Keywords:
zooplankton, genetics, climate change, species identification, fjord, ecosystem shift

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3949609.

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Marine biology

Genetics redraws pelagic biogeography of *Calanus*

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Planktonic copepods of the genus *Calanus* play a central role in North Atlantic/Arctic marine food webs. Here, using molecular markers, we redrew the distributional ranges of *Calanus* species inhabiting the North Atlantic and Arctic Oceans and revealed much wider and more broadly overlapping distributions than previously described. The Arctic shelf species, *C. glacialis*, dominated the zooplankton assemblage of many Norwegian fjords, where only *C. finmarchicus* has been reported previously. In these fjords, high occurrences of the Arctic species *C. hyperboreus* were also found. Molecular markers revealed that the most common method of species identification, prosome length, cannot reliably discriminate the species in Norwegian fjords. Differences in degree of genetic differentiation among fjord populations of the two species suggested that *C. glacialis* is a more permanent resident of the fjords than *C. finmarchicus*. We found no evidence of hybridization between the species. Our results indicate a critical need for the wider use of molecular markers to reliably identify and discriminate these morphologically similar copepod species, which serve as important indicators of climate responses.

1. Introduction

Copepods of the genus *Calanus* are central in North Atlantic and Arctic pelagic food webs. Rich in lipids, they form a key link between primary producers and secondary consumers and predators. Four species of the genus *Calanus* occur throughout the North Atlantic and Arctic Oceans (figure 1): *C. helgolandicus* (*Chel*), *C. hyperboreus* (*Chyp*), *C. finmarchicus* (*Cfin*) and *C. glacialis* (*Cgla*); and there has been considerable effort to document and model their distributional changes [1,2]. Importantly, abundances and dynamics of fish stocks are strongly associated with *Calanus* species composition and abundances [3], and climate-driven changes in their biogeographical distributions (i.e. range shifts) can lead to ecosystem regime shifts and potential collapse of fish stocks such as cod [4]. However, distinguishing *Calanus* species is challenging due to their morphological similarity and lack of diagnostic characters. The

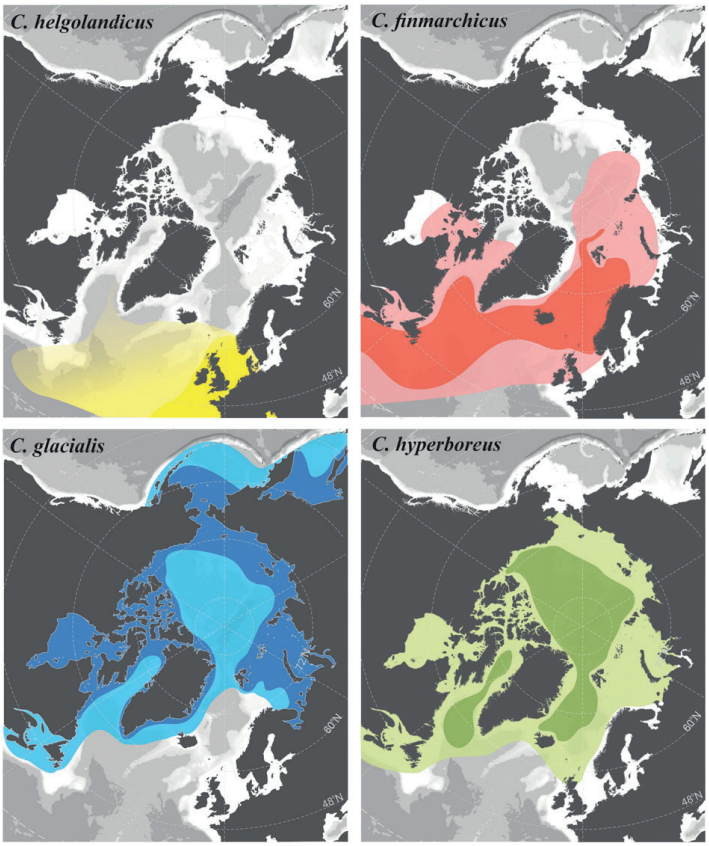

Figure 1. *Calanus* species distributional ranges in the North Atlantic and Arctic Oceans based on morphological identification from previous studies (sources in electronic supplementary material, S8). For each panel, dark-shaded colour represents core area for each species, where reproduction is known to occur; light-shaded colour represents the total described distributional area.

usual method of species identification is body (prosome) length, although this approach has been questioned [5,6]. Misidentification may thus occur, impacting the reliability of our current knowledge of species distributions and preventing accurate assessment of species geographical range shifts in response to climate change.

Here we re-examine the distributional ranges of four co-occurring *Calanus* species in the North Atlantic and Arctic Oceans, using six molecular markers designed to ensure reliable species identification.

2. Material and methods

(a) Sample collection

Zooplankton samples were collected from 83 locations in the North Atlantic and Arctic Oceans (electronic supplementary material, S1) by vertical nets tows with 150–200 µm mesh sizes and preserved in 70–80% ethanol. A Folsom plankton splitter was used to make subsamples containing up to 150 *Calanus* individuals from developmental stage CIV–CVI (electronic supplementary material, S1). No species level morphological identification was performed for any individuals.

(b) Molecular species identification

DNA was extracted from the excised antennae of each specimen using the HotSHOT protocol [7], and molecular species identification of 4434 individuals was achieved using six nuclear markers type InDels (Insertion or Deletion motifs) [8] scored on a 3500xL genetic analyzer (Applied Biosystems). These bi-parentally inherited markers are easy to use and can potentially detect hybridization [9]. Their reliability was confirmed by the traditional, but more cost- and labour-intensive mitochondrial 16S rDNA sequencing (mtDNA) [10,11] of 159 individuals from 53 locations (electronic supplementary material, S2 and S3), following Smolina *et al*. [8]. In addition, 129 individuals from Saltfjord/Skjerstadfjord were measured (prosome length) and sequenced for the 16S (table 1; electronic supplementary material, S4 and S5). Identification of specimens from InDels and 16S rDNA sequences was congruent for all 677 individuals investigated (288 in present study (electronic supplementary material, S2–S4) and 389 in Nielsen *et al*. [9]). InDel markers were also used to test for the presence of hybrids between *Cfin* and *Cgla* [9] (electronic supplementary material, S6).

(c) Population differentiation

Population genetic analysis was carried out to distinguish between fjord resident and drifting (seasonally transient) species [12] (electronic supplementary material, S7). Focusing on *Cfin*

Table 1. Comparison of *Calanus finmarchicus* (*Cfin*) and *C. glacialis* (*Cgla*) identification methods in Saltenfjord/Skjerstadfjord.

Saltenfjord/ Skjerstadfjord	InDel species ID	16S rDNA species ID	markers' congruence	prosome length range (μm)			
				N	stage CV	*N*	stage CVI female
Cfin	89	89	100%	26	1976.64 – 2717.76	14	2406.89 – 2747.02
Cgla	40	40	100%	20	2119.40 – 2623.33	69	2150.68 – 3030.50

and *Cgla* populations, genetic differentiation was measured using the global index of population differentiation, F_{ST} [13], based on 10 microsatellite DNA markers [14,15] assayed for 24 individuals per species from three locations: Isfjord, Saltfjord and Lurefjord.

3. Results and discussion

Identification of *Calanus* species using molecular markers revealed that all four species have much wider distributional ranges than previously reported (figures 1 and 2; electronic supplementary material, S1), as suggested by an earlier molecular study [6]. The distribution of *Chel* was known to extend from the Mediterranean Sea to the North Sea (58° N, figure 1) [16]. Here, we identified *Chel* in several Norwegian fjords and in the Norwegian Sea as far north as 70° N (figure 2). Specimens found in Myken stations (66° N) and near Tromsø (70° N) could result from transport in ocean frontal jet currents running from the North Sea along the Norwegian coast. However, the high prevalence (85%) of the species recorded in the relatively isolated Sognefjord (61° N) may represent a locally established population. It remains to be tested whether *Chel* has always been present in these fjords but misidentified, or whether our findings represent evidence of a recent biogeographical range shift.

Previous reports of the Arctic *Chyp* [17] occurring in the northern Norwegian Sea (figure 1) have been attributed to transport of individuals by Arctic intermediate waters [18]. Here, we detected the species in large proportions along the Norwegian coast, everywhere north of 58° N (figure 2; electronic supplementary material, S1). Whether the southern presence of *Chyp* results from advection from Arctic stocks or from self-reproducing populations remains to be investigated.

Calanus finmarchicus is currently considered to be an indicator species of North Atlantic water masses [17], and our results largely support this view (figure 2). The genetically confirmed species range extends as far north as 87° N and as far east in the Arctic as the eastern boarder of the Laptev Sea (78° N, 113° E, figure 2), regions of the Arctic Ocean affected by Atlantic inflow. It was proposed that *Cfin* may thrive in these Northern regions and replace *Cgla* in response to Arctic warming [19], however, at present the individuals recorded at these most northerly locations were likely transported from southern populations [19].

Calanus glacialis is regarded as a true Arctic shelf species, which serves as a circumpolar indicator of these waters [17] (figure 1). We rarely observed it offshore in Atlantic waters, but documented the species' occurrence in many Norwegian fjords, as far south as 60° N (figure 2), where it usually co-occurred with *Cfin* in fjords with deep basins separated from shelf waters by shallower sills (electronic supplementary material, S1). In several fjords, *Cgla* dominated over other *Calanus* species; we recorded a positive gradient of relative abundance of *Cgla* from the mouth to the innermost areas of some fjords (e.g. Ranfjord, electronic supplementary material, S1).

In the fjords, prosome length of *Cgla* and *Cfin* overlapped completely (table 1; electronic supplementary material, S5), which explains why *Cgla*'s large occurrence has not been reported previously. Furthermore, recent work by our group shows that morphological characters cannot reliably distinguish between *Cfin* and *Cgla* throughout their range [20].

Some zooplankton species are long-term residents of Norwegian fjords, while others are replaced periodically with basin water exchanges [21]. Resident species are expected to show greater genetic differentiation among fjord populations than drifting species [12]. Our analysis found no significant genetic differentiation among fjord populations of *Cfin* ($F_{ST} = 0.004^{n.s.}$), but *Cgla* populations did differ significantly ($F_{ST} = 0.03$*), suggesting lower rates of exchange (i.e. gene flow) for *Cgla* than for *Cfin*. These results support previous descriptions of *Cfin* as a drifting species [12] that is advected into and out of fjords seasonally [22]. Less gene flow—together with the absence of offshore populations—suggests that *Cgla* populations are resident [12]. In both the White Sea [23] and Lurefjord [24], *Cgla* is known to migrate in early summer from warm surface layers to colder deep water. This may explain the species' ability to maintain local populations and avoid transport out of fjords.

Hybridization between *Cfin* and *Cgla* has been suggested in the Northwest Atlantic [14] based on microsatellite markers developed for *C. finmarchicus*. Notably, no first-generation hybrids were found in our survey of 4434 individuals from samples collected throughout the Northeast Atlantic and Arctic Oceans (electronic supplementary material, S6). Based on the nature of the molecular characters (nuclear, co-dominant InDels) used for species identification and careful ground-truthing of our molecular results, we conclude that hybridization between the species, if it occurs at all, is rare or episodic.

4. Conclusion

Marine zooplankton have been regarded as sentinels of climate change [25] due to their short life histories and rapid responses to environmental variation. Development and use of molecular characters that can ensure accurate and reliable identification and discrimination of key indicator species, such as those within the *Calanus* genus, are critically needed. Only then can these species be used to document past, present and future patterns of biogeographical distributions, and detect and track responses of pelagic communities to climate change.

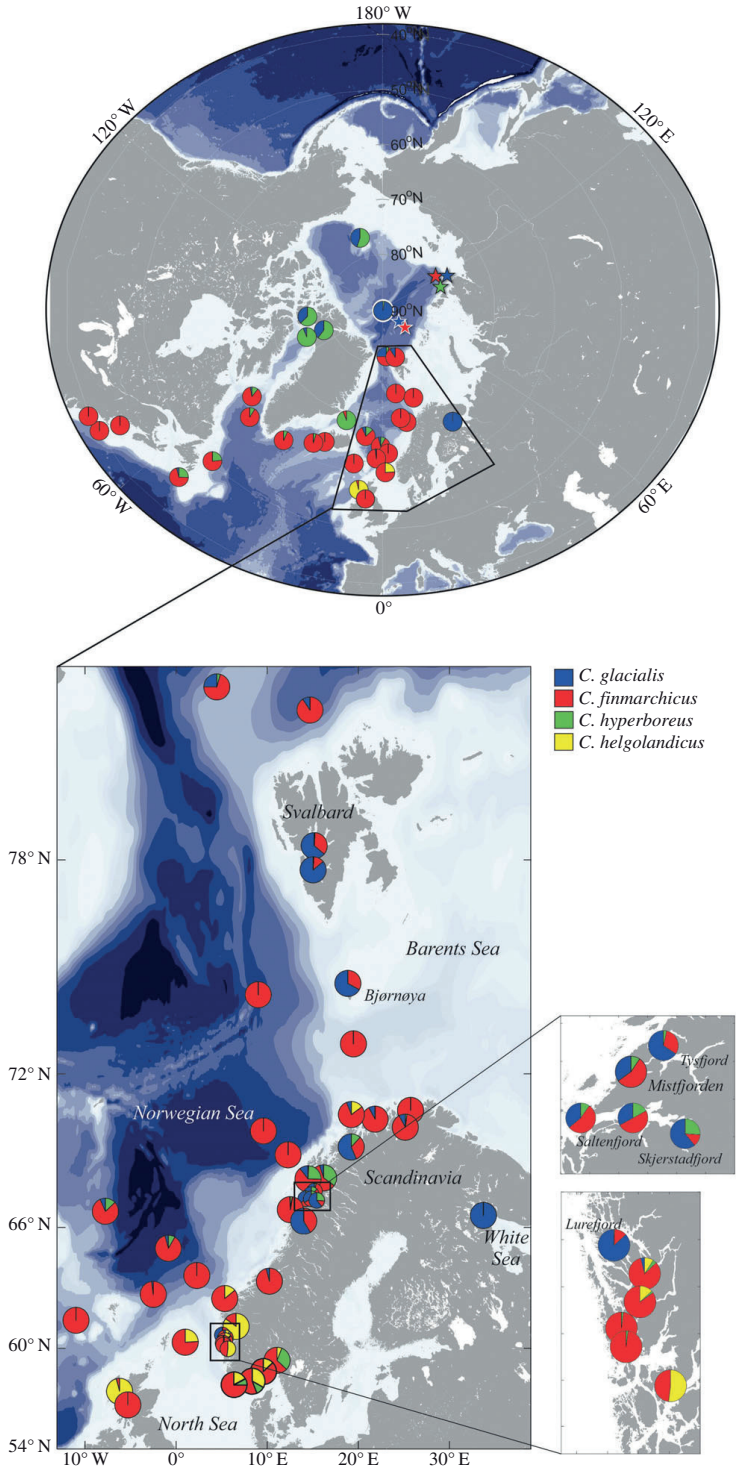

Figure 2. *Calanus* species distributional ranges in the North Atlantic and Arctic Oceans based on molecular species identifications. Pie charts represent relative frequencies of *C. glacialis* (blue), *C. finmarchicus* (red), *C. hyperboreus* (green) and *C. helgolandicus* (yellow) in each sample. Stars indicate non-quantitative species records.

Data accessibility. Protocols are attached as the electronic supplementary material; genotypes and sequences have been deposited to public database, respectively Dryad (http://dx.doi.org/10.5061/dryad.tq71j) [26] and GenBank® (MF959702–MF959730 and MF972920–MF972922).

Authors' contributions. M.C. & G.H. designed the study, collected and analysed data, developed the method and wrote the first draft of the manuscript. M.H., W.M., S.S., K.E., A.B., K.K., J.E.S., A.K.S.D., S.K. and C.S. collected and analysed data. I.S. collected data and contributed to the development of the method. M.D. and V.T. analysed data and made the figures. All authors contributed significantly to the manuscript, approved the final version and agreed to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

Funding. M.C. was supported by the EU (FP7-EURO-BASIN-264933), Norwegian Research Council (216578; 227139; 246747) and Nord University. M.H. was supported by UNIS. K.K. was supported by the Russian Foundation for Basic Research (15-29-02447; 16-04-00375) and the Russian Scientific Foundation (14-50-00095). M.D. was supported by NRC-226417. S.K. was supported by the Polish–Norwegian Research Program (Pol-Nor/201992/93/2014).

Acknowledgements. We thank M. Krogstad, E. Abramova, F. Norrbin, Ø. Leiknes, S. Basedow, T. Dale, T. Falkenhaug, A. Mailli, K. Last, S. Wells and the captains and crews of R/V *Helmer Hanssen* and G.O. Sars for their assistance with sampling. We are grateful to the ARCTOS network for support and useful discussions. We acknowledge two anonymous reviewers for constructive comments.

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Paper II

Daase M, Kosobokova K, Last K, Cohen J, Choquet M, Hatlebakk M, Søreide JE.

New insights into the biology of *Calanus* spp. (Copepoda) males in the Arctic

Marine ecology progress series **607**:53-69

Vol. 607: 53–69, 2018
https://doi.org/10.3354/meps12788

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published December 6

New insights into the biology of *Calanus* spp. (Copepoda) males in the Arctic

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ABSTRACT: Adult males of *Calanus* copepods in the Arctic are mainly observed between late autumn and late spring, and are seldom recorded during summer. Due to logistical constraints, there are still relatively few studies on zooplankton in high-latitude regions during the winter, and subsequently, little is known about *Calanus* males. Here, we present data on abundance, spatial distribution, prosome length, lipid content, respiration and swimming activity of *Calanus* adults, along with adult sex ratios in *Calanus* populations from 5 Arctic fjords in Svalbard, Norway (78–80° N) during the polar night in January 2015, 2016 and 2017. Adult males and females of *Calanus* were observed at all locations and occurred throughout the entire water column. Morphological examination and molecular identification of *Calanus* males proved that all males encountered belong to *Calanus glacialis*, even in the fjords where overwintering copepodite stage CV of *C. finmarchicus* dominated at the time. Adult sex ratios in *C. glacialis* populations varied from 1 male per 4 females to 2 males per female. From 3 to 18% of females carried spermatophores attached to the genital segment. Lipid content in males was slightly higher than in females. Shipboard experiments showed that males had higher swimming activity and respiration rates than females. Our observations indicate that adult males of *C. glacialis* stay active and demonstrate active mating behavior in mid-winter, and that the mating phenology of *C. glacialis* is decoupled from that of *C. finmarchicus* in the study area in January.

KEY WORDS: *Calanus glacialis* · Polar night · Svalbard · Mating · Sex ratio · Metabolism

INTRODUCTION

Calanoid copepods of the genus *Calanus* dominate the mesozooplankton communities of Arctic and sub-Arctic seas in terms of biomass (Kosobokova & Hirche 2009). They play a major role in the Arctic marine ecosystem, converting their algal diet into energy-rich lipid storages and thus facilitating the transfer of energy from primary production to higher trophic level organisms such as fishes, sea birds and marine mammals (Falk-Petersen et al. 2009). Due to their importance in the marine ecosystem, *Calanus* species are probably the most studied copepod taxa, not only in the Arctic but also in sub-Arctic and boreal seas. A number of publications have described the spatial distribution of *Calanus* species in these regions (e.g. Conover 1988, Hirche & Kosobokova 2007, Falk-Petersen et al. 2009, Wassmann et al. 2015, Choquet et al. 2017) and there is a good understanding of different aspects of their life history such as timing of reproduction (Niehoff et al. 2002, Søreide et al. 2010, Daase et al. 2013), vertical migration, juvenile development, and energy requirements for reproduction and growth (e.g. Niehoff 2004, Søreide et al. 2008, 2010, Falk-Petersen et al. 2009).

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Three species of *Calanus* co-occur in the Atlantic-influenced part of the Arctic Ocean: the North Atlantic species *C. finmarchicus*, the arctic shelf species *C. glacialis* and the arctic oceanic species *C. hyperboreus* (Conover 1988, Choquet et al. 2017). The basic life cycle of *Calanus* species in Arctic and Sub-Arctic seas includes a seasonal migration, with the main developmental and growth phase occurring near the surface during spring and summer and an overwintering phase at depth with reduced metabolism (diapause) in winter (Falk-Petersen et al. 2009). The final developmental step towards adulthood takes place sometime between late autumn and spring, with the largest and most lipid-rich CVs moulting to adults first, and males appearing before females (Østvedt 1955, Kosobokova 1999, Bailey 2010). The 3 *Calanus* species have tuned their life-history strategies in relation to the timing and predictability of the spring bloom, ice cover and other factors in their main area of distribution (Falk-Petersen et al. 2009). *C. finmarchicus* is advected to the Arctic mainly with Atlantic water currents. In the northernmost part of its distribution range, *C. finmarchicus* has a 1 yr life cycle and relies on external energy supplied by the spring bloom to fuel reproduction (i.e. income breeding). The ability of *C. finmarchicus* to survive and colonize the Arctic Ocean, however, is hampered by short algae growing seasons and low temperatures (Jaschnov 1970, Tande et al. 1985, Ji et al. 2012), and hence the species largely fails to reproduce in the Arctic Ocean and surrounding shelf seas (Hirche et al. 2006). The larger *C. glacialis* is very productive along the entire shelf break and surrounding shelf seas of the Arctic (Kosobokova & Hirche 2001, Ashjian et al. 2003, Hirche & Kosobokova 2003). *C. glacialis* has a 1–2 yr life cycle (Kosobokova 1999, Søreide et al. 2010, Daase et al. 2013) and is efficient at utilizing the 2 available food sources in seasonal ice-covered seas (ice algae and phytoplankton) for reproduction and growth. The early ice algae bloom is primarily utilized to fuel gonad maturation and egg production (income breeding) while the later phytoplankton bloom supports growth and development of its new generation (Hirche 1989, Tourangeau & Runge 1991, Søreide et al. 2010, Wold et al. 2011). However, egg production can also occur before any algal food is present, being fuelled by internal resources only (i.e. capital breeding). The flexible reproductive strategies observed in *C. glacialis* may explain its wide distribution in seasonally ice-covered Arctic shelf seas (Daase et al. 2013), a region of high inter-annual variability in the timing of ice break-up and bloom phenology. The largest of the 3 species, *C. hyperboreus*, has its centre of distribution in the Greenland Sea and the Central Arctic Ocean, and is specialized to the highly unpredictable timing of the spring bloom in the Arctic Ocean. It is a pure capital breeder, producing eggs at depth in winter decoupled from the spring bloom (Hirche & Niehoff 1996, Hirche 2013).

Given the key role of *Calanus* spp. in the food web, discussions of their fate in a warming Arctic has become a research priority in recent years (e.g. Ji et al. 2012, Kjellerup et al. 2012, Kwasniewski et al. 2012, Grote et al. 2015, Wilson et al. 2016). However, winter studies are still scarce and knowledge on *Calanus* males and their biology is basically non-existent from the Arctic, hindering a thorough understanding of *Calanus* life-history strategies needed to assess their response to Arctic warming.

Calanus males seem to have a rather short life span, similar to males of many other copepod species (Bogorov 1939, Mednikov 1961). Kosobokova (1999) reported that males of *C. glacialis* have only a 3–4 mo life span in the White Sea, and Marshall & Orr (1955) suggested that the life span of *C. finmarchicus* males does not exceed 7 mo even at high latitudes. In comparison, the life span of females of *C. glacialis* may vary from 9–10 mo up to 1.5 yr, and it has been suggested that *C. glacialis* females may even be iteroparous (Kosobokova 1999).

The absence of *C. glacialis* males is noteworthy during the period of most active biological sampling from late spring to autumn (e.g. Kosobokova 1999, Ashjian et al. 2003, Darnis & Fortier 2014), while females are found year-round (e.g. Kosobokova 1999, Wold et al. 2011, Daase et al. 2013). Males start to appear in northern polar waters in early autumn and can persist until May–June, with most studies observing maximum abundance and highest proportion of *C. glacialis* males between December and February (Madsen et al. 2001, Niehoff et al. 2002, Wold et al. 2011, Estrada et al. 2012, Darnis & Fortier 2014). Periods of peak abundance thus coincide with the polar night, a period that is traditionally understudied due to logistical constraints of conducting fieldwork at high latitudes in darkness, extreme low temperatures and in often ice-covered seas. As a result, male abundance, size range and structure, feeding habits, metabolic rates and lipid content have been poorly documented, and their life span remains uncertain.

Here, in order to fill knowledge gaps on *Calanus* spp. reproductive strategies due to the lack of data on males, we collected zooplankton samples in the middle of winter in the Arctic archipelago of Svalbard, during the supposed peak of *Calanus* male abun-

dance (Bailey 2010). Although it is likely that males of all 3 *Calanus* species are present in the studied area, we focused only on *C. finmarchicus* and *C. glacialis* since abundance of *C. hyperboreus* is generally low in the fjords and on the shelf (Daase & Eiane 2007, Blachowiak-Samolyk et al. 2008, Søreide et al. 2008). The vertical distribution, abundance, morphology, activity and physiology of *Calanus* spp. adults were investigated together with the females' gonad maturation state in order to understand and document for the first time the details of the mating phase of *Calanus* spp. in the Arctic.

MATERIALS AND METHODS

Study area

Zooplankton samples were collected in January 2015, 2016 and 2017 in fjords along the western and northern coast of the Svalbard archipelago (Fig. 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/m607p053_supp.pdf) onboard the R/V 'Helmer Hanssen'. In January 2015, samples were collected at 3 stations in Kongsfjorden: the outer (KF1), the middle (KF3) and the innermost part of the fjord close to the glacier front (KF5). In January 2016, samples were collected in Kongsfjorden (KF3 and KF5), Isfjorden (IF), Billefjorden (BF), Smeerenburgfjorden (SMF) and Rijpfjorden (RF), and in January 2017, sampling was repeated at IF, KF3, SMF and RF (Fig. 1, Table S1).

Isfjorden, Kongsfjorden and Smeerenburgfjorden are located on the western coast of Svalbard and may be affected by inflow of Atlantic water from the West Spitsbergen Current (Cottier et al. 2005, Nilsen et al. 2008). Billefjorden is a sill fjord in the inner part of Isfjorden and is largely unaffected by inflowing Atlantic water but dominated by locally formed cold water (less than −0.5°C year-round), providing a refuge for Arctic zooplankton species (Arnkværn et al. 2005). Rijpfjorden is a north-facing fjord dominated by cold Arctic water masses, but inflow of Atlantic water may occur (Wallace et al. 2010). All fjords were ice-free during our study in January 2015, 2016 and 2017.

Hydrography

Measurements of temperature and salinity were obtained at all stations by a ship-board conductivity, temperature and depth profiler (SBE911plus, SeaBird Electronics).

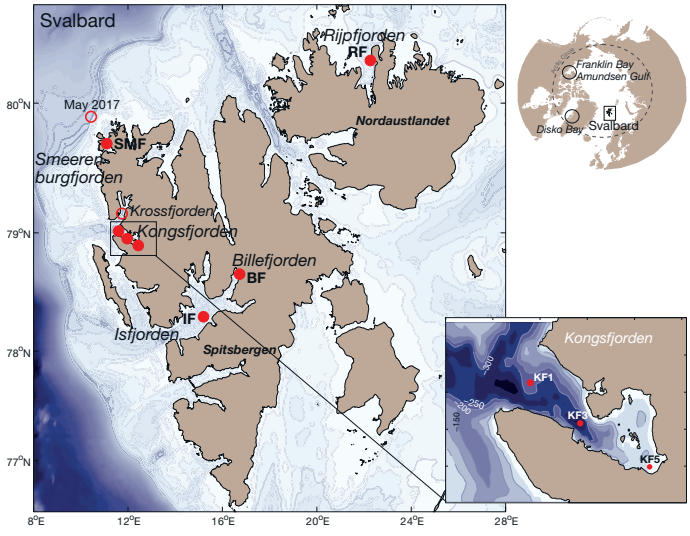

Fig. 1 Map of study area. Red dots show location of main sampling sites in Svalbard fjords. Red circles indicate sampling sites where additional samples were taken. Inset in lower right corner shows locations of stations sampled in Kongsfjorden. Arctic map in upper right corner shows location of Svalbard as well as locations of sampling sites referred to in Fig. 9

Zooplankton abundance and vertical distribution

Zooplankton were sampled by vertical net hauls (towing speed 0.5 m s^{-1}) from close (10–20 m) to the seafloor up to the surface using a multiple opening/closing net (Multinet; Hydrobios: mouth opening 0.25 m^2, mesh size 180 µm). Up to 5 depth strata were sampled at each location (Table S1). Samples were preserved in a 4% hexamethylenetetramine-buffered formaldehyde-in-seawater solution and analyzed under a Leica stereomicroscope at institutional home laboratories. Samples were examined by subsampling with aliquots obtained by 5 ml automatic pipette, with the pipette tip cut at 5 mm diameter to allow free collection of mesozooplankton. Prior to taking subsamples, large (total length >5 mm) organisms were picked out using forceps. The

number of subsamples analyzed was chosen so that at least 150 individuals of *Calanus* copepodites were counted. Samples with low abundances were examined in their entirety. Adult males (AM) and females (AF) of *Calanus* were counted from the entire samples. The prosome length of all counted individuals of *Calanus* spp. was measured from the tip of cephalosome to the distal lateral end of the last thoracic segment with precision of ±50 µm.

Calanus species identification

To distinguish between the morphologically similar *C. glacialis* and *C. finmarchicus* from formalin-preserved samples, we used size classes derived for each developmental stage (copepodites CIII–CVI) from prosome length frequency analyses for the study region (Daase & Eiane 2007) (Table 1), which were readjusted after considering molecular-based studies (Gabrielsen et al. 2012, Choquet et al. 2018, Renaud et al. 2018). These molecular investigations have indicated a much higher overlap in prosome length between the 2 species than previously assumed, resulting in a regionally variable potential for misidentification. In our study area, misidentifications based on size classes seemed, for the most part, to be unidirectional (Gabrielsen et al. 2012, Choquet et al. 2018) and biased towards an underestimation of *C. glacialis* and a comparative overestimation of *C. finmarchicus*. Since prosome length measurements of genetically identified *Calanus* CV and females indicate a discrepancy between previously published size classes for the study area (e.g. Daase & Eiane 2007, Kwasniewski et al. 2003) and observed prosome length (Gabrielsen et al. 2012, Renaud et al. 2018), we adjusted the size classes and defined CV ≥ 2.9 mm and AF ≥ 2.95 mm as *C. glacialis* (Table 1).

In living *Calanus*, the presence or absence of red pigmentation of the antennules has been genetically confirmed as useful to distinguish between CIV, CV and AF of *C. finmarchicus* (pale antennules) and *C. glacialis* (antennules with red pigmentation) (Nielsen et al. 2014, Choquet et al. 2018). This characteristic was used to identify *C. glacialis* from digital images taken to estimate lipid content (see below) and when selecting AF for respiration and swimming activity measurements (see below). The pigmentation of antennules is, however, not present in AM of either species.

The morphology of the 5th thoracic leg (swimming leg P5) can also be used to identify *Calanus* to species following descriptions by Jaschnov (1955), Frost (1974) and Brodskii (1967), although the method has recently been proven to be unreliable for CVs and AF (Choquet et al. 2018). For males, the morphological characteristics are more clearly defined than in females, and we used this morphological feature to identify a subset of *Calanus* males (those sampled in Rijpfjorden) to species as described in Choquet et al. (2018) to check the reliability of size classes derived for AM in this study.

Another subset of *Calanus* males was identified to species using molecular tools. A total of 80 *Calanus* males sampled at KF3 in January 2015 using a MIK net (mouth opening 3.14 m^2, mesh size 1500 µm), and 40 males from BF and 74 males from RF sampled in January 2016 using the Multinet were preserved individually in 96% ethanol. Prosome length of each individual was measured from digital images taken prior to preservation. Individuals were genetically identified to species following procedures described in Choquet et al. (2017). To compare the size structure and species composition of *Calanus* males in January with that of *Calanus* males found in spring, we used the same methods on 42 randomly selected *Calanus* males collected with a WP3 net (1 m^2 mouth opening, 1000 µm mesh size) during a cruise in May 2017 north-west of Svalbard (Fig. 1, Table S1).

Table 1. Size ranges (prosome length, mm) used to differentiate between copepodite stages CIII–CV and adult females (AF) of *Calanus finmarchicus* and *C. glacialis* based on Daase & Eiane (2007). Size classes for CV and AF have been readjusted based on molecular identification (Gabrielsen et al. 2012, Renaud et al. 2018)

	C. finmarchicus	*C. glacialis*
CIII	1.12–1.47	1.47–2.07
CIV	1.6–2.01	2.01–3.63
CV	1.92–2.9	2.9–3.99
AF	2.4–2.95	2.95–4.63

Estimation of lipid content

In 2015 and 2016, additional Multinet samples were taken at BF, KF3, SMF and RF from which live *Calanus* were sorted out to estimate the lipid content of individuals (see Table S1 for sample depth). Digital images (lateral view) of all specimens in subsamples containing at least 100 *Calanus* were taken following procedures described in Daase et al. (2014) using a Leica stereomicroscope with a camera Leica DFC420 or Sony HDR_HC7 video camera. Copepodite stage of each individual was determined while taking the

pictures. The digital images were used to measure lipid sac area, prosome length and prosome area of specimens using ImageJ, an open source graphics program (Rasband 1997–2009). Lipid content of individual *Calanus* specimens was calculated from lipid sac area according to Vogedes et al. (2010).

The variance in lipid content and lipid sac area/prosome area ratio (LA/PA; an indication of the fullness of the body) was not homogenous. We therefore applied the non-parametric Kruskal-Wallis test to test for differences in lipid content and LA/PA between copepodite stages, followed by the post hoc test according to Nemenyi for pairwise multiple comparisons of the ranked data. Statistical analyses were done in RStudio v.1.0.143.

Gonad maturation status and spermatophore counts

The gonad maturation stage (GS) of adult *Calanus* females and CVs were examined using formalin-preserved samples. A total of 30 randomly selected females and CVs from each fjord sampled in 2016 were stained with 2% borax carmine solution (Tande & Hopkins 1981), dehydrated and stored in glycerine. The GS of females was assessed according to the classification scheme suggested by Niehoff & Hirche (1996). Four stages of gonad maturation (GS1–GS4) were distinguished. The gonads in CVs were examined to discriminate between sexually undifferentiated specimens and potential females/males, according to Kosobokova (1998, 1999). The number of AF bearing spermatophores was assess in all Multinet samples collected in January 2015 and 2016.

Swimming activity and respiration

Measurements of swimming activity of *Calanus* AM and AF were taken using a modified LAM10 locomotor activity monitor (LAM; Trikinetics) connected to a laptop computer. The LAM monitors use infrared light beam arrays to detect the motion of animals in test chambers (2.5 ml clear acrylic tubes); beam breaks are recorded on the computer. For activity experiments, animals were collected in January 2017 in Krossfjorden (a side-fjord of Kongsfjorden; Fig. 1) and at RF using a Hydrobios WP2 net (mesh size 180 µm, mouth opening 0.25 m^2), vertically hauled from 100 m to the surface. Net contents were immediately transferred to a shipboard temperature-controlled room at 4.5°C, where sorting was undertaken by stereomicroscope under a dim red light. Sorted animals were individually transferred into the LAM monitor tubes, each containing ~2 ml of 0.5 µm filtered seawater. Animals were left undisturbed and under constant darkness in the shipboard temperature-controlled room at 4.5°C for ~2 d and their activity logged, after which each copepod was photographed to confirm species and stage (see methods described above). Rayleigh's tests were used to determine whether bouts of swimming activity were clustered over the diel cycle, while rank sum tests were used to compare variance of swimming activity between AM and AF at Krossfjorden and RF.

From net collections at RF we also measured weight-specific oxygen consumption rates in individual adult *C. glacialis* males (n = 11) and females (n = 8). Respiration rates were measured in darkness at 4.5°C in 1 min intervals over ~10 h using a 24-well microplate respirometry system (Loligo Instruments). Individual copepods were tested in 200 µl wells, with respiration rates calculated over an interval where partial pressures were 90–80% air saturation in each well, ensuring measurements considered only independent respiration. Copepods were photographed following experiments, from which prosome length was calculated and used to derive dry weight (M. Daase & J. E. Søreide unpubl. data) for correcting respiration by copepod size:

$$DW = e^{2.25} PL^{3.31}$$

where DW is dry weight (mg) and PL is prosome length (mm).

Respiration rates were compared between AM and AF by a rank sum test. While both copepod activity and respiration in the experiments described above could be influenced by tank enclosure effects in these relatively small volumes, we ensured that our methods were consistent between individuals and therefore any differences are very likely due to inherent differences among sexes/sites as opposed to experimental artefacts.

RESULTS

Hydrography

Atlantic and Transformed Atlantic water prevailed in Kongsfjorden and Isfjorden during our studies (Fig. 2). The water column in Kongsfjorden was well-mixed and homogenous in 2015, and stratified, warmer and fresher in 2016 and 2017. Water masses in Isfjorden were similar to Kongsfjorden in 2016 and 2017. Cooler and fresher waters were observed in

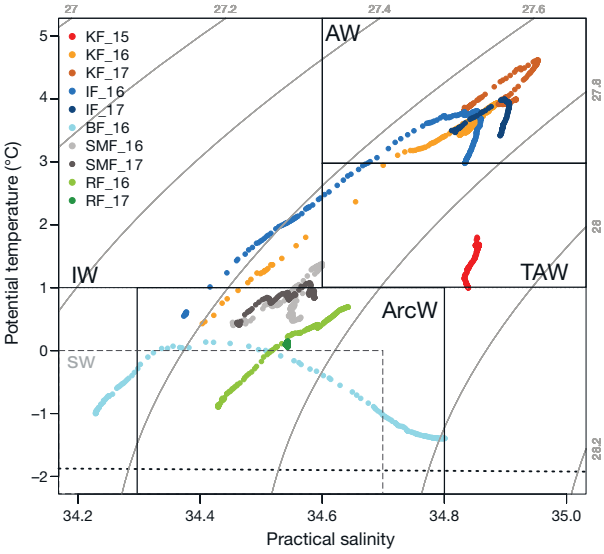

Fig. 2. TS-diagram of water masses in January in Kongsfjorden (KF3): 2015 (KF_15), 2016 (KF_16) and 2017 (KF_17); Isfjorden: 2016 (IF_16) and 2017 (IF_17); Billefjorden: 2016 (BF_16); Smeerenburgfjorden: 2016 (SMF_16) and 2017 (SMF_17); and Rijpfjorden: 2016 (RF_2016) and 2017 (RF_17). AW: Atlantic water; TAW: Transformed Atlantic water; ArcW: Arctic water; IW: intermediate water; SW: surface water (grey dashed box). Black dotted line indicates freezing point. Grey lines show isopycnals at 0.2 intervals. Water mass definitions based on Cottier et al. (2005)

Billefjorden and Rijpfjorden, indicating the presence of Arctic or locally formed water cooled during the winter. Smeerenburgfjorden was warmer than Rijpfjorden and Billefjorden, but not as warm as Kongsfjorden and Isforden.

Calanus stage composition, length frequency and genetics

Abundance of the larger and easily morphologically distinguishable *Calanus hyperboreus* was low in the study area (0.16–2.5 ind. m^{-3}) and we therefore only report data on *C. finmarchicus* and *C. glacialis*, which were abundant in all fjords.

The *Calanus* population in January was dominated by copepodite stages CIV and CV (Fig. 3). CVs dominated at all stations in 2015

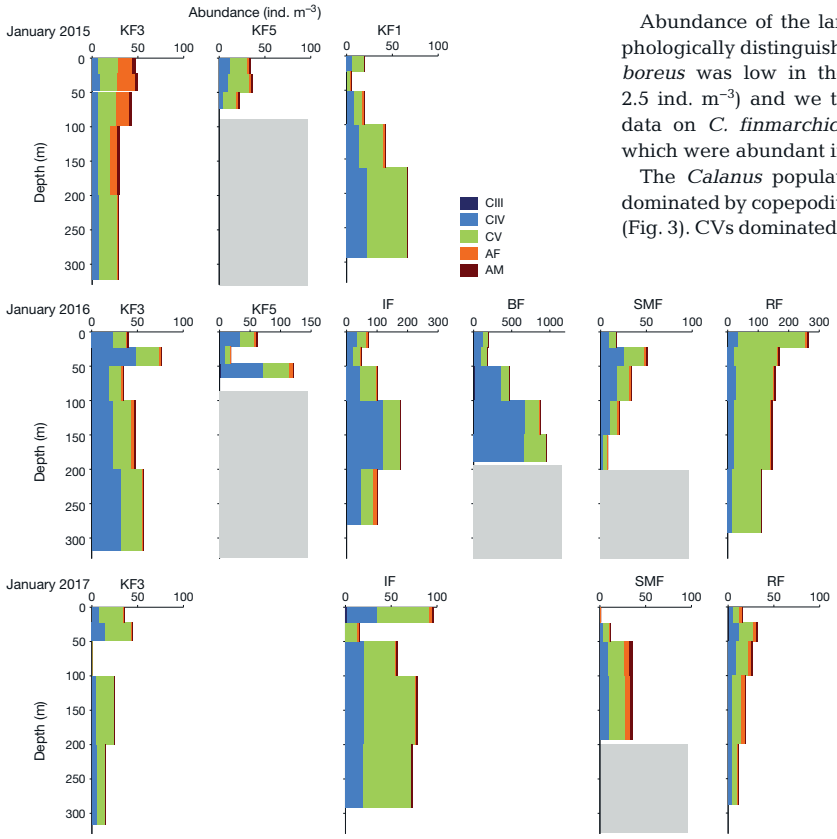

Fig. 3. Vertical distribution, stage composition and abundance of *Calanus* spp. in 5 Svalbard fjords. Note differences in scale of *x*-axis. Gray bars: bottom depth. AF: adult females; AM: adult males

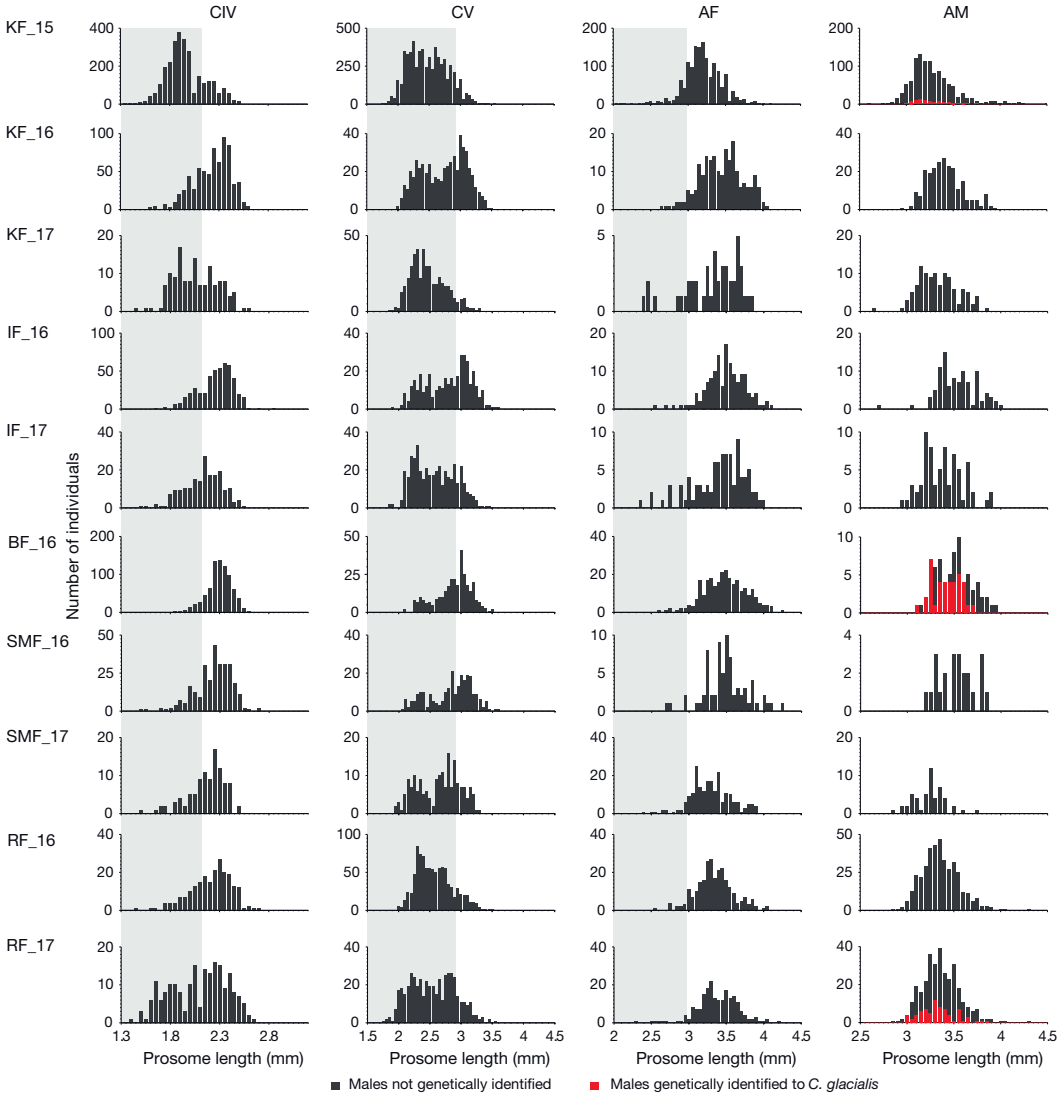

Fig. 4. Station-specific prosome length frequency distributions of *Calanus* spp. copepodite stage CIV and CV, adult females (AF), and adult males (AM) for Kongsfjorden 2015 (KF1, KF3 and KF5 pooled), 2016 (KF3 and KF5 pooled), 2017 (KF3); Isfjorden (IF) 2016, 2017; Billefjorden (BF) 2016; Smeerenburgfjorden (SMF) 2016, 2017; and Rijpfjorden (RF) 2016, 2017. Red bars for AM in KF_15, BF_16 and RF_16 show length frequency distribution of males genetically identified as *C. glacialis*. Gray shaded areas: size range assigned to *C. finmarchicus* (see Table 1)

and 2017, while CIVs were more abundant in 2016, except at RF. The prosome length frequency of copepodite stage IV (CIV) was biomodal but skewed towards larger individuals at almost all locations, indicating a dominance of *C. glacialis* among CIVs (Fig. 4). Exceptions were Kongsfjorden in 2015 and 2017, where the majority of CIV fell into the size classes assigned to *C. finmarchicus*, and Rijpfjorden in 2017, which showed equal numbers of CIVs for both size classes. In contrast, the length distribution of

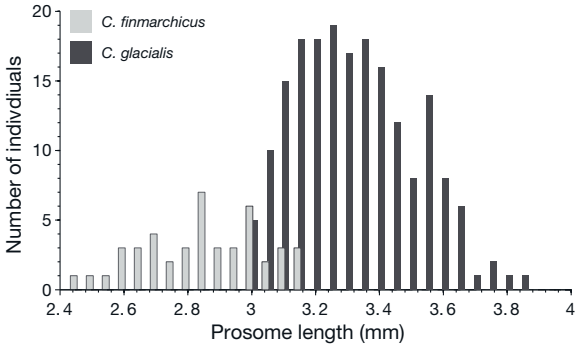

Fig. 5. Prosome length frequency distribution of genetically identified *Calanus* adult males (AM). *C. finmarchicus* AM were sampled in north-west Svalbard in May 2017, and *C. glacialis* AM were collected in Svalbard fjords in January 2015 and 2016 (see also Fig. 3)

CVs was skewed towards smaller sizes, indicating a dominance of *C. finmarchicus* CVs at most locations (Fig. 4), with the exception of BF and SMF in 2016 (Fig. 3). The length distribution of AF was largely unimodal, with only few smaller AF present (Fig. 4). The size range and length frequency distribution pattern of AM closely resembled that of AF, but no AM smaller than 2.7 mm were observed. All 194 AM identified to species using molecular tools (corresponding to 10% of all measured males) were found to be *C. glacialis*. Prosome length of these genetically identified AMs varied from 3.00 to 3.84 mm and the length frequency distribution overlapped entirely with the length frequency distribution of AM not identified genetically (Fig. 4). Morphological examination of the fifth pair of swimming legs (P5) of AM from RF in 2016 indicated that only *C. glacialis* AM were present there, ranging in prosome length from 2.85–4.05 mm. In contrast, *Calanus* AM sampled north-west of Svalbard in May 2017 were smaller than those observed in January. They were all identified as *C. finmarchicus* using molecular tools and displayed a unimodal length frequency distribution with prosome length varying from 2.40–3.14 mm (Fig. 5).

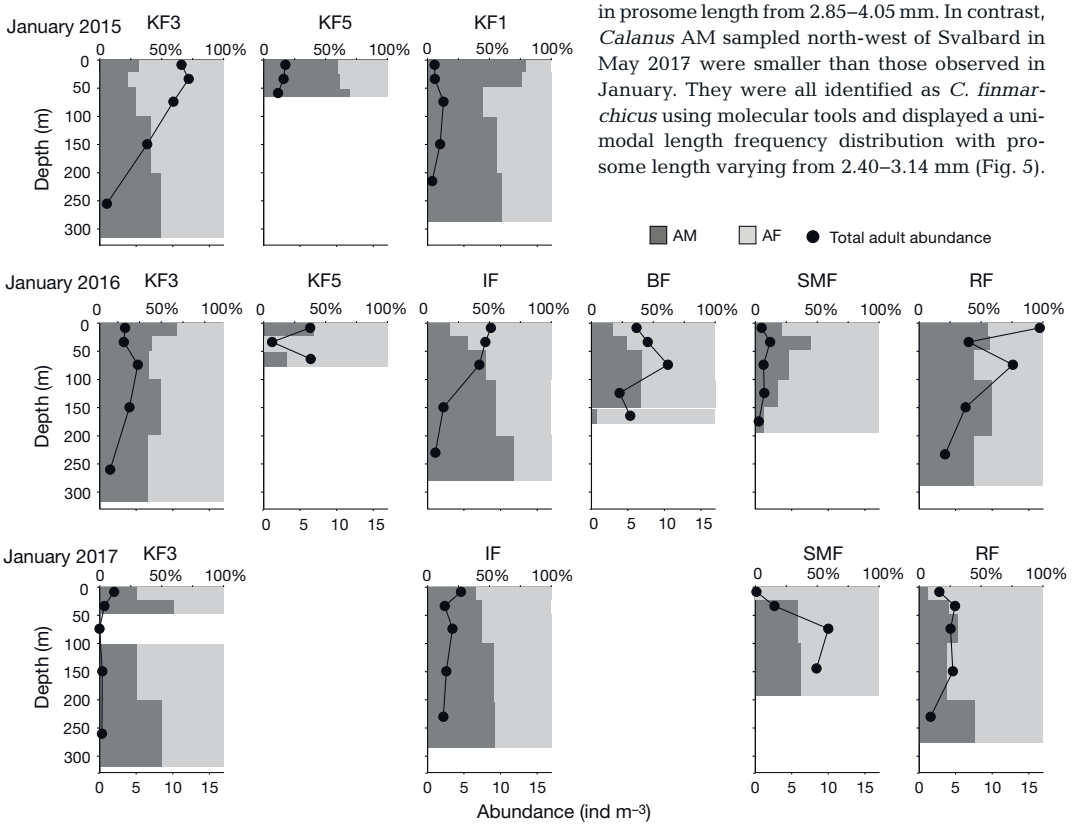

Fig. 6. Vertical distribution of *Calanus glacialis* adult females (AF; light gray) and males (AM; dark gray) at each station in January 2015, 2016 and 2017. Black dots: total adult abundance (ind. m^{-3})

Table 2. Abundance of adult males (AM) and females (AF) of *Calanus glacialis* (ind. m^{-2}), and sex ratios and percentage of *C. glacialis* females observed with spermatophores. nd: not determined

Station	No. AM (ind. m^{-2})	No. AF (ind. m^{-2})	Sex ratio adults males:females	% AF with spermatophores
2015				
KF1	200	276	0.7	13.9
KF3	636	2328	0.3	9.2
KF5	116	128	0.9	17.6
2016				
KF3	384	532	0.7	8.1
KF5	68	176	0.4	nd
IF	356	969	0.4	9.2
BF	256	1048	0.2	3.3
SMF	88	272	0.3	8.3
RF	1240	784	1.6	7.3
2017				
KF3	56	64	0.9	nd
IF	356	328	1.1	nd
SMF	484	784	0.6	nd
RF	260	608	0.4	nd

Calanus vertical distribution and abundance

At almost all stations, the *Calanus* population was distributed throughout the entire water column (Fig. 3). Exceptions were KF1 in 2015 and BF in 2016, where the bulk of the *Calanus* population was concentrated in the deeper layers. Total *Calanus* abundances were highest in RF, BF and IF in 2016 (Fig. 3); low *Calanus* abundance was observed in Kongsfjorden in 2015 (Fig. 4).

Both AM and AF were distributed throughout the entire water column (Fig. 6). The highest abundance of *Calanus* AF was observed in KF3 in 2015 (2328 ind. m^{-2}; Table 2), which was 2–3 times higher than maximum AF abundance recorded in 2016 and 2017. The contribution of *C. glacialis* AM to the total *C. glacialis* population was highest in 2015, at 12–25%. In 2016 and 2017, AM contributed 2–12 and 5–11%, respectively.

Sex ratios and proportion of females with spermatophores

The sex ratio in the *C. glacialis* population varied from 1.6 (ca. 2 AM AF^{-1}) in RF in 2016 to a pronounced prevalence of AF in BF (0.04–0.2 AM AF^{-1}) and SMF (0.1–0.3 AM AF^{-1}) in 2016, especially in the deeper layers (Fig. 6, Table 2).

Between 3 and 18% of the *Calanus* AF carried spermatophores (Table 2). The highest proportion of such females was observed in Kongsfjorden in 2015 (where we also observed the highest AF abundance), and at IF in 2016. The lowest proportion of AF with spermatophores was found at BF in 2016 (Table 2). AF carrying spermatophores were not counted in 2017. Length measurements of AF with spermatophores showed that the majority fell within the size class of *C. glacialis*, with 18% (24 ind., most of them observed in KF in 2015) being slightly smaller (2.6–2.9 mm) but still within a size range that may include AF of *C. glacialis* (Renaud et al. 2018) (Fig. S1 in the Supplement). There was no relationship between the proportion of AF with spermatophores and the number of AM, AF or the sex ratio, but there was a positive correlation between the proportion of AF with spermatophores and the proportion of *C. glacialis* AM relative to the total *C. glacialis* abundance (Pearson correlation, $r = 0.811$, $p = 0.015$).

Gonad status and CV sex ratios

We assessed the gonad stage of AF and the CV sex ratios from samples taken in 2016. The majority of both *C. glacialis* and *C. finmarchicus* AF were immature (>95% with gonad stage GS1) (Table 3). The majority of CVs (75%) in both species were classified as potential females except for CVs from RF and BF, where up to 50% of CV specimens still had sexually undifferentiated gonads (Table 4). Overall, only a small portion (<5%) of CVs were developing male gonads and could be classified as potential males.

Lipids

The adults of *C. glacialis* had higher lipid content and a higher LA/PA compared to CIV and CV in January (Fig. 7). Differences in lipid content and LA/PA

Table 3. Gonad maturation stage (GS) of *Calanus glacialis* (% of adult females [AF] with GS1, GS2 or undifferentiated gonads) in January 2016

	GS1	GS2	Undifferentiated gonads
KF3	99.1	0.9	0.0
IF	100.0	0.0	0.0
BF	98.1	0.0	1.9
SMF	94.0	1.5	4.5
RF	100	0	0

Table 4. Gonad differentiation in *Calanus glacialis* and *C. finmarchicus* CVs in January 2016 (% of CVs with sexually undifferentiated gonads, potential female and potential male gonads). N: number of individuals examined

Station	*C. glacialis*					*C. finmarchicus*				
	N	% undiff.	% female	% male	Sex ratio	N	% undiff.	% female	% male	Sex ratio
KF3	137	5.1	91.2	3.6	0.04	15	26.7	73.3	0.0	0.00
IF	132	9.1	88.6	2.3	0.03	15	53.3	46.7	0.0	0.00
BF	97	38.1	59.8	2.1	0.03	52	78.8	21.2	0.0	0.00
SMF	102	9.8	86.3	3.9	0.05	38	39.5	60.5	0.0	0.00
RF	25	16.0	68.0	16.0	0.24	134	78.4	20.9	0.7	0.04

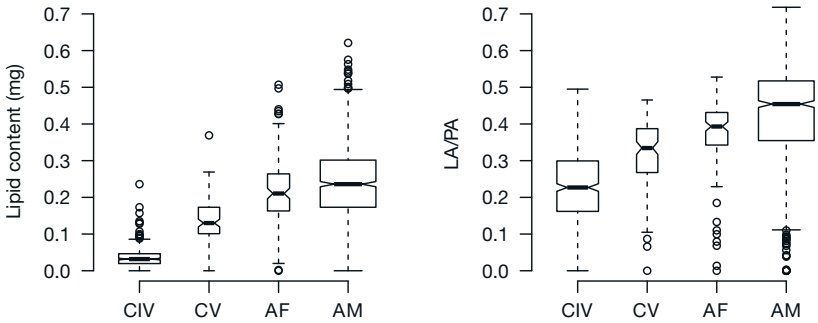

Fig. 7. Box plot of lipid content (mg ind.$^{-1}$) and lipid sac area to prosome area ratio (LA/PA) in copepodite stages CIV, CV, adult females (AF), and males (AM) of *Calanus glacialis* in January 2015 and 2016. Horizontal line: median; bottom and top of the box: 25th and 75th percentiles, respectively; whiskers extend 1.5 times the interquartile range of the sample; values outside this range are marked by circles. The boxes are drawn with widths proportional to the square-roots of the number of observations in the groups. Notches display the variability of the median between samples. If the notches of two boxes do not overlap there is strong evidence that their medians differ at 95% confidence interval (Chambers et al. 1983)

between stages were significant (Kruskal-Wallis, $p < 0.0001$). For lipid content, these differences were due to significant differences between adults and CIV and CV, while there was no significant difference in lipid content between AF and AM (Nemenyi post hoc test, $p = 0.24$). However, LA/PA was significantly different among all stages ($p < 0.001$), i.e. AM had a higher LA/PA ratio than AF. Additionally, there was a higher variability of lipid content in AM than in AF (Fig. 7).

Swimming activity and respiration

Swimming activity in adult *C. glacialis* varied with sex and collection site (Fig. 8). AF from both sites showed little variation in swimming activity over the duration of the experiment. AM from both sites, however, displayed bouts of elevated swimming activity. For Krossfjorden, these activity bouts were clustered at intervals over the diel cycle, while for RF the activity bouts were uniformly distributed (Rayleigh's test, $p < 0.001$ and $p = 0.211$, respectively). The variance of swimming activity in individuals across time was greater for AM than AF, both in Krossfjorden and RF (rank sum tests, $p < 0.001$ and $p = 0.003$, respectively). This is reflected in bouts of swimming activity up to 381 beam breaks per 30 min in AM from Krossfjorden, and 1118 beam breaks per 30 min for AM from RF. Overall, activity levels were higher at RF than Krossfjorden (19–25 beam versus 5–7 breaks per 30 min). Consistent with activity, weight-specific respiration rates were 2.2-fold higher for AM from RF than for AF (38.6 ± 8.6 SE versus 17.5 ± 4.0 pmol O_2 µg^{-1} DW h^{-1}) ($p = 0.019$, rank sum test).

DISCUSSION

Adult males of *Calanus* were present in all 5 Svalbard fjords during our winter studies in January 2015, 2016 and 2017. Given the unimodal length frequency distribution of males, molecular results and additional examination of the morphology of the 5th swimming leg of individuals from RF in 2016, we conclude that only *C. glacialis* AM were present in the study region

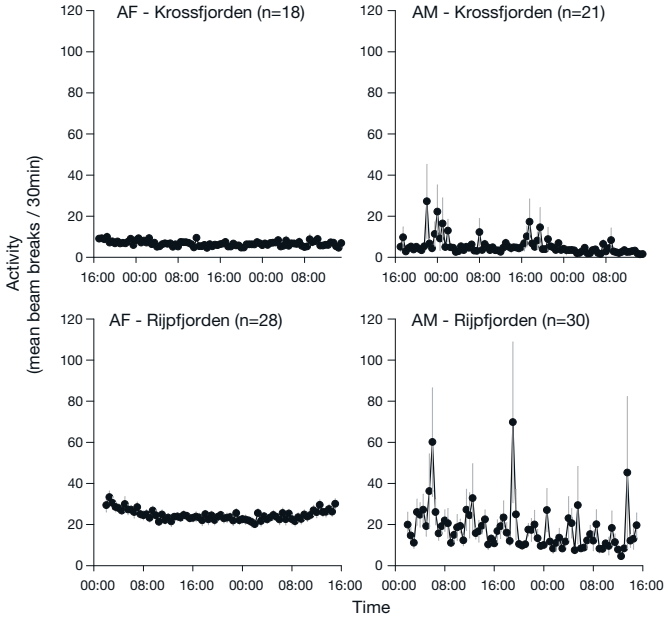

Fig. 8. Swimming activity of *Calanus glacialis* from Krossfjorden and Rijpfjorden in January 2017. Panels are for adult females (AF) and adult males (AM) from each site. Mean (±SE) activity is shown for copepods incubated individually in constant dark conditions over a 2 d period, with activity scored as breaks of infra-red light beam arrays crossing each tube

in January along with a high proportion of AF of the same species. In contrast, *C. finmarchicus* males were absent in January, female abundance was low and late copepodite stages, in particular CV, dominated the overwintering population.

Male size and species identification

There are very few published data on body size of *Calanus* AM (Table 5), presumably because of their scarcity in historic sampling campaigns. Prosome lengths for *C. glacialis* AM measured during this study are similar to those previously obtained in Billefjorden, but they are smaller than records from the North Atlantic, Arctic Ocean and the White Sea (Table 5). The size structure of *C. finmarchicus* AM sampled in May 2017 differed from those identified as *C. glacialis* in January (Fig. 5, Table 5) confirming that we most likely did not encounter *C. finmarchicus* AM during the January campaigns, and that *C. finmarchicus* moults later into AM than *C. glacialis* in Svalbard waters. Similar observations were made in Disko Bay, were AM of *C. glacialis* were present between September and February with highest proportions in December and January, while *C. finmarchicus* AM were found from February to May, with maximum proportions between March and May (Madsen et al. 2001, Niehoff et al. 2002). In the Norwegian Sea, a similar timing of occurrence of AM as in Disko Bay was observed by Østvedt (1955). However, observations from lower latitudes indicate that moulting of *C. finmarchicus* to adults may have

Table 5. Overview of available information on size ranges (mm) for adult males of *Calanus finmarchicus* and *C. glacialis*

Location	*C. finmarchicus* Prosome length (mm)	*C. glacialis*	Reference
Svalbard fjords	–	2.7–4.25 (3–3.84[a])	This study
80° N, western Svalbard	2.43–3.14[a]	–	This study
Greenland Sea, Barents Sea, Norwegian Sea, Central Arctic Ocean	2.34–3.16	3.16–4.1	Frost (1971)
Disko Bay, Greenland	2.16–2.92	2.88–3.62	Swalethorp et al. (2013)
Loch Striven, Clyde area, UK	2.35–2.67	–	Marshall et al. (1934)
White Sea	–	3.5–4.1	Kosobokova (1999)
Billefjorden, Svalbard	–	2.7–3.95	Bailey (2010)
	Total length (mm)		
North Sea (Isle of Man)	2.7–3.2	–	Gunther (1934)
North Sea (L4, UK)	3.01–3.37	–	Russell (1928)

[a]Sizes confirmed by molecular analysis

already begun in December or January. Adults have been recorded in January and February in all regions from the English Channel to East Greenland (Marshall & Orr 1955), and Marshall et al. (1934) observed highest abundance of AM in January–February in the North Sea/Scotland, followed by a low constant presence of AM between April and August. Recently, Choquet (2017) observed AM of both *C. glacialis* and *C. finmarchicus* co-occurring in January and February in 2 northern Norwegian fjords (67° N). This suggests that *C. finmarchicus* moults earlier into males at lower latitudes compared to in Svalbard waters, where this species is at the northern border of its distributional range (Conover 1988, Choquet et al. 2017).

Our molecular results from January and May show that there is an overlap in size between AM of *C. finmarchicus* and *C. glacialis*, with maximum length of 3.14 mm of *C. finmarchicus* AM (Table 5, Fig. 5). A size overlap between these 2 species is common in all copepodite stages and constitutes a challenge when identifying these species (Choquet et al. 2017, 2018). For AM, this problem may be seasonally limited in our study area, since AM of both species did not seem to co-occur in January and May. However, this is likely to differ as soon as *C. finmarchicus* AM start to appear. From our data on prosome length associated with molecular identification (Fig. 5), we suggest *C. finmarchicus* AM may be correctly identified as individuals smaller than 3 mm, and *C. glacialis* as individuals larger than 3.2 mm, which is similar to Frost (1971) and Madsen et al. (2001) (Table 5). A larger data set is needed to improve taxonomic resolution within the overlapping size range.

Male abundance

The presence of AM in the *C. glacialis* population in January confirms previous observations from Svalbard and other high latitude locations (Fig. 9). *Calanus* AM have been observed from September to June in the White Sea (Kosobokova 1999), the Canadian Arctic (Wold et al. 2011, Estrada et al. 2012, Darnis & Fortier 2014) and western Greenland (Madsen et al. 2001, Niehoff et al. 2002), with peak abundance usually observed from November to February (Fig. 9). In Svalbard, *Calanus* AM have been observed between October and May in Billefjorden (Bailey 2010) and in January in Rijpfjorden (Daase et al. 2014), while Leu et al. (2011) did not observe a single male of *Calanus* in Rijpfjorden between March and October. Despite Kongsfjorden being one of the most studied fjords in Svalbard, occurrence of *Calanus* AM has never been reported (e.g. Kwasniewski et al. 2003, 2013, Daase et al. 2013). In our study, we found a high variability in AM abundance among the different fjords and years. Peak abundances observed in Rijpfjorden were comparable to winter abundance previously observed in Billefjorden, while the lower abundance estimates were in the same order of magnitude as estimates from the Canadian Arctic (Fig. 9).

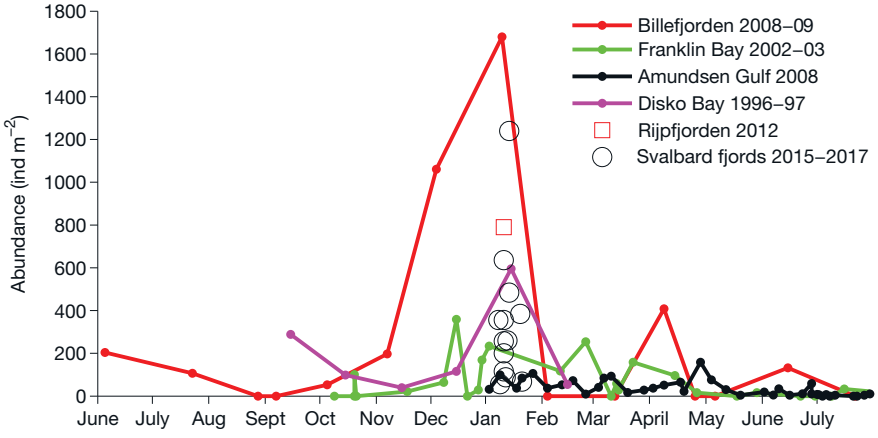

Fig. 9. Seasonal variability of abundance (ind. m^{-2}) of *Calanus glacialis* males across the Arctic: Billefjorden 2008–2009 (Bailey 2010); Franklin Bay 2002–2003 (Fortier & Darnis 2006); Amundsen Gulf 2007–2008 (Wold et al. 2011); Disko Bay 1996–1997 (Madsen et al. 2001); Rijpfjorden 2012 (Daase et al. 2014); and Svalbard fjords January 2015, 2016 and 2017 (this study). Locations of sampling sites are marked in Fig. 1

Sex ratios

Adult sex ratios observed during this study were relatively consistent, varying from 0.3–0.9 in all years (1–3 females male^{-1}). Exception were in RF in 2016, where AM dominated over AF during our study (sex ratio of 1.6, i.e. 1–2 males female^{-1}), and BF in 2016, where AM were rare (sex ratio of 0.2, i.e. 4–5 females male^{-1}). Such high proportions of males have not been observed in the study area during any other season (Bailey 2010). In other northern geographical locations, *Calanus* AF usually substantially outnumber AM in all other seasons (Marshall & Orr 1955, Conover 1965, Crain & Miller 2000). For *C. glacialis*, the only record of seasonal variability in the sex ratio is from the White Sea, where Kosobokova (1999) reported maximum ratio of 0.5–0.6 males:females in October and November, which is comparable to our observations (Table 3).

The sex ratios of the pre-adult stage CV were clearly skewed towards females during our study. According to published data, moulting of CVs to AM precedes moulting of CVs to AF (Marshall & Orr 1955, Kosobokova 1999), and males seem to generally differentiate from the largest CVs (Grigg et al. 1985, 1987, Miller et al. 1991). This is supported by our observations. The prevalence of potential females in CVs indicates that sex differentiation in males and moulting of potential CV males to adults was largely completed by the time of our January sampling period, with the remaining CVs in the overwintering population presumably developing and moulting to AF later in the season.

The high proportion of sexually undifferentiated CVs in BF indicate that the reproductive phenology may have been delayed in BF compared to the other 4 fjords in 2016. The majority of the sexually undifferentiated CVs from BF were *C. finmarchicus* (Table 4, Fig. S2 in the Supplement). However, 38% of the CVs in the size range of *C. glacialis* were also sexually undifferentiated at the time of sampling. Billefjorden also had the lowest percentage of females with spermatophores and the lowest adult sex ratio. Of all the fjords, the *C. glacialis* population in Billefjorden is the most isolated, since it resides in the inner basin of Billefjorden which experiences reduced water exchange with the outer fjord system and the lowest water temperature (less than −0.5°C). The delay of maturation and moulting of *Calanus* CVs in this fjord may therefore be due to low water temperatures compared to the other locations, as development time is known to increase with decreasing temperatures (Campbell et al. 2001).

In our study, *C. finmarchicus* males were absent in January and the abundance of *C. finmarchicus* females was very low while the proportion of sexually undifferentiated *C. finmarchicus* was high (e.g. in Rijpfjorden). This indicates that the time of moulting into adults and mating does not coincide between *C. finmarchicus* and *C. glacialis*. These elements suggest there is a very low potential for inter-species mating and consequently hybridization is unlikely, which supports results from recent molecular-based studies (Nielsen et al. 2014, Choquet et al. 2017).

Females with spermatophores

In 2015 and 2016, 3–18% of *Calanus* AF had spermatophores attached, indicating that active mating occurred during the studied period. Copulation usually occurs at an early stage in the female's gonad development with a male attaching a spermatophore to the genital segment of a female (Marshall et al. 1934, Marshall & Orr 1955). Our data support these observations, as we found mainly immature *C. glacialis* AF bearing spermatophores in January. The spermatophore is retained by *Calanus* AF only for a short time, while sperm may be stored in spermatheca for a rather long period (i.e. several months), and eggs are presumably fertilized while spawning (Marshall et al. 1934, Marshall & Orr 1955). The highest proportion of *C. glacialis* AF with spermatophores occurred in Kongsfjorden in January 2015, where abundance of AF was also highest (Figs. 4 & 5), as was the relative contribution of AF (28–46%) and AM (12–25%) to the total *C. glacialis* population. In January 2016, AF and AM contributed only 1–7 and 0.3–10%, respectively, to the total *C. glacialis* population, as a much higher proportion of overwintering CIVs was observed (Fig. 4). The high proportion of AF and AM in Kongsfjorden in 2015 likely increased the encounter rate between AM and AF, thus leading to a higher percentage of AF bearing spermatophores that year.

Overall, 18% of females bearing spermatophores fell within the size range defined as *C. finmarchicus* (<2.95 mm). However, the length frequency distribution of females bearing spermatophores was unimodal, with the smallest individuals being 2.6 mm (Fig. S1). Given that *C. glacialis* females may also be of that size (Choquet et al. 2018, Renaud et al. 2018), we find it likely that all females bearing spermatophores were indeed *C. glacialis*.

Vertical distribution and metabolism

It has been proposed that in some calanoid species with extended seasonal migrations AMs concentrate in mesopelagic layers, which ensures high encounter rates and copulation success when AF pass through this layer on their ascent from the overwintering depths (Spiridonov & Kosobokova 1997). Tsuda & Miller (1998) suggested that AF and AM of *Calanus* spp. would benefit from gathering in rather narrow layers of the water column to attract and search for a mate, and that pycnocline or thermohaline stratification would provide favorable conditions for 'painting' pheromone tracks. However, we found that adults of *C. glacialis* did not concentrate at any particular depths in January. They seemed to avoid the very deepest layer but were otherwise present throughout most of the water column. Such a distribution pattern suggests that both sexes are actively swimming in search of a mate for copulation rather than waiting at a certain depth for a mate to swim by. However, males did display higher swimming activity levels and respiration rates than females, indicating that males engage more than females in actively seeking a mate. This fits with observational (Tsuda & Miller 1998, Kiørboe & Bagøien 2005) and theoretical (Kiørboe 2008) studies in other calanoids suggesting that ritualized, directed swimming of males facilitates locating females.

Interestingly, the lipid content of AM was slightly higher than that of AF and much higher than that of the overwintering stages CV and CIV. AM also had significantly higher lipid sac area relative to their body area compared to AF, CVs and CIVs. The relatively high lipid content of AMs observed in January suggests that AMs just recently started to actively mate and thus had not yet depleted their lipid reserves. It may also indicate that AMs may start out with a higher lipid content after moulting to adults compared to AFs, supporting observations from the White Sea that the largest and most lipid-rich CVs are the ones that moult to AMs (Kosobokova 1999). Future studies need to assess the seasonal variability in lipid content between CVs, AM and AF, especially during the moulting period.

Winter abundance and distribution data for *Calanus* from the Arctic are still scarce. Our data on vertical distribution of both *C. glacialis* and *C. finmarchicus* confirm recent observations from the polar night in Svalbard (Daase et al. 2014, Berge et al. 2015, Błachowiak-Samołyk et al. 2015) that overwintering stages and adults of *Calanus* are distributed throughout the entire water column in January instead of being concentrated at depth. These observations were made in the same study area as our study (Rijpfjorden, Kongsfjorden) but also in the off-shelf waters north of Svalbard, indicating that such a distribution pattern is not characteristic to fjord populations only. For adults, this may be an indication of a mid-winter ascent from overwintering depth in order to search for a mate. It also suggests an earlier seasonal ascent of the overwintering population from depths than traditionally believed. There is a marked increase in ambient light from the winter solstice to mid-January that may be sufficient enough to function as a visual cue for *Calanus* to trigger the seasonal ascent (Båtnes et al. 2015, Cohen et al. 2015).

CONCLUSIONS

Males of *Calanus glacialis* were much more abundant in mid-winter in Svalbard fjords compared to all other previously studied seasons. The absence of *C. finmarchicus* AM, low abundance of AF and a high proportion of sexually undifferentiated CVs indicate a distinct reproductive phenology in the 2 species, reducing the likelihood of their interbreeding and hybridization in the study area. The presence of *C. glacialis* AF with spermatophores and elevated swimming activity levels in AM relative to AF revealed that active mate seeking and mating occurs in mid-winter. Furthermore, *Calanus* populations were distributed throughout the water column and not confined to overwintering depths, corroborating recent studies showing that the polar night is a much more biologically active period than previously assumed (Berge et al. 2015, Ludvigsen et al. 2018). It is apparent that for *C. glacialis* the polar night is an important reproductive period. We suggest that further studies with increased seasonal and vertical resolution address gaps in our understanding of the life-history strategies of northern *Calanus* males. Specifically, a better understanding of the timing and energetic costs of the moult to adult, mating and spermatophore production, and the sensitivity of these costs and activities to increased winter temperatures, is now required.

Acknowledgements. We are grateful to Gerald Darnis for providing *Calanus* male abundance data from Franklin Bay and Amundsen Gulf. We appreciate the help of Captain and crew of RV 'Helmer Hanssen'. This study was funded by the Norwegian Research Council (NRC) through the projects Marine Night (226417), Arctic ABC (244319) and COPPY (227139). K.K. was supported by the Russian Foundation for Basic Research (Project Nos. 15-29-02447 and 16-04-00375),

the Russian Science Foundation (Project No. 14-50-00095) and performed within the framework of the state assignment of FASO Russia (theme No. 0149-2018-0035). K.S.L. and J.H.C. received additional funding from the MASTS pooling initiative (The Marine Alliance for Science and Technology for Scotland), which is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions. This study is a contribution to the ARCTOS research network (http://arctos.uit.no).

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Editorial responsibility: Sigrun Jónasdóttir, Charlottenlund, Denmark

Submitted: January 31, 2018; Accepted: October 15, 2018
Proofs received from author(s): November 22, 2018

Paper III

Hatlebakk M, Graeve M, Boissonnot L, Søreide JE.

Lipid storage consumption and feeding ability of *Calanus glacialis* Jaschnov, 1955 males

Journal of experimental marine biology and ecology **521:** 151226

Journal of Experimental Marine Biology and Ecology 521 (2019) 151226

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Lipid storage consumption and feeding ability of *Calanus glacialis* Jaschnov, 1955 males

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ARTICLE INFO

Keywords:
Arctic zooplankton
Lipid storage
Ingestion
Assimilation
Survival
^{13}C

ABSTRACT

Calanus is one of the best studied genera of Arctic zooplankton, but still we know very little about the males since they are short-lived and mainly present in winter. Their short life-span compared to females is assumed to be a combination of high mating activity, no feeding and consequential depletion of lipid stores. In this study we tested 1) if the life span of male *Calanus glacialis* is limited by their lipid storage reserves and 2) if males are capable of feeding and utilize food if present. We ran two separate experiments from January to March; one on starvation and one on feeding. In the 39-days long starvation experiment we followed the lipid sac size of individually incubated males until their time of death. On average the total lipid (TL) content decreased by 2.6 to 4.5 μg day^{-1}, but despite this males had substantial amounts of lipids left (131.4 μg, SD 44.0) when they died. This strongly suggests that the depletion of lipid reserves is not the main reason for males' short life span which in this study was measured to be up to 73 days. In the feeding experiment, we fed both *C. glacialis* males and females ad libitum with ^{13}C labelled microalgae. Both males and females were capable of feeding and assimilate the diatom monoculture, but females responded faster to the sudden favourable food conditions, and produced more and larger fecal pellets than the males. Assimilation of ^{13}C labelled 20:5(n-3), an essential polyunsaturated fatty acid (PUFA), from the diatom diet was traceable in both males and females on day 21, and then with a higher enrichment in females than males. Morphological investigations of the feeding appendages showed some differences between sexes, suggesting males to be more omnivorous than females. In conclusion, lipid storage depletion is not the cause of death for male *C. glacialis*, and males may even compensate for some of the mating energy costs by feeding. In future, we recommend further studies on the role of essential fatty acids (FA) for sperm formation and aging as determining factors for males' relatively short life span.

1. Introduction

The mesozooplankton community of Arctic and Sub-Arctic seas, in terms of biomass, are dominated by copepods of the genus *Calanus* (Kosobokova and Hirche, 2009). In Svalbard shelf seas and fjords this genus comprise up to 80% of the mesozooplankton biomass (Blachowiak-Samolyk et al., 2008; Søreide et al., 2008), and they are key actors in the Arctic marine Ecosystem. When light returns in spring, it triggers first an ice algae bloom followed by a short and intense pelagic bloom which lasts for a couple of weeks before fading into a smaller summer and fall production (Leu et al., 2015). *Calanus* spp. plays a major role in harvesting and transferring the energy from these primary producers to higher trophic levels (Falk-Petersen et al., 2009).

Copepods have developed three different foraging strategies: ambush feeders which waits passively for prey to come within range (Kiørboe et al., 2009), cruise feeders which encounter and catch prey as they swim through the water (Kjellerup and Kiørboe, 2011) and feeding-current feeders which create a feeding current and harvest their catch in the current (Koehl and Strickier, 1981). The different foraging strategies entails different levels of activity, which has consequences for encounter rates with food, predators and mating partners and trade-offs between these. The passive ambush feeders have lower risk of being eaten, but at the cost of lower feeding efficiency (Henriksen et al., 2007; Kiørboe et al., 2010) while the active feeders benefit from high feeding efficiency, but at the cost of higher risk of encountering predators (Gonçalves et al., 2014; Van Someren Gréve et al., 2017). *Calanus* spp.

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https://doi.org/10.1016/j.jembe.2019.151226
Received 3 November 2018; Received in revised form 3 September 2019; Accepted 5 September 2019

belongs to the feeding-current feeders, using their antennules and feeding appendages to create currents around the body (Koehl and Strickier, 1981; Huntley, 1988). They can be very effective in filtering water for food, though it varies with season and size of available prey (Tande and Båmstedt, 1985; Levinsen et al., 2000).

In the European Arctic three species of *Calanus* co-exist; the big Arctic *C. hyperboreus* with core distribution in the Greenland Sea (Conover, 1988), the intermediate sized *C. glacialis* which mainly inhabit the Arctic shelf seas, and the slightly smaller *C. finmarchicus*, a north Atlantic expatriate species carried to the Arctic with Atlantic water currents (Falk-Petersen et al., 2009). *Calanus* spp. efficiently accumulates and de novo biosynthesises lipids from its microalgae diet, storing the surplus in a lipid sac which can fill up to 80% of the body cavity of older copepodite stages (Vogedes et al., 2010). Especially essential polyunsaturated fatty acids (PUFA), which are important for physiological processes and reproduction (Sargent and Falk-Petersen, 1988; Kattner and Hagen, 2009; Koski et al., 2012) are preferably retained from the diet (Graeve et al., 2005). This efficient accumulation of lipids makes *Calanus* spp. key species in Arctic marine food webs, ensuring efficient energy transfer of essential fatty acids (FA) and other lipids to higher trophic levels (Falk-Petersen et al., 2007).

The three *Calanus* species are morphologically similar, and are primarily separated to species level by their differences in body size (Daase and Eiane, 2007) despite that recent molecular studies show that *C. glacialis* and *C. finmarchicus* have overlapping size distributions (Gabrielsen et al., 2012; Choquet et al., 2017). At high latitudes, however, live specimens can be distinguished with a high degree of confidence to species level by size (prosome length) combined with red pigmentation (Choquet et al., 2018). Females of *C. glacialis* tend to have red antennules and genital segment, and *C. finmarchicus* tend to have pale antennules and genital segment (Choquet et al., 2018). Live males of *Calanus* are not pigmented, but genetic studies confirm that males appearing in January in Svalbard with prosome lengths between 2.9 and 3.4 mm are *C. glacialis* (Daase et al., 2018). Males of *C. finmarchicus* are smaller (2.4–3.1 mm) and first seem to appear in mid-February/March (Daase et al., 2018). *C. hyperboreus* are only found in low numbers in Svalbard fjords (Scott et al., 2000; Arnkværn et al., 2005), hence we focused on *C. glacialis* in this study.

C. glacialis efficiently build up its lipid storages during the short, but intensive spring bloom period. The females utilize previous summer's stored lipid resources to fuel early maturation and egg production the following year to enable the offspring to hatch and develop in time for the phytoplankton bloom (Søreide et al., 2010). The young copepods develop and grow to the overwintering copepodite stages CIV or CV and descend to deeper waters in autumn as soon as they have built up sufficient lipid storages. The primary storage lipids in *Calanus* spp. are wax esters (WE), characterised by the long-chain monounsaturated fatty acids (MUFA) and fatty alcohols (FAlc) 20:1 (n-9) and 22:1 (n-11) (Lee et al., 1971; Graeve and Kattner, 1992; Lee et al., 2006). As the microalgae season narrows down towards higher latitudes the proportions of WE in copepods increases (Lee and Hirota, 1973; Lee et al., 2006), and may constitute > 90% of the TL content in the Arctic *C. glacialis* (Conover, 1988; Lee et al., 2006). This extensive energy storage combined with reduced metabolism during fall and winter makes it possible for *C. glacialis* to survive the long food-poor Arctic winter. Individuals spending the winter as CIV will first be capable to moult to CV after one more feeding season in spring for so to overwinter as CV the following winter (Kosobokova, 1999). The individuals which overwinter as CV will moult to adults and mate during the winter to ensure early reproduction. Males moult a few weeks earlier than females, and are only present for a few months with peak abundance in December–January (Kosobokova, 1999; Daase et al., 2018). In comparison, females of *C. glacialis* may be iteroparous i.e. being able to survive another winter and reproduce again (Kosobokova, 1999).

The shorter life span for males compared to females is typical among calanoid copepods (Gilbert and Williamson, 1983). Short life span may not necessarily be due to predation or energy depletion, but also due to the physiological aging being faster in males than females (Rodríguez-Graña et al., 2010; Kiørboe et al., 2015). Kiørboe et al. (2015) found that trade-offs between various life history traits and behaviours of small, copepods are consistent with the disposable soma theory (Kirkwood, 2002). In short it states that the investment in maintaining somatic (non-reproductive) tissue in good health should not exceed the life expectancy in the wild. Van Someren Gréve et al. (2017) demonstrated the relationship between behaviour and predation risk in pelagic copepods. Higher risk means shorter life expectancy. As males are typically the most active part in mate search, they are generally at higher predation risk (Kiørboe, 2008). Thus male copepods benefit most on investing any spare resources in reproduction rather than self-maintenance and growth (Hirst and Kiørboe, 2014). Even at high latitudes when the light is practically absent in winter, the predation risk may be considerable since recent studies found unexpectedly high activity levels in pelagic communities during the polar night (Berge et al., 2015a).

The live-fast-die-young approach and trade-off between mate search and food search have led some calanoid males to not invest in fully developed mouth parts, rendering them less efficient feeders or unable to feed (Schnack, 1989). However, this has not been observed within the *Calanus* genus (Bradford and Jillett, 1974), so they may potentially capture food particles they encounter (e.g. Cleary et al., 2017). Due to the lack of microalgae during the dark Arctic winter, it is hypothesized that males most likely do not actively feed and that their short life span is due to lipid storage depletion (Raymont and Gross, 1942; Kosobokova, 1999). However, we know very little about the ecology of *Calanus* males, since Arctic winter data is scarce (Kosobokova, 1999; Madsen et al., 2001; Daase et al., 2018). Recent studies have shown that the long, dark winter is not void of biological activity as previously assumed (Berge et al., 2015b and references therein), and more winter-studies are urgently needed to better understand polar zooplankton life strategies and their capability to adapt to a rapidly changing Arctic (e.g. Madsen et al., 2001; Berge et al., 2012; Zamora-Terol et al., 2013).

To increase our knowledge on *Calanus* life strategies we investigated lipid storage consumption and feeding capability of male *C. glacialis* in parallel with the much better known *C. glacialis* female as a reference. Following research questions were targeted: (1) is the life span of males determined by their lipid storage reserves? We know their life span is shorter than females, but there are uncertainties to why. Do they die because they run out of energy in the unproductive winter or are there other factors connected to their life strategy? (2) do males actively feed? And finally (3) can males utilize this food? Males don't need to make a trade-off between mate search and feeding, since actively searching for females increase their food encounter rate. It may therefore be beneficial for males to take advantage of present food, especially if they encounter algal blooms which may start as early as March (Leu et al., 2015). These questions were addressed in two experimental studies. First, a starvation experiment where the lipid sac sizes of individually incubated males were followed by image analyses from early winter until time of death. Secondly, a feeding experiment with males and females offered ad libitum ^{13}C labelled microalgae, to study their capability to ingest and assimilate dietary lipids.

2. Materials and methods

2.1. Sampling

Copepods for the experimental work were collected in Billefjorden (78° 39′N, 016°44′E) and Rijpfjorden (80°17′ N, 022°18′E) (Fig. 1) in January 2013 and 2016 onboard R/V Helmer Hanssen. Billefjorden is situated in the innermost part of Isfjorden in Western Spitsbergen. It is a threshold fjord with a sill depth of 50 m and an inner basin of 190 m depth, which restricts the influence of the warmer and saltier Atlantic water from the West Spitsbergen Current to enter the fjord (Nilsen et al., 2008). Local cold water formation due to winter cooling and sea

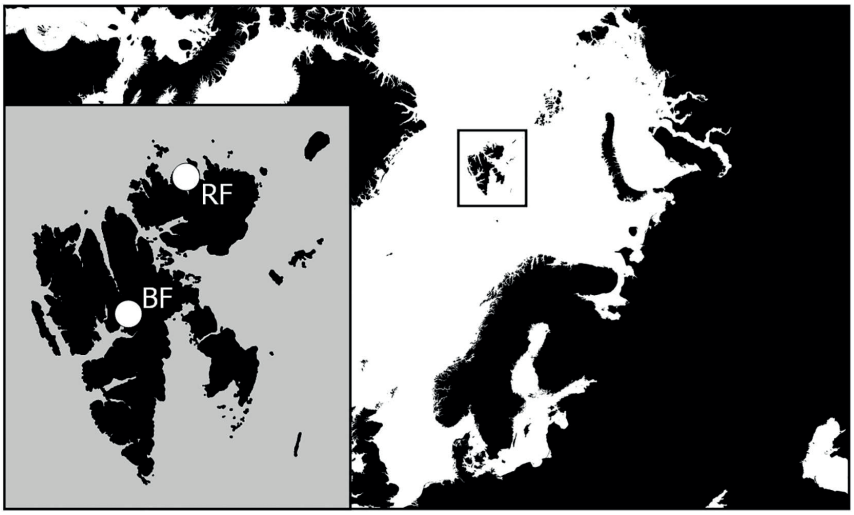

Fig. 1. Map showing the sampling stations Billefjorden (BF) and Rijpfjorden (RF) in Svalbard archipelago (Map data: Norwegian Polar Institute and thematicmapping.org).

ice formation result in water masses similarly cold (< -1 °C) and saline ($\leq$ 34.8 psu) as Arctic water year-round below the threshold depth in Billefjorden (Nilsen et al., 2008). Rijpfjorden is a north facing fjord on Nordaustlandet in NE Svalbard. It is considered to be a high-Arctic fjord with its extensive seasonal sea ice cover 6–8 months a year (Wallace et al., 2010). Detailed knowledge on the bathymetry of Rijpfjorden is sparse, but there are at least two basins (Howe et al., 2010) with a maximum depth of 250 m, and no sill separating the fjord from the wide shelf outside (Leu et al., 2011).

For experiments, copepods were collected vertically by a WP3 net (1000 µm, 1 m^2 opening) with a large non-filtrating cod end at slow speed (0.5 m s^{-1}). Samples were quickly and gently transferred to 30 L barrels, diluted with surface sea water and kept in the dark in a cold room (2 °C) until start of the experiments.

2.2. Starvation experiment

The copepods for the starvation experiment were collected from bottom to surface in Billefjorden (180 to 0 m) and Rijpfjorden (270 to 0 m) in January 2013 and kept in large 30 L barrels in a temperature regulated cold room (2 °C) on board RV Helmer Hanssen for eight and six days respectively before being transferred to the cold room laboratory at the University Centre in Svalbard (UNIS). There they were kept in 1 µm filtered sea water (Sartopure PP2 capsule, Sartorius stedim biotech) in 30 L barrels at −1 °C for another 28 days until the experiment was started at February 15. Five males from Billefjorden and 38 males from Rijpfjorden were sorted out from the barrels, giving a total of 43 males that were incubated individually in incubation chambers of 150 mL filled with GF/F filtered sea water (Whatman GF/F: 0.7 µm, GE Healthcare). The incubation was done in darkness at −1 °C to simulate the in situ winter conditions. Every three to four days, when the water was renewed, the males were photographed under a dissecting microscope at 16× magnification from lateral view for lipid sac size estimates. This interval was chosen as a compromise between handling stress and relatively frequent lipid sac size measurements. TL content was estimated from the area of the lipid sac following Vogedes et al. (2010). Images were taken with a SONY video camera (HDR-HC7) with an ocular adapter and analysed using ImageJ software 1.48v (Rasband 1997–2009).

2.3. Feeding experiment

Males and females were collected from Rijpfjorden (100 to 0 m) during the polar night cruise with R/V Helmer Hanssen in January 2016. The feeding experiment was running for three days on board before it was transferred to the cold lab at UNIS where it was run for another 18 days.

The ^{13}C labelling of the microalgae culture started 2 weeks prior to start of feeding experiment to ensure the microalgae to be sufficiently labelled (Boissonnot et al., 2016). Algae culture of *Porosira glacialis* (Grunow) Jörgensen, 1905 *(size class 30–35 µm ⌀*, calculated carbon content 774 pg $cell^{-1}$ (Menden-Deuer and Lessard, 2000)) was kept at ~5 °C in a light:dark cycle of 16:8. *P. glacialis* is a centric diatom with bipolar distribution (Hasle, 1976) and it is in the food size range typically preferred by *Calanus* (Levinsen et al., 2000). The algae culture was diluted regularly to keep it growing in an exponential phase with Guillards f2 medium dissolved in sterile sea water, enriched with silicate (0.1 µmol L^{-1}) and labelled with ^{13}C sodium bicarbonate (1.5 mg L^{-1}). On day 0, 11 and 21 of the feeding experiment, triplicates of the algal cultures (100 mL each) were filtered on burnt GF/F filters and quickly frozen at −80 °C for later FA and compound specific stable isotope analyses at Alfred Wegener Institute, Bremerhaven, Germany.

The copepods were incubated in 1 L borosilicate bottles: 4 bottles with 20 females each and 4 bottles with 20 males each. This number was a compromise between space on the plankton wheel and sufficient number of individuals for lipid samples throughout the feeding experiment. Incubations were done in darkness at 2–3 °C, slightly higher than the in situ sea temperature in Rijpfjorden (mean 0 °C; range − 1 to 1 °C). The incubation bottles with the copepods were kept on a rotating plankton wheel (1.5–2 RPM) to keep the microalgae in constant suspension. The copepods were fed with ^{13}C labelled *P. glacialis* at a concentration of 1200 cells mL^{-1} (~929 µg C L^{-1}), and water and microalgae were renewed every 2–3 days by diluting parts of the culture in filtered sea water to the right concentration. When water was changed, the content of each bottle was filtered through a 90 µm mesh. The animals were inspected and the number of dead animals was counted. Live animals were transferred back to the bottle with renewed water and microalgae. The fecal pellets collected on the mesh were transferred to a petri dish and counted under dissecting microscope, and the fecal pellet production was used as a relative indicator of ingestion rate. Fecal pellet volume was measured on day

7 by image analyses using ImageJ software 1.48v (Rasband 1997–2009). In total, 46 fecal pellets from males and 168 fecal pellets from females were collected in petri dishes and photographed at 40× magnification with a SONY video camera (HDR-HC7) with an ocular adapter. Length and width of the fecal pellets were measured and volume calculated assuming a cylindrical shape. At day 0, 2, 11 and 21, copepods from each bottle were collected for lipid composition and compound specific stable isotope analyses. On day 0 triplicates of 10 random males and females were sampled, on day 2 and 11 five individuals were collected from each incubation bottle giving quadruplicates for both males and females, and on day 21 the remaining animals were collected as one replicate per bottle (1, 2 and 3 individuals for male triplicate, and 3, 4 and 6 individuals for female triplicate). Animals were rinsed in filtered seawater and frozen in glass vials at −80 °C.

Of specific FA we were particularly interested in the diatom FA markers 16:1(n-7) and the essential PUFA 20:5(n-3) (Dalsgaard et al., 2003). In addition, the most abundant saturated FA; 16:0 and 18:0 and the essential PUFA 22:6(n-3), known to be an appropriate dinoflagellate fatty acid trophic marker (FATM) were focused upon.

2.3.1. Lipid composition and stable isotope analyses

TL was extracted by homogenizing animal tissues and filters in a solution of dichloromethane:methanol (2:1, v:v), modified after Folch et al. (1957). As internal standard, a known amount of the tricosanoic acid methyl ester (23:0) was added to each sample. A 0.88% solution of KCl (potassium chloride) was added to easily differentiate the biphasic system. Transesterification of the lipid extracts was performed by heating the samples with 3% sulfuric acid H_2SO_4 in methanol for 4 h at 80 °C under nitrogen atmosphere. Fatty acid methyl esters (FAME) were extracted with cyclohexane. FAME and FAlc were determined using a gas chromatograph (HP 6890 N, Agilent Technologies Deutschland GmbH & *Co*. KG) equipped with a 30 m × 0.25 mm i.d. wall-coated open tubular capillary column (film thickness: 0.25 μm; liquid phase: DB-FFAP), a split/splitless injector (250 °C) and a flame ionization detector (280 °C), according to the method of Kattner and Fricke (1986). The oven program was set from 60° to 160 °C with a rate of 30 °C min^{-1}, reaching a final temperature of 240 °C at 1.5 °C min^{-1}. Helium 5.0 was used as carrier gas at a flow rate of 1.0 mL min^{-1}. To identify unknown peaks, additional GC-mass spectrometry runs were carried out. The chromatograms were evaluated using the ChemStation software from Agilent. TL mass per individual was calculated by summing up FA and FAlc masses. The percentage of WE in TL was calculated from the proportion of FAlc on a mole basis, assuming that copepods contain no free FAlc (Kattner and Krause, 1989).

2.3.2. Carbon isotopic ratios

The ^{13}C isotopic enrichment in FA and FAlc was measured using a Thermo GC-c-IRMS (gas chromatography-combustion-isotope-ratio-mass spectrometry) system, equipped with a Trace GC Ultra gas chromatograph, a GC Isolink operated in combustion mode at 1000 °C and a Delta V Plus isotope ratio mass spectrometer connected via a Conflo IV interface (Thermo Scientific Corporation, Bremen, Germany). FAME and FAlc, dissolved in cyclohexane, were injected (1 μL) in splitless mode and separated on a DB-FFAP column (60 m, 0.25 mm I.D, 0.25 μm film thickness). The column flow was set to constant flow mode. Helium 5.0 was used as carrier gas at a flow rate of 1.6 mL min^{-1}. Injector and detector temperature was set to 250 °C. Temperature programming started at 80 °C for 2 min, increased by 20 °C min^{-1} to 160 °C, and with 2 °C min^{-1} to the final temperature of 240 °C, with a final hold for 15 min.

Linearity and precision of the mass spectrometer were checked with a series of reference gas pulses (CO_2). The isotopic composition of different amounts of reference gas (CO_2, δ 35.08 vs PDB) within a concentration interval resulting in a response of mass 44 from 400 to 6000 mV were measured in five to seven repetitions per concentration step. For each analytical run, two reference gas pulses were used for data calibration at the start and at the end together with the internal 23:0 FAME (δ −32.50 vs PDB). The chromatographic peak areas and carbon isotope ratios were obtained with the instrument-specific software (Isodat 3.0) and the reference standards 14:0 and 18:0 FAME (Iowa University) were used with known δ-values for further calculations.

Isotopic ratios of each FA and FAlc are normally expressed in δ notation according to the formula (1).

$$\delta^{13}C\,(‰) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

where R is the ratio $^{13}C/^{12}C$, and the commonly used standard is Vienna Pee Dee Belemnite (V-PDB): $R_{standard} = 0.0112372$.

For this study, δ-values of labelled samples were converted to atom percent, which is more appropriate than relative values to express isotope data in terms of isotope concentrations. Conversion was made according to the following Eq. (2):

$$AT\,(atom\ percent) = \frac{R_{sample}}{R_{sample} + 1} \times 100 \quad (2)$$

This equation's result includes the atom percent of enriched samples as well as their natural background (Brenna et al., 1997).

2.3.3. Morphology of mouthparts

Because differences in ingestion of food between males and females were observed during the feeding experiment in this study, dissection of mouthparts was done to compare the morphology for a possible explanation of these observations. Males and females collected in Rijpfjorden in January 2016 were picked from 4% formaldehyde preserved community samples and dissected under a stereo microscope in a 1:1 glycerol:distilled water solution on watch glass. Due to limited material only one male and one female was successfully dissected for further analysis. Mouthparts were mounted in glycerol and photographed using a Canon EOS 750D camera mounted on a Leica DM 1000 LED light microscope at 20× magnification. Pictures of the mandibular gnathobases were measured using ImageJ software 1.48v (Rasband 1997–2009) to calculate Itoh's edge index (Ie) (3) for the male and female (Itoh, 1970; Giesecke and González, 2004).

$$\text{Ie} = \sum \left(\frac{w_i}{W} \times \frac{h_i}{H} \times 10^4 \right) / N \quad (3)$$

Where w_i is the distance between adjacent cusps, W is the total length of the cutting edge, h_i is the depth of inter-cusp depression, H is the height of the ventral tooth and N is the number of teeth on the mandible blade. Based on the calculated Ie the copepods could be categorized as either herbivorous (Ie < 500), omnivorous (500 < Ie > 900) or carnivorous (Ie > 900) (Itoh, 1970).

2.4. Statistical analyses

Statistical analyses were done in Sigmaplot (14.0, Systat Software, San Jose, CA). One way ANOVA was applied to test the null-hypothesis of no variation between time points in the experiments. If the null-hypothesis was rejected, a post hoc pairwise comparison using Holm-Sidak method was applied to test for when a significant change from time 0 had occurred. When the data failed to pass the equal variance test (Brown-Forsythe), Kruskal-Wallis was applied instead of ANOVA to test for significant differences, and the post hoc pairwise comparison of the groups was done using the Dunn's method. For comparison of the fecal pellet production of males and females over time, a two way repeated measure ANOVA was applied, followed by Bonferroni *t*-test comparing males vs. females for each day of the experiment. When comparing only two groups, a student's t-test was applied. Significance level was set to $p \leq .05$ in all tests.

3. Results

3.1. Starvation experiment

In total, 43 males were incubated individually without food, of which seven males died soon after the start of the experiment (day <

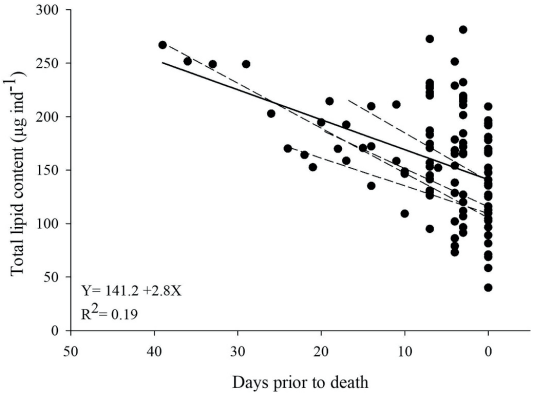

Fig. 2. Total lipid content of males of *Calanus glacialis* during the starvation experiment run at constant temperature (2–3 °C) in the dark from February to March. The time of death (TOD) is set as Day 0. The longest lived male survived for 38 days. The solid line shows the regression line for all data points. Dashed lines show regression lines for the four individuals with six or more time points (R^2 = 0.73 to 0.85).

3). These were not included in the calculations since we could not rule out the effect of handling as cause of death. The male starvation experiment was run until the last male died, in total 39 days from February to March. The prosome length of the males varied little 3.3 mm (SD 0.3) and was within the core size range of *C. glacialis* males (Daase et al., 2018). During the experiment, the TL content decreased by 2.8 µg day^{-1} per individual (linear regression, $R^2 = 0.19$, $p < .001$), and at the time of death the males had on average 131.4 (SD 44.0) µg TL per individual (Fig. 2). TL decrease rate differed between individuals, hence individual linear regression was run for the four individuals with six or more time points. Lipid decrease rates for these four individuals varied between 2.6 and 4.5 µg (mean 3.7 µg) day^{-1} per individual (linear regressions, $R^2 > 0.73$, $p < .03$) (Fig. 2). The longest survival of *C. glacialis* males in this study was 73 days, which was calculated from the day of capture to date of death. The average life span was 23 days (SD 7.3) for the males incubated, calculated from their day of capture.

3.2. Feeding experiment

During the feeding experiments in January–February 2016 both males and females were found to ingest and assimilate the microalgae, and green guts were observed (Fig. 3). Remaining microalgae in the incubation water was observed when exchanging water. From a visual comparison of the male and female side by side their mouthparts appeared to be very similar, but it was observed that the setae of the maxillipeds were longer for the female than for the male (Supplementary Fig. S1). The cutting edge of the mandible were also slightly different as indicated by Itoh's edge index which was calculated to Ie = 659 for the male and Ie = 508 for the female, categorizing them as omnivorous and borderline herbivorous-omnivorous, respectively (Itoh, 1970). The mandibular gnathobase from both the male and female (supplementary, Fig. S2) had two relatively big ventral teeth clearly separated from the other teeth. On the male gnathobase, another seven teeth were identified, with the dorsal most tooth being slightly longer than the others. On the female gnathobase another eight teeth were identified and like the male, the dorsal most tooth was slightly longer. The widths of the gnathobases were 164 µm for the male and 197 µm for the female. The teeth of the male gnathobase appeared more distinct than those at the female gnathobase.

Fig. 3. Male *Calanus glacialis* with clearly visible green gut at day 21 of the feeding experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.1. Fecal pellet production

Both males and females had low fecal pellet (FP) production in the beginning (day 0 to 2) of the feeding experiment (0.16 FP ind^{-1} day^{-1}, SD 0.15 and 0.74 FP ind^{-1} day^{-1}, SD 0.26, respectively) but it steadily increased with time (Fig. 4). Two-way repeated measures ANOVA found a difference between sexes over time, with the males consistently producing fewer fecal pellets (Fig. 4, supplementary, Table S2) and fecals of smaller size than females (Fig. 5). The fecal pellet volume measured on day 7 was on average 2.6 $\times 10^5$ µm^3 (SD 0.9) for males and 9.3 $\times 10^5$ µm^3 (SD 5.2) for females. Females reached a maximum fecal pellet production on day 16 (Fig. 4). On day 21 males and females had similarly high fecal pellet production, but by the end of the experiment the number of individuals in each incubation bottle was very low (1–3 ind. per bottle for males and 3 to 6 ind. per bottle for females).

3.2.2. FA composition and stable isotope analyses of male and female copepods

To follow the incorporation of dietary lipids, the copepods were fed ^{13}C labelled diatoms, a monoculture of *P. glacialis*. The microalgae appeared to be in a healthy condition throughout the experiment with high Chl *a* values, comparable to spring bloom concentrations (4–10 µg Chl *a* L^{-1}) with modest Chl *a*: phaeophytin ratios (~ 1:1) (Supplementary, Table S1). Dominant (> 10%) FA in *P. glacialis* was 16:0, 16:1(n-7), 18:0 and 20:5(n-3) (Table 1). For the ^{13}C labelling, the

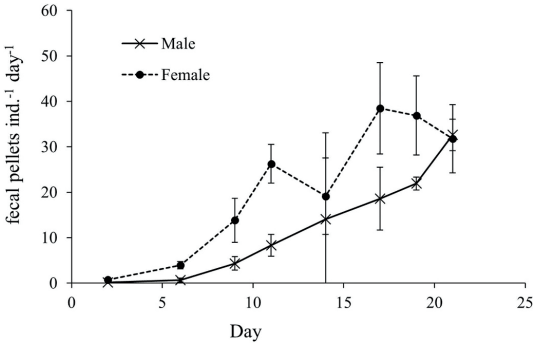

Fig. 4. Fecal pellet production (mean ± SD) of *Calanus glacialis* males and females fed ad libitum with a diatom monoculture of *Porosira glacialis* in winter.

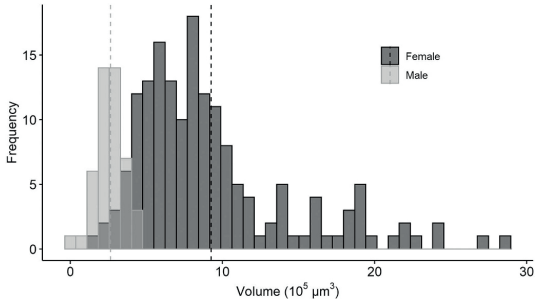

Fig. 5. Histogram showing the volume distribution of fecal pellet of *Calanus glacialis* males (light grey) and females (dark grey) at day 7 of the feeding experiment. Overlapping data in medium grey.

analyses showed enrichment, albeit poorly for the microalgae (Supplementary Fig. S3). However, it was sufficient to prove incorporation of the most important algae FA in the lipids of the copepods. On Day 11, the highest ^{13}C enrichment in algal lipids was found in 20:5(n-3) at 1.3 AT.%, followed by 14:0 (1.2 AT.%), 16:0 (1.1 AT.%) and 16:1(n-7) (1.2 AT.%) (Supplementary, Fig. S3).

For the male *C. glacialis* the TL content kept stable throughout the feeding experiment (Kruskal-Wallis, $H_3 = 2.9$, $p = .41$) with an average TL weight of 147.3 µg ind^{-1} (SD = 23.4). Major FA (> 10%) in the males were, in descending order, 20:1(n-9), 16:1(n-7), 22:1(n-11) and 14:0. Major FAlc were 20:1(n-9) and 22:1(n-11) which constituted 83–84% of the FAlc for the duration of the experiment (Table1). The proportion of WE did not change, but were stable at an average 87.3% (One way ANOVA, $F_{3,9} = 2.35$, $p = .14$) for the duration of the feeding experiment.

The TL content in *C. glacialis* females were stable around 160 µg ind^{-1} (SD 16.4) throughout the feeding experiment (One way ANOVA, $F_{3,11} = 1.84$, $p = .20$) (Table 1). Major FA in the females were, in descending order, 20:1(n-9), 16:1(n-7), 22:1(n-11), 14:0 and 16:0. Another important FA was 22:6(n-3) which increased from 3.6% (3.2 µg ind^{-1}) on day 0 to 6% (5.0 µg ind^{-1}) on day 21. Major FAlc were 20:1(n-9) and 22:1(n-11) which constituted 82–85% of the FAlc for the duration of the experiment. The proportion of WE in females varied through the experiment (One way ANOVA, $F_{3,11} = 10.21$. $p = .002$) (Table 1) fluctuating between 74.9% (SD 2.3) and 90.0% (SD 5.0) with no clear trend throughout the experiment.

Feeding on ^{13}C labelled diatoms resulted in a significant increase of this heavier C isotope in the PUFA 20:5(n-3) in both males and females. On day 0 the natural background of ^{13}C of 20:5(n-3) in the copepods was $\delta\ ^{13}C = -27.1$ for males and $\delta^{13}C = -28.3$ for females. Both for the males and the females a ^{13}C enrichment in 20:5 (n-3) was found with time on day 21 (Kruskal-Wallis, $H_3 > 8.0$, $p < .046$) (Fig. 6) and the ^{13}C enrichment was higher in females ($\delta\ ^{13}C = 29.5$, SD 13.0) than males ($\delta\ ^{13}C = -4.2$, SD 10.5) on day 21 (Student's *t*-test, $t_4 = 3.5$, $p = .025$).

4. Discussion

4.1. The life span of male Calanus glacialis

In the Arctic, *Calanus* males are mainly present during the winter. The lack of feeding combined with active mating behaviour and rapid depletion of lipid resources have been regarded as the most likely reason for the short life span of *Calanus* males (Raymont and Gross, 1942; Kosobokova, 1999). From the starvation experiment we observed that *C. glacialis* males had considerable amount of lipids (131.4 µg ind^{-1}) left at time of death. In contrast, similar experiment with females by Hatlebakk (2014) showed that females completely depleted their lipid sac and still survived. Respiration rates of *Calanus* males in January in Svalbard suggest a carbon demand equivalent to a consumption of 6.3 µg carbon day^{-1} (Daase et al., 2018), which is twice as high as the estimated lipid decrease rate of 2.8 µg TL day^{-1} (range 2.6–4.5 µg TL day^{-1}) in this study. However, high individual variability in the respiration rates was found and was only measured for the most active males in the study to Daase et al. (2018). It could be that males in our lipid decrease study had lower metabolic activity as it has been found that male copepods kept away from females tend to live longer, which may be connected to lower activity levels (Burris and Dam, 2015). Assuming roughly 10% of TL to be structural lipids (Lee, 1975), these rates suggest that males had on average 19 to 42 days' worth of lipids left at the time of death. This indicated that their death was not a direct cause of lipid reserve depletion. The longest life span of males recorded in this study was 73 days (average 43 days, SD 6), from capture in January to death at the end of March. To our knowledge, this is the first record of *C. glacialis* male life span under laboratory conditions. Peak male abundance has been recorded to be December/January (Bailey, 2010) in a seasonal study in Billefjorden, Svalbard with end of male appearance in late February. This suggests that the life span of *C. glacialis* males do not exceed 100 days and that most males live much shorter.

4.2. Feeding activity of male and female Calanus glacialis

In this study we fed our *Calanus* unrealistically high microalgae concentrations for being the winter season, although ice algae may start to grow as early as beginning of March in Svalbard (Hegseth, 1998). Reasons for these high algal concentrations were to ensure data above detection limit for studying males' capability of feeding and to assimilate ingested food. Feeding ability to *Calanus* females, on the other hand, are well-known. Females manage to utilize the early growing sea ice algae which may precede the phytoplankton bloom by 2 months (Søreide et al., 2010). Inclusion of females in our feeding experiment thus gave valuable comparative data and a "female-reference" knowing that females are capable to quickly respond to changes in the algal food environment during the winter-spring transition (Wold et al., 2011; Daase et al., 2013). Clearly visible green guts in both males and females showed that both sexes were capable of ingesting the microalgae they were offered in mid-winter. Moulting and onset of feeding characterise the final phase of diapause for the sibling species *C. finmarchicus* (Hirche, 1996). The slow start and the steadily increase in ingestion rate throughout the feeding experiment showed that both females and males in this study were in a transition state and had not completely terminated diapause at the start of the feeding experiment. Our results for the females are in agreement with Toxværd et al. (2018) who measured fecal pellet production in *C. glacialis* females in March–April when they were fed after an overwintering period without food. Also in this experiment the FP production started out low and increased with time, but the initial response in Toxværd et al. (2018) was steeper and the copepods here reached a stable high FP production quicker than in our study. Main reason for this may be the difference in timing of these two experiments with females' being less "dormant" in March–April than in February (Freese, 2015).

Compared to the females, males needed more time to adjust and optimize to the new, sudden favourable food conditions in our study. They had consistently lower fecal pellet production, with the exception of the very last day of the experiment. The volume of the fecal pellets was also smaller for males than females with a female to male ratio of 3.5 to 1. The fecal pellets appeared to be intact, hence we did not consider fragmentation (coprorhexy) and consumption (coprophagy) of fecal pellets to have influenced the results. Our results are in agreement with the experimental findings from Raymont and Gross (1942) for the sibling species *C. finmarchicus*. Through several feeding experiments they found that females produced 2–10 times as many fecal pellets as males and that the volume of the fecal pellets in the experiments with the diatoms *Skeletonema* and *Ditylum* had a female to male ratio of 5.5 to 1 and 6.5 to 1, respectively. Fecal pellet size has been connected to food concentration, with low concentrations leading to smaller fecal pellets since the copepods are not able to ingest sufficient food to fill the

Table 1
Fatty acid and alcohol composition per individual *Calanus glacialis* female and male at days 0, 2, 11 and 21 of the feeding experiment run in January–February 2016. Values given as mass% of total fatty acids and alcohols, respectively, unless otherwise specified.

Time(day)	Females				Males			
	00 (n = 3)	02 (n = 4)	11 (n = 4)	21 (n = 3)	00 (n = 3)	02 (n = 4)	11 (n = 4)	21 (n = 3)
Fatty acids								
14:0	11.4	10.9	11.4	7.1	10.2	11.0	13.1	7.8
15:0	2.0	1.6	1.4	1.4	1.5	1.7	2.0	1.6
16:0	9.7	9.0	8.0	10.4	8.7	9.1	9.5	8.8
16:1(n-5)	0.6	0.2	0.6	0.7	0.6	0.6	0.6	0.4
16:1(n-7)	13.0	14.0	17.8	10.3	15.0	12.6	12.3	9.7
16:2(n-4)	0.9	0.5	0.9	0.8	0.9	0.7	0.8	0.7
16:3(n-4)	0.4	0.1	0.2	0.3	0.3	0.3	0.3	0.3
16:4(n-1)	0.7	0.1	0.2	0.1	0.2	0.2	0.1	0.2
17:0	–	–	–	0.2	–	–	–	–
18:0	0.9	0.7	0.8	1.0	0.7	0.9	0.9	1.8
18:1(n-5)	0.8	0.7	0.6	1.0	0.8	0.8	0.8	0.9
18:1(n-7)	1.0	1.0	1.0	1.4	0.9	1.0	0.8	0.9
18:1(n-9)	7.6	7.6	5.9	8.4	6.6	7.1	7.4	8.2
18:2(n-6)	–	1.9	1.3	1.7	1.3	2.0	1.7	1.6
18:3(n-3)	2.0	1.8	1.2	1.8	1.4	1.3	1.4	1.4
18:3(n-6)	0.4	0.1	0.2	0.1	–	–	0.2	–
18:4(n-3)	3.8	2.8	1.4	0.6	1.8	2.0	1.4	0.7
20:0	0.3	0.1	0.3	0.1	0.0	–	0.2	0.1
20:1(n-7)	1.0	0.3	0.5	0.7	0.9	1.0	0.8	0.5
20:1(n-9)	15.3	21.3	20.1	20.7	20.8	20.9	20.0	22.9
20:2(n-6)	1.1	0.7	0.2	2.5	0.6	1.5	0.8	–
20:3(n-6)	0.7	0.3	0.4	0.1	0.3	0.5	0.5	1.2
20:4(n-6)	–	0.1	0.2	0.1	–	–	0.1	0.1
20:3(n-3)	–	–	–	–	–	–	–	–
20:4(n-3)	1.1	0.5	0.8	1.1	0.9	0.9	1.0	1.1
20:5(n-3)	7.4	7.0	7.3	8.3	6.2	5.5	5.0	5.9
22:1(n-7)	–	0.2	0.3	0.1	–	–	0.2	0.3
22:1(n-9)	1.9	2.2	2.0	1.8	1.9	1.8	1.8	2.0
22:1(n-11)	10.8	10.6	10.4	9.8	11.9	11.5	11.3	14.4
22:5(n-3)	0.4	0.1	0.2	0.1	0.0	0.2	0.4	0.2
22:6(n-3)	3.6	3.0	3.3	6.0	4.3	3.7	3.5	5.0
24:1(n-9)	1.2	0.6	1.1	1.5	1.1	1.2	1.1	1.3
Alcohols								
14:0	1.6	2.1	1.7	1.1	1.5	1.7	1.5	1.5
16:0	8.6	9.4	7.9	7.1	7.7	7.7	7.5	7.7
16:1(n-7)	1.8	2.5	2.7	1.2	2.2	2.2	1.7	1.3
18:1(n-9)	2.9	3.0	2.4	2.7	2.7	2.8	2.7	3.0
18:1(n-7)	2.0	2.2	2.3	2.2	1.9	2.1	1.8	1.6
20:1(n-9)	54.2	52.6	54.1	57.0	53.6	55.3	53.1	57.7
22:1(n-11)	27.9	25.7	28.1	28.1	29.7	27.6	30.7	26.5
22:1(n-9)	0.9	2.6	0.9	0.5	0.8	0.6	0.9	0.7
Sum								
Total (μg ind^{-1})	161.4	157.6	181.2	134.9	135.5	147.8	184.5	121.6
MUFA	53.1	58.6	60.4	56.3	60.5	58.7	57.1	61.4
PUFA	22.5	18.9	17.6	23.4	18.3	18.6	17.2	18.2
SFA	24.3	22.4	21.9	20.2	21.2	22.7	25.6	20.2
FAlc (μg ind^{-1})	72.8	59.8	76.1	50.3	60.7	62.8	78.0	54.6
Wax ester %	90.2	75.8	84.0	74.6	89.6	85.0	84.5	89.9

n = number of replicates; – = below detection limit.

gut before defecating (Dagg and Walser Jr, 1986). This argues for females being more efficient than males in grazing on the *P. glacialis* in our study. Reasons for this may be related to sex differences in physiological state (e.g. Hallberg and Hirche, 1980) or possibly due to females being more herbivorous than the males (see below).

In this feeding experiment the number of individuals per 1 L bottle was rather high with potential negative impacts on the individual clearance rates (e.g. Levinsen et al., 2000). However, we feel confident that sufficient microalgae concentrations were provided. The low grazing rates as suggested by the low fecal pellet production in the beginning of the feeding experiment was more likely a result of the copepods needing time to adjust to the new favourable food conditions than reduced clearance rates due to food limitation (Morata and Søreide, 2015; Toxværd et al., 2018). This was also indicated by the increase in number of fecal pellets with no distinct reduction in number of copepods per bottle the first 11 days. Females of *C. glacialis* ingest approximately 40 μg C fem^{-1} d^{-1} in a bloom setting when their feeding activity is at the highest (Levinsen et al., 2000). We provided the copepods with approximately 929 μg C bottle^{-1} which would support 8–12 actively feeding females for 3–2 days assuming these max bloom ingestion rates determined by Levinsen et al. (2000). Based on Seuthe et al. (2007) we estimated that males egested between 0.002 and 0.41 μg C ind^{-1} day^{-1} and females egested between 0.03 and 1.69 μg C ind^{-1} day^{-1} during the feeding experiment. Assuming the same and constant respiration rates as measured by Daase et al. (2018), this means a minimum carbon demand of 6.71 μg C ind^{-1} day^{-1} and 4.49 μg C ind^{-1} day^{-1} for males and females respectively. Since the TL levels remained stable throughout the experiment the net carbon budget should be close to I = E + R, where I is ingestion, E is egestion and R is respiration. If we apply this to the longest sampling interval, where algae was not replenished for four days, and assume maximum number of individuals in a bottle (n = 20), the total carbon demand

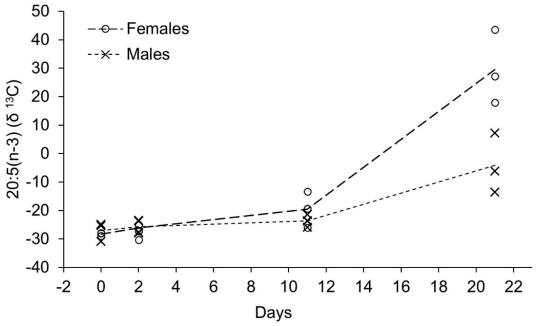

Fig. 6. ^{13}C enrichment of the essential polyunsaturated fatty acid (PUFA) 20:5(n-3) in male and female *Calanus glacialis* throughout the feeding experiment run in winter.

amounts to 536.8 μg carbon or 58% of the carbon they were offered. In addition, microalgae were still abundant (M. Hatlebakk pers. observation) when water was renewed. We are therefore confident that the copepods grazing rates were not limited by the experimental set up.

4.3. Utilization of ingested microalgae

The ^{13}C labelling of the *P. glacialis* algae culture in the feeding experiment was poor. It was detectable only in the measurements from day 11 of the experiment at a maximum enrichment of 1.3 AT.% (20:5(n-3)) which is low compared to similar experiments, which reached an average enrichment of 15.3 AT.% (Boissonnot et al., 2016) and 37 AT.% (Graeve et al., 2005). Reasons for this is not known, but something may have happened with the storage and shipment of the lipid samples since the FA composition of the microalgae samples showed various signs of being degraded with its high proportions of 18:0 (up to 60%) and poor PUFAs (Dalsgaard et al., 2003) despite that the Chl *a* concentrations measured were high (4.1–9.5 μg Chl *a* mL^{-1}; Supplementary Table S1). Despite the apparently poor labelling of the microalgae, we were able to follow an increase in the ^{13}C enrichment in copepods' FA with time. The essential PUFA 20:5 (n-3) is selectively retained by the copepods (Graeve et al., 2005), and a ^{13}C enrichment of this important PUFA was detected with time both in the females and the males by the end of the feeding experiment. The females appear to have been quicker to assimilate the microalgae dietary lipids and showed a higher enrichment in 20:5 (n-3) on day 21 than the males. The reason for this slow or delayed assimilation of the algal food most likely corresponds to the time it takes for the copepods to respond to the change in food conditions and up-adjust their digestive enzyme activity as shown by Freese (2015). As mentioned above, the females and males were most likely not fully awake from the winter dormancy at the start of the feeding experiment, meaning that the metabolism was still reduced. Freese (2015) studied the regulation of digestive enzyme activity in CV of *C. glacialis* under different light and food conditions in autumn, and even if the copepods were offered surplus algal food it took minimum 10 days before there was a significant increase in digestive enzyme activity. This delay was explained by the copepods already being in diapause when captured in field and that they needed time to mobilize again. Though it took some time before the copepods in our feeding experiment incorporated dietary ^{13}C into their lipids, they did appear to sustain their basic metabolic needs during the experiment since no reduction in TL content was observed in either females or males from day 1 to day 21. Based on the lipid consumption rates estimated for males from the starvation experiment (Sections 3.1 and 4.1), the males should have spent approximately ~65 μg of their lipid reserves over the course of the feeding experiment (21 days), almost half of the measured TL at day 0. Looking at the carbon budget (Section 4.2) we see that the copepods were offered more carbon than they spent and the stable TL content over the duration of the feeding experiment thus reflects that they were able to sustain themselves on the food offered. Even if the TL content of the females kept stable, the amount of FAlc decreased over the course of the experiment, from 45.1% to 37.3%, indicating a 16% reduction in the amount of WE of TL. WE are the primary long-term storage lipids of copepods and these are used extensively by the females during gonad maturation, even if they are actively feeding (Hirche and Kattner, 1993). For female *C. glacialis* the WE content was reduced by 20% when fed and 31% when starved, with the steepest WE decline seen during gonad maturation and not during egg production (Hirche and Kattner, 1993). In our study several of the females had well developed gonads (Hatlebakk, pers. obs.) at the end of the feeding experiment in February, which is rather early, but access to food had probably sped up the gonad maturation (Rey-Rassat et al., 2002).

The twice as high respiration rates in males versus females in winter (Daase et al., 2018) confirms that males are the most active part in mating, spending energy searching for the females (Kiørboe, 2008). Higher swimming activity results in higher risk of predator encounter, but also higher chance of encountering food particles. From the feeding experiment we observed that females had a higher feeding efficiency than males, which made us investigate potential sexual dimorphism in the mouth parts as part of the explanation. Though it was not originally planned in this study, we were able to investigate the mouthparts of a few specimens collected at the same time and site as those used in the feeding experiment. When we studied the mouth parts of males and females the setae on the maxillipeds of males were slightly shorter than those for the females, a trait that has been connected to omnivorous copepods (Schnack, 1989). In addition, Itoh's edge index categorized the males as omnivores, while it for females suggested females to be less omnivorous, on the borderline herbivores-omnivore (Itoh, 1970). Itoh's index is considered a simple indicator of diet (Giesecke and González, 2004). Nevertheless, it is interesting that the Arctic *Calanus* males may be better equipped for an omnivorous diet than the females. Though access to microalgae is poor during the polar night, the waters are not void of food particles for filter feeders like *Calanus*. The winter protist community in Svalbard are typically made up of *Gymnodinium* (Dinophyceae) and unidentified nanoflagellates, as well as a clear presence of Bacillariophyceae cells, most likely introduced from the sediments through strong vertical mixing. Even if the overall biomass (0.001–0.1 g C m^{-2}) in winter is low (Kubiszyn et al., 2017), there is a presence of organisms that could potentially be consumed by e.g. *C. glacialis* males.

4.4. Aging - another potential cause of death?

Kirkwood (2002) identifies extrinsic mortality as the principal driver for length of life, meaning that if external factors greatly limit the life expectancy, there will be no selection for maintaining costly body processes to counter act aging. Male *C. glacialis* are dependent of stored resources since they are only present in winter, and they expose themselves to higher risk of predator encounter by actively searching for females (van Duren and Videler, 1996), factors that contribute to high extrinsic mortality. Other male copepods, e.g. *Paraeuchaeta norvegica*, which primarily utilize stored resources, have been shown to only produce a limited amount of high quality spermatophores (Hopkins, 1978; Burris and Dam, 2015). These males do not benefit from a longer life, but are better served by quick investment in reproduction (Bonduriansky et al., 2008; Ceballos and Kiørboe, 2011). This suggest that even though the males have not depleted their lipid storage, important FA, such as essential PUFAs (e.g. Docosahexaenoic acid (DHA) or Eicosapentaenoic acid (EPA)), could have been limited because they have been prioritized for sperm production and thus hindered the copepod from maintaining bodily functions. DHA and EPA are vital components of the cell membrane and play various roles in mediating and controlling several physiological processes (Sargent and Falk-Petersen, 1988; Ahlgren et al., 2009). Unfortunately, the FA composition of the males at day 0 and time of death was not analysed in

the starvation experiment so further studies are needed to investigate this hypothesis closer.

4.5. Concluding remark

Depletion of the energy storage, the oil sac, was not the main cause for the short life span of *C. glacialis* males. Further, males were also found to be capable to feed, although less efficiently on microalgae than females, which may be due to males being more omnivorous combined with slower digestive enzyme recovery than females. Main reasons for males short life span is thus not resolved and we recommend more research on the role of essential FA and aging as determining factors for males' life span.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.151226.

Acknowledgement

This study is funded by the Norwegian Research council, Norway through the projects CLEOPATRA II (216537), IMOS (246747) and Arctic Field Grant (227438). We are grateful to the captain and crew on board RV Helmer Hanssen for their sampling support, the projects Marine night (226417) and Arctic ABC (244319) for organizing the polar night cruises and to UiT The Arctic university of Norway for the opportunity to participate. This study is a contribution to the ARCTOS research network (http://arctos.uit.no).

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Paper IV

Hatlebakk M, Niehoff B, Eide H, Daase M, Choquet M, Wold A, Hoarau G, Søreide JE.

Year-round metabolic and digestive activity in *Calanus glacialis* and *C. finmarchicus* in a warm Arctic climate

Manuscript in preparation

Seasonal population dynamics and enzyme activity of co-occurring *Calanus glacialis* and *C. finmarchicus* in the high-Arctic

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Abstract

The mesozooplankton biomass of Arctic shelf seas is dominated by the copepod *Calanus glacialis*. Its North Atlantic sibling *Calanus finmarchicus* is expanding northwards, but it is uncertain whether *C. finmarchicus* will compete and finally replace *C. glacialis.* In a comprehensive monthly study, we studied population structures and physiologically adaptations of co-occurring *C. glacialis* and *C. finmarchicus* in high-Arctic off-shore and fjord environments. Off-shelf north of Svalbard and in Isfjorden, *C. glacialis* mainly overwintered as CIV (74 %) and *C. finmarchicus* as CV (65 %), confirming a primarily 2 and 1 year life cycle, respectively. Adult males and females of *C. glacialis* appeared as early as December-January, up to two months earlier than in *C. finmarchicus*, with a corresponding 1 month earlier peak in recruitment for *C. glacialis.* The seasonal regulation of anabolic and catabolic enzyme activities were overall similar for the two species, but with some ontogenetic species specific differences. Wake-up from overwintering and reproduction started earlier in adults of *C. glacialis* than *C. finmarchicus,* and onset of dormancy earlier for the overwintering stages of both species. Furthermore, *C. glacialis* was found to be more specialized in efficiently building up lipids, as reflected by an earlier and higher mobilization of lipase enzyme activities. Enzyme activities are normally assumed to be very temperature dependent. Comparisons of seasonal HOAD enzyme activities which reflect catabolism of internal lipid resources indicated, however, that increased temperature during overwintering had no direct negative impacts and was comparable between the two species, although large individual differences were found which most likely are a result of large individual variability.

In contrast to *C. glacialis, C. finmarchicus* timed its reproduction closer to the phytoplankton bloom and continued to reproduce throughout the summer. Population structures revealed that primarily the early-borned *C. finmarchicus* managed to develop to CV in time for successful overwintering while those that are born later in the season may fail to reach overwintering stage. Thus, *C. finmarchicus* is likely dependent on continuous supply of advected specimens to keep sustain high abundances far North. Alternating between 1 and 2 year life cycle, early reproduction and more efficiently lipid anabolism gives *C. glacialis* an advantage over *C. finmarchicus* in high-Arctic unpredictable environments with short-pulsed primary production regimes. As long as the season for primary production remain short and the temperatures do not exceed what *C. glacialis* thrives in, *C. glacialis* will most likely remain the best adapted and most numerous species in high Arctic shelf seas.

Keywords: Calanoid copepods, Svalbard, reproduction, metabolism, enzyme activities

Introduction

Calanoid copepods of the genus *Calanus* dominate the mesozooplankton community in Arctic and sub-Arctic seas in terms of biomass (>60%) (Jaschnov 1970; Blachowiak-Samolyk et al., 2008; Kosobokova et al., 2011) and constitute the key link between primary producers and higher trophic levels (Falk-Petersen et al., 2007). *Calanus* are filter feeders, primarily feeding on microscopic algae, but do also consume alternative preys such as eggs, fungi and heterotrophic microorganisms outside the peak productive spring and summer season (Cleary et al., 2017; Frank-Gopolos et al. 2017). The conversion of their food to energy-rich lipids is one major reason for the rapid and efficient transfer of energy and essential fatty acids from primary producers to higher trophic levels in Arctic marine ecosystems (Dahl et al., 2003, Falk-Petersen et al., 1990). For these relatively large calanoid copepods, lipids may comprise up to 70 % of their dry weight (Sargent and Falk-Petersen, 1988, Lee et al., 2006), which they acquire during short periods of primary production lasting from two to eight weeks at high latitudes (reviewed by Leu et al., 2015). The shorter the season for primary production, the more challenging it is for these primarily herbivorous copepods (Falk-Petersen et al., 2009; Banas et al., 2016. In the most extreme environment – the deep Arctic Ocean – these copepods must be capable of surviving up to 10-11 months without food. There the lack of sun during the 4 month polar night as well as the ice cover may efficiently block the incoming solar light for most of the year. To survive these long periods of food scarcity, *Calanus* spp. perform seasonal vertical migrations, descending to depth and reducing their metabolism to a minimum when winter approaches, a physiological state of hibernation called diapause that is often also referred to as over-wintering (Hirche, 1996). During this period, *Calanus* spp. sustain themselves using their lipid reserves; and the larger and more lipid-rich the copepods are the longer periods they can withstand the lack of food (Falk-Petersen et al., 2009). Furthermore, lipid content in *Calanus* is positively correlated to body size (Vogedes et al., 2010, Renaud et al., 2018) and thus lipid content, generally increase with latitude (Falk-Petersen et al., 2009; Renaud et al., 2018).

Three species of the genus *Calanus* co-occur in the European Arctic: the two Arctic species *Calanus hyperboreus* and *C. glacialis*, and the North Atlantic *C. finmarchicus* (Conover, 1988, Choquet et al., 2017). These three species are morphologically similar but have evolved slightly different life strategies fitting the environment of their core distribution area, which differs among the three species. The large Arctic deep-water species *C. hyperboreus* is associated with the most extreme environment. Its life cycle varies from 2 to 4 years and it reproduces in winter using internal resources only (i.e. capital breeding) (Hirche and Niehoff, 1996; Varpe et al., 2009). The smaller Arctic shelf species *C. glacialis* has a 1-2 year life cycle and performs both capital and income breeding, i.e. fueling egg production by ingesting food (Hirche and Kattner, 1993). The smallest of the three species, the boreal *C. finmarchicus*, has a 1 year life cycle at its northernmost distribution and is considered to be a primarily income breeder (Plourde and Runge, 1993; Kjellerup et al., 2012). All three species develop through 6 naupliar (N1-N6) stages, followed by 5 copepodite stages (CI-CV) before they reach

adulthood (CVI) (e.g. Kosobokova, 1999). Adult males of *Calanus* have a relatively short life span compared to females (Hatlebakk et al., in press), thus the timing of their presence, and consequently mating, is an important aspect in the *Calanus* life cycle (Daase et al., 2018). Because they need a certain amount of lipids and thus body size to successfully overwinter, they have to reach a certain development stage before they are ready to overwinter (Falk-Petersen et al., 2009). Consequently, the large *C. hyperboreus* can overwinter already as CIII, *C. glacialis* as CIV and *C. finmarchicus* primarily as CV (Falk-Petersen et al., 2009).

Of these three species, *C. finmarchicus* and *C. glacialis* are regarded as important climate beacons in the Svalbard-Barents Sea region (Wassmann et al., 2015). High proportion of *C. finmarchicus* over *C. glacialis* reflects a high influence of Atlantic water and a relatively warm Arctic climate while the opposite indicates a colder Arctic climate. A substantial borealization of the Arctic zooplankton community is predicted with global warming (Hays et al., 2005), and in the surrounding path of the North Atlantic Current into the Fram Strait and the Barents Sea an increase in abundances of the boreal *C. finmarchicus* and other North Atlantic species has already been recorded (Hop et al., 2019, Aarflot et al., 2017, Wassmann et al., 2006). Because of their morphological similarity, *C. finmarchicus* and *C. glacialis* have often been distinguished from one another using differences in body sizes (e.g. Daase and Eiane 2007). However, recent molecular analyses revealed a significant underestimating of *C. glacialis* when using body size for species identification (Gabrielsen et al., 2012, Choquet et al., 2017, Choquet et al., 2018), challenging their use as valid climate indicator species. Studies on individual level revealed that *C. glacialis* may not grow larger than *C. finmarchicus* if it completes its life cycle within 1 year (Renaud et al., 2018). A change in species phenology to shorter life cycles and thus overall smaller body sizes as the climate gets warmer and the open water productive season becomes longer, resulting in higher turnover rates, may actually also compensate for less lipids per unit mass (Renaud et al., 2018). To better predict consequences of the rapid warming on species fate in the Arctic, better knowledge on species physiological adaptations to the strong seasonal Arctic environment is needed. Baseline information on the natural seasonal variability in metabolism is important to identify physiological responses directly linked to the ongoing climate change (e.g. Møller et al., 2016).

Diapause is controlled endogenously and is a physiological reaction to reoccurring adverse environmental conditions such as low food availability in winter (Dahms, 1995). Diapause is commonly performed by specific ontogenetic stages in crustaceans (Guppy and Withers, 1999), which for *Calanus* spp. are their respective overwintering stages (see above). Five phases of diapause have been described in copepods: 1) preparatory phase where the organisms accumulate lipids (energy reserves) and arrest further development and growth, 2) the induction phase where the organisms stop feeding and reduce their metabolism, 3) the refractory phase where the organisms are torpid and reach minimum metabolic activity, 4) the activation phase when organisms regain their ability to develop, and, finally 5) the termination phase when the organisms reach full metabolic activity again (Hirche, 1996).

Depending on environment, latitude and season, dormancy strategies and ontogenetic migrations may vary profoundly among species and even within populations of the same species (Hirche, 1996, Hirche, 1998, Darnis and Fortier, 2014).

The seasonal changes in metabolic activity can be determined by measuring the relative activity-level of enzymes taking part in central metabolic pathways (e.g. Freese et al., 2017). For instance, citrate synthase (CS) and malate dehydrogenase (MDH) which catalyze different reactions in the citric acid cycle are regarded as good indicators of the aerobic potential of an organism and thus its overall metabolism (Torres and Somero, 1988, Meyer et al., 2002, Teschke et al., 2007). MDH is strongly correlated with respiration (Meyer et al., 2010), but the advantage of measuring MDH rather than respiration by incubations is the minimum laboratory handling effect (Ohman et al., 1998). CS on the other hand has also been found to correlate with egg production (Kreibich et al., 2008). Catabolism of stored lipids, which occurs mainly Januray-March for *C. glacialis* (Freese et al., 2017), is positively correlated to actvity of 3-hydroxyacyl-CoA dehydrogenase (HOAD), a key enzyme of the β-oxidation of fatty acids (Auerswald and Gäde, 1999, Hassett, 2006). Further, the HOAD activity is inversely related to digestive enzyme activities (Freese et al., 2017). The major components of the copepods diet are proteins and lipids, which are processed by proteinase and lipase/esterase, respectively. They are both groups of several enzymes with variations in the specific mechanisms, but in general, proteinases break down proteins by hydrolyzing the peptide bonds between the amino acids (Mayzaud, 1986), and lipases/esterases break down lipids by cleaving the ester bonds of carboxylic acids (Luppa and Andrä, 1983). Peak digestive enzyme activities in *C. glacialis* are found when algal food is plentiful for so to gradually decrease throughout the autumn-winter (Freese et al., 2017). Overall the metabolism of *C. glacialis* is reduced to half in winter, which suggest that this species is not going into a proper diapause as defined by Hirche (1996) (Freese et al., 2017). *C. glacialis* is considered a shelf species with shallower overwintering depth than the oceanic species *C. finmarchicus* and *C. hyperboreus* which have been observed to be torpid in winter (Hirche, 1983, Auel et al., 2003), suggesting that the dormancy in the latter two are more intense. However, respiration rates of all three species in winter have been found to be reduced by a similar amount (40–65%; *C. finmarchicus/C. helgolandicus*: Hirche, (1983); *C. glacialis*: Morata and Søreide (2015); *C. hyperboreus*: (Conover and Corner, 1968; Auel et al., 2003) and do not suggest large species differences in the overwintering metabolic states. How *Calanus* regulate their physiology to best schedule their complex life history to the strong seasonality at high latitudes is poorly understood. In this study we were particularly interested in the timing of physiological changes in co-occurring *C. glacialis* and *C. finmarchicus*, and how such changes related to life history and the environment. Possible species specific differences may provide new insights into why *C. glacialis* is specially adapted to the Arctic while *C. finmarchicus* thrives better under more temperate North Atlantic conditions.

We studied the monthly population development of co-existing *C. glacialis* and *C. finmarchicus* and their corresponding physiology in terms of general metabolism (CS and

MDH), digestion of incoming food (proteinase and lipase) and utilization of stored lipid resources (HOAD). Samples were taken over a 15 months period in Isfjorden, a high-Arctic (78°N) fjord in Svalbard with relatively warm sea temperatures (>1°C) and no seasonal ice. For comparison, and to prevent misinterpretation of results due to specific local fjord adaptations, we also collected samples off-shelf north of Svalbard. We were particularly interested in the following questions: (1) Do the population structure and timing of the seasonal migration and reproduction differ between species and between off-shelf and fjord populations? (2) Does the general metabolism follow the same seasonal variability in the two *Calanus* species and in off-shelf vs fjord populations? (3) Do they utilize the short, but intensive spring bloom with the same efficiency? And finally (4) do *C. glacialis* and *C. finmarchicus* utilize stored lipids to the same extent?

Material and Methods

Sampling area and sample processing

Calanus glacialis and *C. finmarchicus* were collected monthly from June 2015 to August 2016 at Karlskronadjupet (78°19'N; 015°10'E) (IsK Fig. 1), a 274 m deep basin in the central part of the Isfjorden system, Svalbard. The mouth of Isfjorden is open towards the shelf and slope area along west Spitsbergen, with no sill limiting the inflow of Atlantic and Arctic water reaching this area via the West Spitsbergen Current (WSC) and the East Spitsbergen Current, respectively. Both these currents are flowing from south to north along the shelf break and shelf respectively (Nilsen et al., 2008). In addition, samples were collected off-shelf north of Svalbard (Fig. 1, supplementary table S1). The main sampling period off-shelf was from February to June during the N-ICE 2015 campaign when RV Lance was frozen into the ice. During the drift, RV Lance passed over the Yermak plateau, an area influenced by the north flowing WSC current (Meyer et al., 2017). Additional off-shelf sampling north of Svalbard was conducted in late summer 2015 and 2016, and January 2016.

Sampling was conducted from larger vessels (R/V Helmer Hanssen, K/V Svalbard, R/V Dalnie Zelentsy, R/V Lance) and smaller boats (R/V Viking Explorer and UNIS Polaris). Data on the properties of the water column were collected using a SAIV SD204 CTD or a SBE Seabird Electronics CTD; and identification of water masses were based on Cottier et al. (2005) for the fjord station, and on Meyer et al. (2017) and Rudels et al. (2000) off-shelf north of Svalbard. Fluorescence data from fluoremeters attched to the CTDs were used to estimate the average Chlorophyll a concentration in the depth intervals zooplankton was sampled (see below). Comparisons of flurorscense data with Chl *a* concentrations, using methanol as an extraction solvent, measured fluorometrically with an AU10 Turner Fluorometer (Turner Design, Inc.) were comparable and captured well the seasonality in Chl *a* concentrations as described in Eide (2016) for Isfjorden and in Assmy et al. (2017) for off-shelf north of Svalbard.

Stratified zooplankton samples for *Calanus* community composition were collected with a Multi Plankton Sampler (MPS, 0.25 m^2 mouth opening, mesh size 200 µm, Hydro Bios) or a WP2 closing net (200 µm, Hydro Bios) and fixed in 4 % buffered formaldehyde sea water solution. Samples were collected at the depth intervals from bottom-200-100-50-20-0 m or from bottom-600-200-50-20-0m if bottom depth was deeper than 600m when using the MPS and bottom-100-50-20-0 when using the WP2 net (Supplementary, table S1). Additional samples were collected from the layers where the majority of the population resided and sorted for enzyme analyses. Females for egg incubation were collected from 50-0 meters using a WP3 net (1000 µm, Hydrobios). When sampling from bigger vessels, sorting was conducted on board immediately after sampling. When this was not possible, sorting was done immediately at return to the University Centre in Svalbard (UNIS) within 12 hours of sampling. In either case, sorting was conducted in a temperature controlled room at 2-4 °C to avoid temperature stress on the copepods. The dominating stages of *C. glacialis* and *C. finmarchicus* were sorted in triplicates à 10 and 15 for both species (supplementary table S1). Individuals were quickly rinsed in distilled water, blotted briefly by touching them to a tissue paper and transferred to cryo vials before they were snap frozen in liquid nitrogen. Samples were stored at -80°C and transported to Alfred Wegener Institute, Bremerhaven, Germany either in dry shipper or on dry ice for further analyses.

Morphological identification of *Calanus* spp.

The different species of *Calanus* were distinguished by prosome length according to Daase and Eiane (2007) with adjustments for CV and adult females after Gabrielsen et al. (2012) as presented in Daase et al. (2018). From copepodite stage CIV, *C. hyperboreus* was also distinguished by the presence of an acute spine on the fifth thoracic segment.

On live samples, pigmentation can be used as an additional indicator of species where *C. glacialis* tend to have more red pigmentation on the antennules and genital segment of the urosome (females only) compared to *C. finmarchicus* (Nielsen et al., 2014, Choquet et al., 2018).

Calanus population

Abundance was analyzed from the formalin preserved samples. The samples were drained, rinsed in filtered sea water and the soaked in filtered sea water to wash out the formalin, prior to transferring the sample to a beaker and dilute it with filtered seawater to a known volume. Subsamples of 2-5 ml were taken using a macropipette with enlarged opening and analyzed under a stereo microscope. All *Calanus* spp. in the subsample were enumerated and identified morphologically to species and development stage. Subsamples were taken until a minimum of 200 *Calanus* individuals was reached.

Weighted mean depth (WMD) was calculated for total population of C. *glacialis* and *C. finmarchicus* as well as copepodite stages within the species according to the following equation

$$\frac{\sum_{i=1}^{n}(a_i d_i)D_i}{\sum_{i=1}^{n} a_i d_i}$$

Where a_i is the number of individuals per m^3 of species a in depth stratum i, d_i is the sampled distance in depth stratum i, D is average depth in depth stratum i and n is the number of depth strata at a station.

Egg production

Adult females of *C. glacialis* (n = 30) and *C. finmarchicus* (n = 30) were sorted from the WP3 sample collected from 50 to 0 m and incubated individually. The incubation chambers were 125 mL and equipped with a false bottom of mesh size 300-500 µm that allowed the eggs to sink through, separating them from the female to avoid predation (Basedow and Tande, 2006). Incubation was started within four hours of sampling and lasted for 24 hours at *in situ* temperatures. After the 24 hours the eggs were transferred to a petri dish and counted under a stereo microscope in a cold lab (4-6 °C).

Analyses of enzyme activity

Analyses of enzyme activity were done at Alfred Wegener Institute in Bremerhaven, Germany. Activities were measured for both *C. glacialis* and *C. finmarchicus* in triplicates for two digestive enzymes: Proteinase and Lipase/Esterase, and three metabolic enzymes: Citrate synthase (CS), Malate Dehydrogenase (MDH) and 3-Hydroxyacyl-CoA dehydrogenase (HOAD). Analyses of proteinase, lipase/esterase and CS activity were done from the same extract of 10 individuals, MDH activity were analyzed from extract of 2 individuals and HOAD activity were analyzed from extract of 3 individuals. Individuals for MDH and HOAD were collected from the triplicates à 15 individuals, and the remaining 10 in each vial was kept for back up.

Metabolic enzymes

Citrate synthase (CS)

Citrate synthase activity (EC 4.1.3.7.) was measured after Stitt (1984) and Saborowski et al. (2002), but with a different buffer system. In a semi-microcuvette, 20 µL 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, D8130), 20 µL Acetyl-CoA (Acetyl-Coenzyme A trilithium salt, Roche Diagnostics, 13893324), 20 µL sample, or buffer for controls, and 520 µL 0.1 M Tris/HCl (supplemented with 10mM $CaCl_2$) buffer at pH 7.0 was mixed together. The mixture was pre-incubated in the spectrophotometer (Thermo Scientific, UV1), kept at 25 °C using a Peltier element (Krüss Optronic), for 5 minutes before 20 µL Oxaloacetic acid

(Sigma-Aldrich, O4126) was added to start the reaction. The absorbance was measured regularly in the spectrophotometer for 3 minutes at 405 nm, and the measurements were recorded with the software VisionLite (version 2.2).

Malate dehydrogenase (MDH)

Measurements of the Malate dehydrogenase activity (EC 1.1.1.37) was modified after Teschke et al. (2007) as described in Freese et al. (2017). The frozen copepod samples were transferred to 1.5 ml eppendorf tubes in triplicates á 2 individuals and homogenized by hand with a micropestle in 40 µl 0.1 M potassium phosphate buffer at pH 7.0. The Homogenates were centrifuged at 15 000 *g* and 4 °C for 15 minutes and the liquid face transferred to a new 1.5 ml eppendorf tube. The sample was diluted 1:10 by adding 6 µl of the sample to a vial with 54 µl of buffer. Measurements of enzyme activity were done in a 96 well plate. 180 µl buffer, 6.7 µl NADH (Roche Diagnostics 10107735001) and 6.7 µl sample was mixed together and incubate at 25 °C for 5 minutes. The reaction was started by adding 6.7 µl Oxaloacetic acid and the absorbance was measured for 5 minutes at 25 °C and 340 nm in a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev.21. The slope of the curve from 1-4 minutes was used to calculate the enzyme activity.

3-Hydroxyacyl-CoA dehydrogenase (HOAD)

Measurements of 3-Hydroxyacyl-CoA dehydrogenase activity (EC 1.1.1.35) was modified after Auerswald and Gäde (1999) as described in Freese et al. (2017). The frozen copepod samples were transferred to 1.5 ml Micro centrifuge tubes in triplicates á 3 individuals and homogenized by hand with a micropestle in 180 µl 107 mM triethanolamine/HCl buffer (supplemented with 5.3 mM EDTA) at pH 7.0.The Homogenates were centrifuged at 15 000 *g* and 4 °C for 15 minutes and the liquid face transferred to a new 1.5 ml eppendorf tube. Measurements of enzyme activity were done in a 96 well plate. 180 µl buffer, 6.7 µl NADH (Roche Diagnostics 10107735001) and 6.7 µl sample was mixed together and incubate at 25 °C for 5 minutes. The reaction was started by adding 6.7 µl Acetoacetyl-CoA (Sigma A-1625) and the absorbance was measured for 8 minutes at 25 °C and 340 nm in a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev.21. The slope of the curve from 3-7 minutes was used to calculate the enzyme.

Digestive enzymes

The frozen copepod samples were transferred to 1.5 ml eppendorf tubes in triplicates á 10 individuals and homogenized by hand with a micropestle in 200 µl 0.1 M Tris/HCl (supplemented with 10mM $CaCl_2$) buffer at pH 7.0. The Homogenates were centrifuged at 15 000 *g* and 4 °C for 15 minutes. The liquid face was transferred to a new 1.5 ml eppendorf tube and the remaining tissue was stored at -80 °C for later molecular verification of species.

Proteinase activity

Total proteinase activity (EC 3.4.21-24) was measured after Saborowski et al. (2004), modified after Kreibich et al. (2008). 20 µL of sample, or buffer for controls, was pipetted

into 1.5 ml eppendorf tubes and pre-incubated at 30 °C for 5 minutes on a thermo shaker at 400 rpm. 5 µl azocasein (1 % in deionized water, Fluka BioChemika, 11615) was added to each vial before incubation for another 60 minutes. The reaction was stopped by adding 50 µl trichloroacetic acid (TCA, 8 %) and the vials were centrifuged at 15 000 *g* and 4 °C for 15 minutes. The supernatant was transferred to an ultra-microcuvette (Hellma 105.203-QS). The optical density of the supernatant was measured with a spectrophotometer (Thermo Scientific, UV1) at 366 nm (dE_{366}) and recorded with the software VisionLite (version 2.2).

Lipase/esterase activity

Lipase/esterase activity (EC 3.1.1.) was measured after Knotz et al. (2006). 20 µl sample, or buffer for controls, was diluted in 470 µL 0.1 M Tris/HCl (supplemented with 10mM $CaCl_2$) buffer at pH 7.0. 10 µl 4-methylumbelliferyl butyrate dissolved in dimethyl sulfoxide (5 mmol L^{-1}, MUF-butyrate, Fluka BioChemica, 19362; DMSO, AppliChem A3608) was added to each vial and incubated in the dark at 25 °C for 30 minutes on a thermo shaker at 400 rpm. Fluorescence was measured on a NanoDrop 3300 at 360 nm (excitation) and 450 nm (emission) and recorded with the software ND-3300 V 2.7.0.

Molecular identification of species

Because of the uncertainty of differentiating between *C. glacialis* and *C. finmarchicus* based on morphology (Choquet et al., 2018), the remaining pellet of tissue after the first batch of enzyme extraction was kept and stored at -80 °C for molecular species identification. The pellets were transported in a dry shipper to Nord University in Bodø, Norway where the analyses were conducted. The remaining tissue pellets from the second batch of analyses were also kept, but currently not analyzed, thus 48 samples are still awaiting ID.

Pooled species composition was genetically assessed by analysis of species-specific profiles of polymorphism in 6 nuclear molecular markers characterized by motifs of insertion or deletion (i.e. InDels) (Smolina et al., 2014), following Choquet et al. (2017). In short, DNA was extracted from the remaining pellet of *Calanus* tissue, following the HotSHOT DNA extraction method (Montero-Pau et al., 2008). Six InDel markers were amplified by Polymerase Chain Reaction (PCR). The resulting PCR amplicons were scored on a 3500xL Genetic Analyzer (Applied Biosystems), generating either a “pure” species-specific profile characteristic of *Calanus finmarchicus* or *C. glacialis* (see Smolina et al., 2014) in case of only one species present within a pool, or a profile containing mixed signals from both species in case of the two species being present within a pool.

Dry mass and CN content

For each sampling, 24 individuals of both *C. glacialis* and *C. finmarchicus* were collected for dry weight and CN analyses. The animals were rinsed quickly in distilled water and before being placed individually in pre-weighed tin capsules (IVA analysentechnik, SA76981102) and dried at 60 °C for 24 hours. Dry mass (DM) was determined by weighing the tin capsules and subtracting the pre-weight. After weighing, the tin capsules were packed tight and analyzed

for carbon and nitrogen content with an element analyzer (vario EL cube, Elementar) and the accompanying software.

Statistical analysis

Statistical analyses were done in Sigmaplot (14.0, Systat Software, San Jose, CA). T-test was applied to investigating potential difference in enzyme activity between either species, stages or dates. Correlations between changes in enzyme activities and different physical (temperature, light) and biological (chl *a*, WMD) environmental factors, as well as among enzyme activities, were done with Pearson correlations when linear relationships and normal distributed data occurred, and Spearman's rank-order correlation when not. Significance level was set to α=0.05 in all tests.

Results

ENVIRONMENTAL CONDITIONS

Sea ice and Hydrography

In the consolidated pack ice north of Svalbard, during N-ICE, the hydrography was characterized by a cold relatively fresh and deep mixed surface layer of Polar Surface Water down to 100 m and warmer and more saline Atlantic Water and Modified Atlantic Water were located between 100-500 m (Meyer et al., 2017). Hydrographic conditions changed significantly after 25th May, when the Atlantic Water was found closer to the surface and the mixed surface layer were thinner, fresher and warmer (Fig. 2a). Additional samples north of Svalbard were collected in August 2015 and September 2016, as well as January 2016 (Fig. 1). At these additional locations similar water mass composition were seen with an upper Polar Surface Water (~100 m) and warmer and more saline Atlantic water/Modified Atlantic water below.

In Isfjorden proper (Stn. IsK, Fig. 1), local water prevailed in June and July 2015 and was then replaced by warm transformed Atlantic water in August 2015, with a strong influx of Atlantic water in December 2015 seen as a distinct + 3°C increase in temperature at around 100 m depth (Fig. 2b). This warm (1-3 °C) transformed Atlantic water which prevailed in the entire water column (~300 m), persisted until the end of the sampling campaign in August 2016. Sub-zeros temperatures were only reached in the upper 20 m for a brief period of extensive surface cooling in March 2016, whereas maximum temperatures of 6 to 7 °C) were only found in the upper 10-20 m in the summer (July-August).

Chlorophyll *a*

Off-shelf, north of Svalbard low winter algal biomasses (< 0.01 µg Chl *a* L^{-1}) prevailed until mid-May in 2015 during the N-ICE drift campaign (Fig. 3a). Peak phytoplankton bloom concentrations (8 µg Chl *a* L^{-1}) were recorded in early June and remained relatively high (4.7-6.3 µg L^{-1}) until late June when the last measurement was done (2.4 µg L^{-1}).

North of Svalbard in September 2015 and August 2016 the chlorophyll a concentrations were elevated in the upper 20 m with respective 1.4 µg Chl *a* L^{-1} (15 m) and 1.3 µg Chl *a* L^{-1} (14 m). In January 2016, no fluorescence was detectable.

In Isfjorden, the spring bloom had commenced (1.5 µg Chl *a* L^{-1}) at the start of the sampling campaign in June 2015, but relatively high Chl *a* concentrations persisted throughout July and August (2.2 and 2.4 µg L^{-1}, respectively) (Fig. 3b). From October 2015 to April 2016 typically low winter Chl *a* values (< 0.01 µg Chl *a* L^{-1}) were found with an increase first recorded in early May (2.1 µg Chl *a* L^{-1}), and peak Chl *a* values between mid-May to early June (>7.0 µg Chl *a* L^{-1}). In July, similarly high Chl *a* values were found (2.1 µg Chl *a* L^{-1}) as previous summer for so to drop in late August (0.73 µg Chl *a* L^{-1}) (Fig. 3b).

CALANUS POPULATION DYNAMICS

Both off-shelf north of Svalbard and in Isfjorden the proportions of *C. glacialis* and *C. finmarchicus* in the populations varied, but with an overall dominance of *C. finmarchicus* north of Svalbard and an overall dominance of *C. glacialis* in Isfjorden (Fig. 4). North of Svalbard, *C. finmarchicus* was 0.6 to 18.6 times more abundant than *C. glacialis* (Fig. 4a) while in Isfjorden *C. glacialis* was 0.8 to 9.0 times more abundant than *C. finmarchicus* (Fig. 4b). *Calanus finmarchicus* abundance was similar north of Svalbard (0.5 - 76.8 $\times 10^3$ ind. m^2) and in Isfjorden (3.9 - 53.9 $\times 10^3$ ind m^2), while *C. glacialis* was much more numerous in Isfjorden (5.5 – 340.4 $\times 10^3$ ind. m^{-2}) than off-shelf north of Svalbard (0.4 – 16.24 $\times 10^3$ ind m^{-2}). For both species, however, large seasonal variability in abundance were found with particularly low abundances during the winter-spring transition from March to May, followed by peak populations numbers in summer and autumn (Fig. 4). However, for the two sampling dates in March 2015 off-shelf, it was not possible to sample deeper than 1000 m. Deep dwelling copepods could have been missed on these dates and the data may not be fully representative of abundance and WMD (Fig. 4).

Both species performed strong seasonal migration with monthly WMD comparable for the two species (Fig. 4). Both species descended to deeper waters during August and remained deep (WMD > 175 m) until they started to ascend a bit further up in the water column (WMD ~ 125 m) in January-March. By the time the spring bloom started in May the populations were concentrated in the upper 50 m (Fig. 4). The overwintering community of *C. glacialis* was largely dominated by CIV (74%), followed by CV (17%). Adult females were always present but became more dominant from December when adult males started to appear (Fig. 5a). Some ontogenetic differences in WMD were found. In January, females and males of *C. glacialis* were primarily found in the upper 100 m (WMD = 51 ± 48 m and 107 ± 65 m, respectively) while *C. glacialis* CIV was still below 100 m (WMD = 148 ± 51 m) (Suppl. Fig. S1c). The abundance of young copepodite stages (CI-CIII) was highest in June and July, and by August the population was located at depth (WMD = 217 ± 40 m) and comprised mainly of the overwintering stages CIV and CV (Fig. 5a).

The overwintering community of *C. finmarchicus* was dominated by CV (65 %), followed by CIV (29 %). For *C. finmarchicus*, adult females and males started to increase in numbers in February, two months later than for *C. glacialis*. Females peaked in abundance in May, and the first CI was also observed at this time. The community was dominated by the young copepodite stages (CI-CIII) in June and July, but by August the overwintering stages CIV (29%) and CV (68 %) dominated the *C. finmarchicus* population (Fig. 5b). In contrast to *C. glacialis*, *C. finmarchicus* was still found higher up and more spread in the water column in late August (WMD = 141 ± 96 m).

Egg production

C. glacialis produced the first eggs in March (1.4 ± 4.4 eggs $female^{-1}$ day^{-1}) and reached peak egg production during the spring bloom in mid-May in terms of per individual (58.9 ± 42.4

eggs female^{-1} day^{-1}) and in terms of total eggs per females in the surface (1213.4 eggs day^{-1}, Fig. 5c). By June the egg production had declined significantly (5.8 ± 9.6 eggs female^{-1} day^{-1}) along with the number of females in the population (Fig. 5).

C. finmarchicus produced the first eggs in early April (0.3 ± 1.1 eggs female^{-1} day^{-1}), with increasing numbers in early May (8.3 ± 14.6 eggs female^{-1} day^{-1}). From May to July the egg production rate was stable at around 20 eggs female^{-1} day^{-1}, for so to decrease in mid-August (7.5 ± 13.5 eggs female^{-1} day^{-1}) and to cease end of August. Due to the abundance of females peaking in June, the total egg production of the females in the surface also had a clear peak then (2211.3 eggs day^{-1}, Fig. 5c).

Success of morphological species identification for enzyme samples

From the 138 enzyme samples, 90 (>65%) were tested for molecular species ID. Of these 90 samples, two samples were inconclusive due to failure to extract DNA and/or amplify the InDel markers. Of the molecularly tested samples, only two of 64 samples from Isfjorden showed a mixture of *C. glacialis* and *C. finmarchicus*, while four out of 26 samples from north of Svalbard showed a *Calanus* species mixture. The remaining samples were pure *C. finmarchicus* or *C. glacialis* samples. This gave an overall correct morphological species identification of 97% for Isfjorden and 85% for the off-shelf stations. The mixed samples were discarded from further analyses, while those enzyme samples not tested were assumed to be correctly identified.

ENZYME ACTIVITIES

Metabolic enzyme activity

The CS and MDH activity followed the same seasonal trend for both species, with high levels in spring and summer and low levels in autumn and winter (Fig. 6). For both enzymes, the activity was low prior to the bloom and increased rapidly for a short period, peaking in activity in mid-June (Fig. 6). When looking at activity per individual, *C. glacialis* females increased the activity prior to the bloom, while *C. finmarchicus* CV increased activity simultaneously with the build-up of the bloom (Supplementary, Fig. S2).

CS enzyme activity levels and the timing of peak MDH potential differed between the two species. *C. finmarchicus* had higher CS activity (2.6 U mg DM^{-1}) in spring-summer than *C. glacialis* (1.6 U mg DM^{-1}), but the CS activity levels decreased to the same low levels (~1 U mg DM^{-1}) during fall and winter for both species (Fig. 6b). For MDH no difference was observed in relative activity between species, except that peak MDH activities were reached one month earlier in *C. glacialis* than in *C. finmarchicus* (Fig. 6a). For both species minimum MDH activity levels were recorded in January (3.7-4.9 U mg DM^{-1}) and maximum MDH levels were recorded in early May (23.3 U mg DM^{-1}) for *C. glacialis* and June (24.4 U mg DM^{-1}) for *C. finmarchicus* (Fig. 6a). The CS and MDH activity for *C. glacialis and C. finmarchicus* in Isfjorden were strongly positively correlated to day length (as well as to Chl *a*) for *C. finmarchicus*, but not for *C. glacialis* (Table 1). For activity per individual the general patterns

were similar, but a clear difference was seen in CS and MDH activity level between CV and adult *C. glacialis* in January. In January, particularly high CS activity was found in males (0.62 U ind^{-1}) which was higher than in females (0.42 U ind^{-1}) (t-test, p=0.001) which in turn was higher than for CV (0.24 U ind^{-1}) (t-test, p<0.001). For MDH, the activity level was similarly high for males (6.56 U ind^{-1}) and females (5.24 U ind^{-1}) in January (t-test, p=0.13), but much lower for CV (1.66 U ind^{-1}), (t-test, p=0.002 and p=0.006, respectively).

Digestive enzyme activity

Strong positive correlations between Chl *a* biomass and digestive enzyme activities were found for both species (Table 1, Fig. 8). In Isfjorden the proteinase activity of *C. finmarchicus* varied between a minimum of 1.59 dE_{366} h^{-1} mg DM^{-1} in October and maximum of 15.36 dE_{366} h^{-1} mg DM^{-1} in early May (Fig. 8a). For *C. glacialis* in Isfjorden, highest proteinase activity was also observed in early May (8.47 dE_{366} h^{-1} mg DM^{-1}), but this was roughly half of the maximum proteinase activity measured for *C. finmarchicus* (t-test, p<0.001). In winter and early spring *C. finmarchicus* had a consistently higher relative proteinase activity than *C. glacialis* (Fig. 8a). Minimum proteinase activity for *C. glacialis* was measured in early November (0.2 dE_{366} h^{-1} mg DM^{-1}), and was nearly 8 times lower than the minimum proteinase activity levels found for *C. finmarchicus* in October (t-test, p=0.01). Looking at activity per individual, the two species follow each other more closely, but the minimum activity for *C. glacialis* (0.03 dE_{366} h^{-1} ind^{-1}) was still lower than for *C. finmarchicus* (0.39 dE_{366} h^{-1} ind^{-1}) (t-test, p=0.01) and peak activity of *C. finmarchicus* (4.13 dE_{366} h^{-1} ind^{-1}) was still higher than for *C. glacialis* (3.36 dE_{366} h^{-1} ind^{-1}) (t-test, p=0.01) (Supplementary, Fig. S4a)

The lipase activity of *C. finmarchicus* was at its minimum in February (28.94 nmol h^{-1} mg DM^{-1}) and maximum in May (280.51 nmol h^{-1} mg DM^{-1}). For *C. glacialis* minimum lipase activity was found in March (33.96 nmol h^{-1} mg DM^{-1}) and maximum in May as for *C. finmarchicus*, but with three times as high maximum lipase activities (927.22 nmol h^{-1} mg DM^{-1}) than recorded for *C. finmarchicus* (t-test, p<0.001) (Fig. 8b). The same lipase patterns were also found when looking at activity per individual, with similar low activity in winter in both species, and much higher peak activity in *C. glacialis* (621.24 dE_{366} h^{-1} ind^{-1}) compared to *C. finmarchicus* (103.79 dE_{366} h^{-1} ind^{-1}) in mid-May (t-test, p<0.001) (Supplementary, Fig. 4b).

Off-shelf, a similar positive correlation as in Isfjorden between Chl *a* max and the activity of both proteinase and lipase was observed for *C. finmarchicus* (Table 1). Off-shelf proteinase activity of *C. finmarchicus* was at a minimum in early March (1.0 dE_{366} h^{-1}mg DM^{-1}) and increased to peak activity on May 31st (10.21 dE_{366} h^{-1} mg DM^{-1}). Lipase activity in off-shelf *C. finmarchicus* had a similar pattern as proteinase, with minimum value measured in early March (189.65 dE_{366} h^{-1} mg DM^{-1}) and peak activities in mid-June (1458.98 dE_{366} h^{-1} mg DM^{-1}) (Fig. 8).

C. glacialis was only sampled pre-bloom, when no variations in digestive enzyme activity were observed. Proteinase remained stable at 1.2-1.6 dE_{366} h^{-1} mg DM^{-1} and lipase activity at 109.3-136.8 nmol h^{-1} mg DM^{-1} (Fig. 8).

Catabolism of body lipids

Peak HOAD activity occurred in March for both *C. glacialis* (1.98 U mg DM^{-1}) and *C. finmarchicus* (1.63 U mg DM^{-1}) with minimum activity in late August for *C. glacialis* (0.02 U mg DM^{-1}) and in mid-September for *C. finmarchicus* (0.08 U mg DM^{-1}) (Fig. 7). For *C. finmarchicus*, HOAD was negatively correlated ($r = -0.57$, $p<0.05$) with Chl *a*, but no such correlation ($r = -0.04$) was found for *C. glacialis* (Table 1). Overall the two species did not seem to differ much in HOAD activity except that *C. glacialis* appeared to have higher activity in February compared to *C. finmarchicus* both in terms of activity per DM (1.24 U mg DM^{-1} and 0.43 U mg DM^{-1}, respectively) and per individual (0.37 U ind^{-1} and 0.12 U ind^{-1}, respectively). Unfortunately only a single data point for *C. glacialis* was available from February so no statistical tests were possible. Interestingly, *C. glacialis* appear to have had higher HOAD activity in late October than *C. finmarchicus* (0.32 U ind^{-1} and 0.16 U ind^{-1}, respectively) (Fig. S3).

Discussion

Life history: similarities and dissimilarities in co-occurring *C. glacialis* and *C. finmarchicus*

Population sizes and structures

Off-shelf north of Svalbard*, C. finmarchicus* dominated over *C. glacialis*. This may be surprising since this area was covered by consolidated ice until May. However, the N-ICE drift campaign was located over the core area of the West Spitsbergen Current (WSC) (Meyer et al., 2017), only overlaid by a 100 m polar halocline surface layer. *C. finmarchicus* is continuously transported north into the study area within the WSC from its source population in the Norwegian Sea (e.g. Wassmann et al., 2015). While the inflow may vary with season due to seasonal changes in vertical distribution, recent observations have shown that the inflow of *C. finmarchicus* is high even in winter, thus the populations are replenished even during the low productive season (Basedow et al., 2018). So far there is little evidence for *C. finmarchicus* being capable of sustaining its own population so far North (Hirche and Kosobokova 2007), and the populations advected into the Arctic are likely doomed (Wassmann et al., 2015). In Isfjorden *C. glacialis* dominated over *C. finmarchicus*, particularly in summer (>90%), suggesting that *C. glacialis* was reproducing more successfully here than *C. finmarchicus*. The population size of *C. glacialis* in Isfjorden was large, even larger than maximum abundances reported from other Arctic fjords in Svalbard, with seasonal ice cover and access to early growing ice algae (Arnkværn et al., 2005; Leu et al., 2011). North of Svalbard, however, the abundance of *C. glacialis* was low and comparable to that found in the Arctic Ocean where it is considered to be an expatriate (Kosobokova et al., 2011). The Arctic Ocean has an even more unpredictable environment than Arctic shelf seas, and normally the onset of the ice algae and phytoplankton bloom is initiated later and of less magnitude since being of shorter duration (Leu et al., 2011; Leu et

al., 2015). The similar population sizes and population structures for *C. finmarchicus* off-shelf north of Svalbard and in Isfjorden support a similar origin (e.g. Wassmann et al., 2015). Recent long-term comparisons of the zooplankton community along the West Spitsbergen slope show that the zooplankton communities South and North are highly similar (Gluchowska et al., 2017). The particularly strong inflow of Atlantic water into Isfjorden the winter 2015-2016 (Fig. 1) was identified to be an open situation meaning that Atlantic water had open access into Isfjorden (Skogseth et al., in review). *Calanus glacialis*, on the other hand, is likely to be of more local origin. The sampling location in Isfjorden was selected since this is the deepest part of the Isfjorden proper and *C. glacialis* is known for seeking out deeper trenches and depressions to overwinter, leading to particularly high concentrations in such areas (Søreide et al., 2003) when the seasonal descent is more or less completed in autumn.

The main overwintering stage for *C. finmarchicus* was CV and for *C. glacialis* CIV, reflecting their predominantly one and two year life cycle respectively. A rather high proportion (~30 %) of *C. finmarchicus* CIV was also found in winter, but this has also been observed for the population in the NE Norwegian Sea (e.g. Arashkevich et al., 2004). These CIVs most likely comprise of late-born *C. finmarchicus*, or potentially Generation 2 (G2). In the Norwegian Sea the overwintering population (G0) reproduce sometimes in March (Generation 1) which develope and reproduce by end of summer (Generation 2), but that only manage to develop to CIV before overwintering (Arashkevich et al., 2004). It is relatively easy to identify the G0 and G1 generations from population structure data, but not so easy to identify a possible G2 generation (Aksnes and Blindheim, 1996). Particularly advective influence complicates the identification of new generations. In addition, recent molecular studies have highlighted the challenges we have to determine *C. finmarchicus* and *C. glacialis* correctly to species and the gaps in our original understanding of how the *Calanus* species are distributed geographically (Choquet et al., 2017; 2018). We can therefore not rule out that many of these CIVs may have been misidentified *C. glacialis* CIV (e.g. Gabrielsen et al., 2012; Choquet et al., 2017) which complicates it even further to derive species specific differences in population structures and life cycle durations.

Both *C. glacialis* and *C. finmarchicus* reproduced over an extended time period, but with slightly different timing. The early January appearance of *C. glacialis* males and females is consistent with the findings of Daase et al. (2018), and the appearance of *C. finmarchicus* adults February-March correspond well with population structures of *C. finmarchicus* further South (Arashkevich et al., 2004). The different timing in adult occurrence and thus reproduction is one distinct difference in *C. glacialis* and *C. finmarchicus* life histories (Kjellerup et al., 2012; our study) and we will discuss this further below, in relation to their physiology.

Physiological adaptations to the abiotic and the biotic environment

Seasonal migration and metabolism – closely linked?

We still have a poor understanding of which external and internal cues that trigger and regulate metabolism of *Calanus*, and particularly the onset and termination of diapause. However, recent studies on the seasonal variability in ion concentration (Freese et al., 2016), enzymes (e.g. Freese et al., 2016; 2017) and gene expressions (Häfker et al., 2018), together with life history modelling (Banas et al., 2016; Ejsmond et al., 2018; Varpe and Ejsmond, 2018) have provided new insights into diapause regulation. While most studies assume that deep-dwelling copepods enter a dormant state (i.e. diapause), there does not seem to be a simple on-off switch for diapause (e.g. Häfker et al., 2018). Specimens are gradually lowering and increasing their metabolic rates throughout the year (Freese et al., 2017) which has been well described by Hirche (1996) when identifying the five different phases of diapause in copepods. Diapause may be endogenous regulated (Häfker et al., 2018), or simply a gradually response to ceased feeding as suggested for *C. glacialis* by Freese et al. (2017), and most likely a complex combination of several factors.

Overall, the seasonal regulation in metabolism followed the same seasonal pattern for *C. glacialis* and *C. finmarchicus*, and MDH which is a considered a valid proxy for respiration and thus overall metabolism, correlated well with day length for both species. North of Svalbard, however, enzyme activities were not correlated to day length (Table 1) which can be explained by consolidated sea ice with snow on top which resulted in very low irradiances in sea until late May (Assmy et al., 2017). The solar angle changes profoundly throughout the year at 78-80 °N with almost 4 months of complete darkness and 4 months with midnight sun. This strong seasonality in incoming solar radiation is predictable and will not change with global warming. However, sea ice and snow cover can strongly modulate the underwater light between years and regions. The relatively consistent seasonal patterns in MDH independent of the organisms inhabiting the ice free Isfjorden or north of Svalbard with consolidated sea ice may suggest that the organisms have evolved a circannual clock that creates an endogenous rhythm with a period of ~365 days as suggested by Häfker et al. (2018), but that this circannual clock is not exclusively regulating the overall metabolism but in combination with external triggers such as light and food (e.g. Morata and Søreide et al., 2015) . For advected *C. finmarchicus*, a slightly different circannual clock could be expected in Svalbard, but our study did not allow investigating the seasonal metabolism on a finer scale than 3-4 weeks. In Isfjorden, where we had the longest (15 months) and most consistent data set, we found a negative correlation between MDH and WMD for both *C. glacialis* and *C. finmarchicus* supporting the assumption that *Calanus* reduce their overall metabolism as they descent to depth (Hirche, 1996). Following the five phases described by Hirche (1996), we can assign the preparatory and induction phases to start sometime in July-August when the copepods start their seasonal descent. The refractory phase, i.e. when lowest anabolic enzyme activities were recorded, occurred between September and January, followed by the activation phase sometimes between January-April, dependent on species,

ontogenetic stage and presumable also body size (see below). The final termination phase was shorter and took place in April-May. In Isfjorden, similarly high and low MDH activities were found for *C. glacialis* and *C. finmarchicus* (Fig. 6). When comparing the most active with the most dormant state an 80% reduction in metabolism was seen for both species. This was ~20% stronger reduction in metabolism than that determined for *C. glacialis* in Billefjorden, a colder seasonal ice covered fjord, close by in Svalbard (Freese et al., 2017). Reasons for this discrepancy are not known. We did detect an error in the calculations in Freese et al. (2017). The MDH (and HOAD) enzyme activities were not adjusted correctly to the number of individuals in the samples, but this error was consistent over the year resulting in consistently two times higher MDH activities than found in our study. After correcting the MDH activities in Freese et al. (2017) similar maximum levels for MDH were found for *C. glacialis* in Billefjorden as in our study, but the lowest recorded MDH in Billefjorden (November: 8.9 U mg DM^{-1}) was higher than the lowest recorded MDH potential found for *C. glacialis* in Isfjorden (January: 4.9 U mg DM^{-1}). *Calanus glacialis* may reach the refractory phase only for a brief period which presumable can vary extensively dependent on developmental stage and individual fitness (i.e. lipid content) (Hassett, 2006; Varpe and Ejsmond, 2018), which also the large individual variability in MDH activities showed (Fig. 6). CS is also a proxy for the overall metabolism, and for this enzyme similar seasonal activity patterns were found for *C. glacialis* in Isfjorden and the colder Billefjorden, supporting the 50% reduction in metabolism as previously estimated by Freese et al. (2017). For *C. finmarchicus* similarly low and high seasonal CS activities were found as for *C. glacialis*, except for *C. finmarchicus* females. For females three times higher CS activities were found for *C. finmarchicus* than its Arctic sibling when comparing CS activities per DM. When comparing CS activities per individual, however, such species specific differences were not found. CS positively correlates to egg production (Kreibich et al., 2008) and we will continue discussing reasons for different CS activities when discussing reproduction below.

Catabolism of internal lipid resources and seasonal wake-up

Adults appeared roughly two months earlier in *C. glacialis* than in *C. finmarchicus*, which confirms that *C. glacialis* were "programmed" to reproduce earlier than *C. finmarchicus,* which further was supported by an earlier peak abundance in both *C. glacialis* eggs and young copepodite stages (CI-CIII) (Fig. 5). A combination of internal (endogenous) and external abiotic and biotic cues, operating at species, population and individual level, seemed to regulate when a specimen molted, matured and reproduced since it was a continuous appearance of adults over time (2 to 3 months) for both species. Gonad maturation is very energy demanding, even more energy demanding than egg production (Jónasdóttir et al., 1999). Interestingly, particularly high CS activities was found in in *C. glacialis* males in January which likely indicate active sperm formation, which can further be supported by the high female:male sex ratio (0.4) with 10% of the females observed with attached spermatophores in January (Daase et al., 2018). We only managed to get sufficient number of males for enzyme analyses in January, which supports that they are short-lived (Hatlebakk et al., *in press*). When investigating the gonads of CVs in January, only 2% of them

were identified to be males while almost 90% were females, the rest (~9%) was not possible to sex. In other words, new *C. glacialis* males were not expected to appear, most were already molted to adults. In comparison, most CVs of *C. finmarchicus* (>53%) could not be sexed since they still had undifferentiated gonads in January (Daase et al., 2018).

One main concern of increased water temperatures due to global warming is the effect it will have on secondary production. As metabolic rates increase with temperature, increased water temperatures (particularly in winter), will put strains on the energy demand of herbivorous copepods during overwintering and this will likely have an impact not only on winter survival but also the reproductive output (e.g. Varpe and Ejsmond, 2018). From September to January, low metabolism was generally seen for both species, but a gradual increase in HOAD enzyme activities were seen from September to January. HOAD activity peaked in February-April, indicating preparation of molting and energy demanding gonad maturation (Jónasdóttir, 1999). Interestingly, *C. glacialis* inhabiting the colder Billefjorden had consistently higher HOAD activities (Freese et al., 2017) than *C. glacialis* in Isfjorden. HOAD catabolize body lipids to fuel the metabolism when food is absent. The generally higher HOAD activity for overwintering *C. glacialis* in the colder Billefjorden may simply be due to pure enzyme kinetics. At colder temperatures, each enzyme typically operates slower. One way of compensating for that is to synthesis more of that enzyme (Johnston and Dunn, 1987), suggesting that *C. glacialis* has the capability to adjust for temperature when mobilizing enzymes. Whether it is "expensive" to mobilize HOAD is not known, but no correlation between MDH and HOAD were found for the two species neither in Isfjorden or off-shelf north of Svalbard. For *C. glacialis* CIV which is not known to molt in winter, a longer dormant phase than for *Calanus* CV and adults can be expected. We had, however, too few enzyme data of *C. glacialis* CIV to state if this is true, but in general *C. glacialis* CIV had relatively low MDH and CS enzyme activities until March.

Mobilization of digestive enzymes and utilization of the spring bloom

CS is positively correlated to egg production (Kreibich et al., 2008), and we found peak CS activity was correlated strongly to chlorophyll a concentrations for *C. finmarchicus,* but not *C. glacialis.* Peak CS activity was almost twice as high in *C. finmarchicus* as in *C .glacialis* females. This may be a species specific adaptation in *C. finmarchicus* to optimize an income rather than a capital breeding strategy. However, a closer investigation revealed that CS enzyme activities per individual did not show species specific difference in maximum CS activities. Females of *C. glacialis* were twice as heavy as *C. finmarchicus* females in mid-May (Table 1) and had up to three times larger lipid sac area (Eide, 2016). The lipid sac is assumed to be a metabolic inactive tissue. Thus enzyme activities may be better related to protein content than to dry weight or per individual (e.g. Hassett, 2006). Protein content was not measured in this study, but should be considered in future studies. The enzyme activities indicate the potential rather than the *in situ* enzyme activity, which is important to remember when interpreting the data. Lipase and proteinase were fully mobilized during the spring bloom, and earlier in *C. glacialis* than *C. finmarchicus*. *Calanus* has been found to

have torpid gut epithelium during overwintering (Hallberg and Hirche, 1983). To efficiently utilize the often short and intense Arctic spring bloom the specimens are dependent on start mobilizing their digestive enzymes well ahead of the bloom since it may take 2-3 weeks (Freese, 2016). Interestingly, the lipase activities were up to three times higher in *C. glacialis* than for *C. finmarchicus* both in terms of lipase activity per DM and per individual. For proteinase, the reverse was seen with consistently higher proteinase activities in *C. finmarchicus* than *C. glacialis*, but only when relating proteinase activity per DM and not per individual. This suggests that both species have similar potential for utilizing proteins, but not for lipids. The particularly strong mobilization of lipase in *C. glacialis* may be a special physiological adaptation for Arctic species to fully utilize the generally short primary production window in the high-Arctic compared to lower latitudes (Leu et al., 2015). For the digestive enzymes lipase and proteinase similar high enzyme activities were found for *C. glacialis* in Billefjorden and the warmer Isfjorden (Freese et al., 2016; this study). Reasons for this may simply be that specimens were already mobilizing these enzymes at the maximum possible rates. Several studies have shown that *C. glacialis* is resilient to changes in temperatures, but only to a maximum temperature which for high latitudes has been suggested to be +6°C (e.g. Niehoff and Hirche, 2005). Such high temperatures were only for a brief time period reached in our study and only in the very surface waters. As long as *C. glacialis* can find a colder refugee by migrating down to deeper parts they can inhabit regions experiencing up to 15°C in surface waters (e.g. Kosobokova, 1999, Niehoff and Hirche, 2005).

Concluding remarks

Even slight differences in timing of life history events can have large impacts on a species success (e.g. Varpe, 2012). In our study, *Calanus glacialis* and *C. finmarchicus* had largely similar life histories and physiology, but some distinct differences in timing (i.e. reproduction) and build-up and break-down of lipids were revealed. In strong seasonal environments the onset of the spring bloom may be highly unpredictable due to dynamic sea ice conditions, and for such environments early reproduction seem to be the key to success. To be able to reproduce early, females have to prioritize to grow large to be capable of capital breeding. The earlier the better is stated when it comes to *Calanus* and reproduction (e.g. Varpe et al., 2007), and this seems to be largely true for *C. glacialis* in seasonal ice covered shelf seas. The largest and most lipid-rich individuals are those that molt to adults first. This has been found in both *C. glacialis* (Kosobokova, 1999; Bailey, 2010; Daase et al., 2018) and *C. finmarchicus* (Arashkevich et al., 2004). The ability to perform capital breeding is often coupled to body size (Varpe et al., 2007, Varpe and Ejsmond, 2018). However, large body sizes also expose specimens more easily to visual predation (Varpe and Ejsmond, 2018). In other words, there is a trade-off between body size and reproductive success, and optimal strategies are regulated by both bottom-up (e.g. food availability and accumulated lipid resources) and top-down processes (Varpe and Ejsmond, 2018). The balance between these two trade-offs is reached at different body sizes depending on the

primary production and predatory regimes the specimen inhabits (Berge et al., 2012; Ejsmond et al., 2018). In fish dominated systems it may be optimal for *Calanus* spp. to reduce its body size and potentially life cycle (e.g. *C. finmarchicus* in the North Atlantic) while in the Arctic Ocean where darkness prevails and the abundance of visual predators is low, it may be more beneficial to invest in longer life cycles and thus grow larger before reproducing (e.g. *C. hyperboreus;* Hirche (1996). *C. glacialis* ability to perform both capital and income breeding, and to alter between a 1 or 2 year life cycle makes the species very flexible. *C. glacialis* seem to have evolved a life history somewhere between the large Arctic Ocean specialist *C. hyperboreus* and the smaller North Atlantic *C. finmarchicus.* Less sea ice and warmer sea temperatures are assumed to change the algal bloom phenology in the Arctic (Leu et al., 2015). The change from seasonal ice covered to ice free waters, combined with warmer year-round sea temperatures (+2 °C), as is the case in our Isfjorden study area, did not seem to negatively impact the *C. glacialis* population, but rather strengthen it. *C. finmarchicus* was also found to have some flexibility in their life history strategies, but so far it does not seem to be flexible enough to tackle the still rather short-pulsed primary production widow, followed by the long unproductive winter (Møller et al., 2016; this study).

Acknowledgement

We kindly acknowledge the captains and crew of RV Lance, RV Helmer Hanssen, RV Dalnie Zelentsy, RV Viking Explorer and UNIS logistics for making it possible to conduct thus study's high resolution, year round sampling. We are very grateful to A. Baily during the N-ICE campaign and other fellow colleagues for all their support and help during fieldwork. This study was supported by the Centre for Ice, Climate and Ecosystems (ICE) at the Norwegian Polar Institute, the Ministry of Climate and Environment, Norway, the Research Council of Norway (projects COPPY no. 227139 IMOS no. 246747 and Boom or Bust no. 24464) and FRAM - High North Research Centre for Climate and the Environment, Flagship ArcticOcean, project FADE no. 66009.

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Fig. 1: Map of the study area. Main station IsK, Isfjorden, Svalbard marked with star. Off shelf sampling spots marked with circles (N-ICE campaign in white, other campaigns in grey). (Map data: NPI, GEBCO, GADM)

Fig. 2: Temperature from (a) Off-shelf north of Svalbard from January to June, 2015, during the N-ICE 2015 campaign and (b) Isfjorden from June 2015 to August 2016.

Fig. 3: Fluorescence from (a) Off-shelf north of Svalbard from January to June, 2015, during the N-ICE 2015 campaign and (b) Isfjorden from June 2015 to August 2016. Note the difference in scale.

Fig. 4: Total abundance and weighted mean depth of *C. finmarchicus* (red) and *C. glacialis* (blue) at Karls Krona Deep, Isfjorden from June 2015 to August 2016. Asterisks indicate dates when it was not possible to sample from bottom to surface for community samples.

Fig. 5: (a) Relative copepodite stage composition of *C. glacialis*, (b) relative copepodite stage composition of *C. finmarchicus* and (c) total egg production *in situ* at Karls Krona Deep, Isfjorden from June 2015 to August 2016.

Fig. 6: Specific malate dehydrogenase activity (a) and Citrate synthase activity (b) for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where darker green means higher Chl *a*. White background means no data on Chl *a* max.

Fig. 7: Specific 3-hydroxyacyl-CoA dehydrogenase activity for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where darker green means higher Chl *a*. White background means no data on Chl *a* max.

Fig. 8: Specific proteinase activity (a) and lipase activity (b) for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where lightest green is <0.01 µg L^{-1} and the darkest is 8 µg L^{-1}darker green means higher Chl *a*. White background means no data on Chl *a* max.

Table 1: Correlation between the enzyme activity and the environmental factors Chlorophyll a max in water column (Chl a), Weighted mean depth of community (WMD), temperature at weighted mean depth (WMD temp) and Day length in Isfjorden, Svalbard. Correlations are calculated for all measurements done through the sampling year. Pearson correlation in normal font and spearman in bold font. p value indicated as following: *: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001.

Table 2: Correlation between enzyme activity and environmental factors Chlorophyll *a* max in water column (Chl a) and Day length off shelf north of Svalbard. Correlations are calculated for all measurements done from March to August 2016. Pearson correlation in normal font and spearman in bold font. p value indicated as following: *: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001.

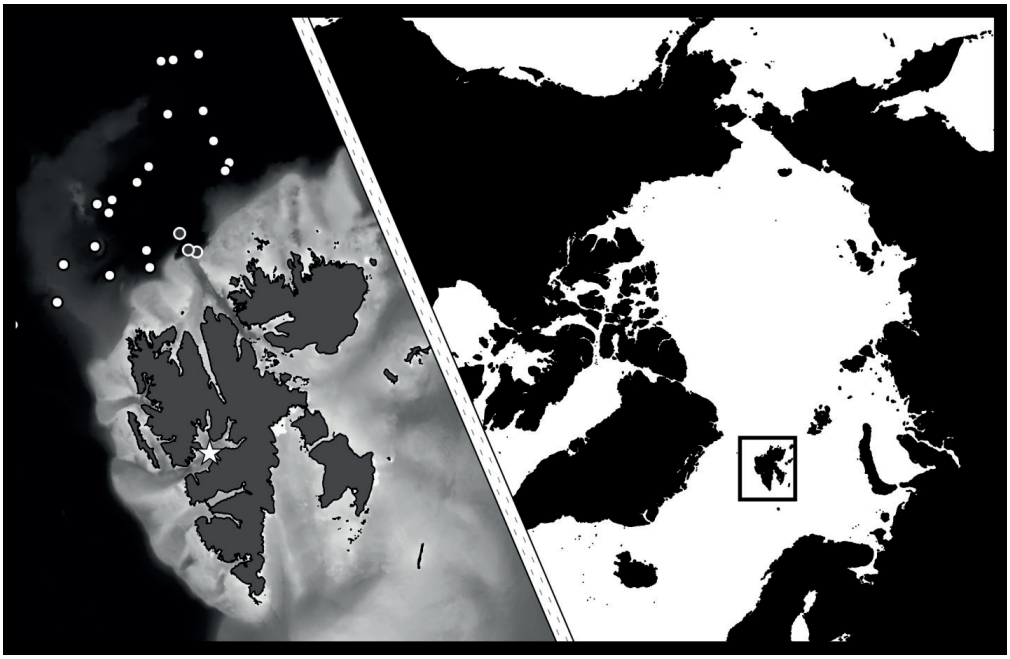

Fig. 1

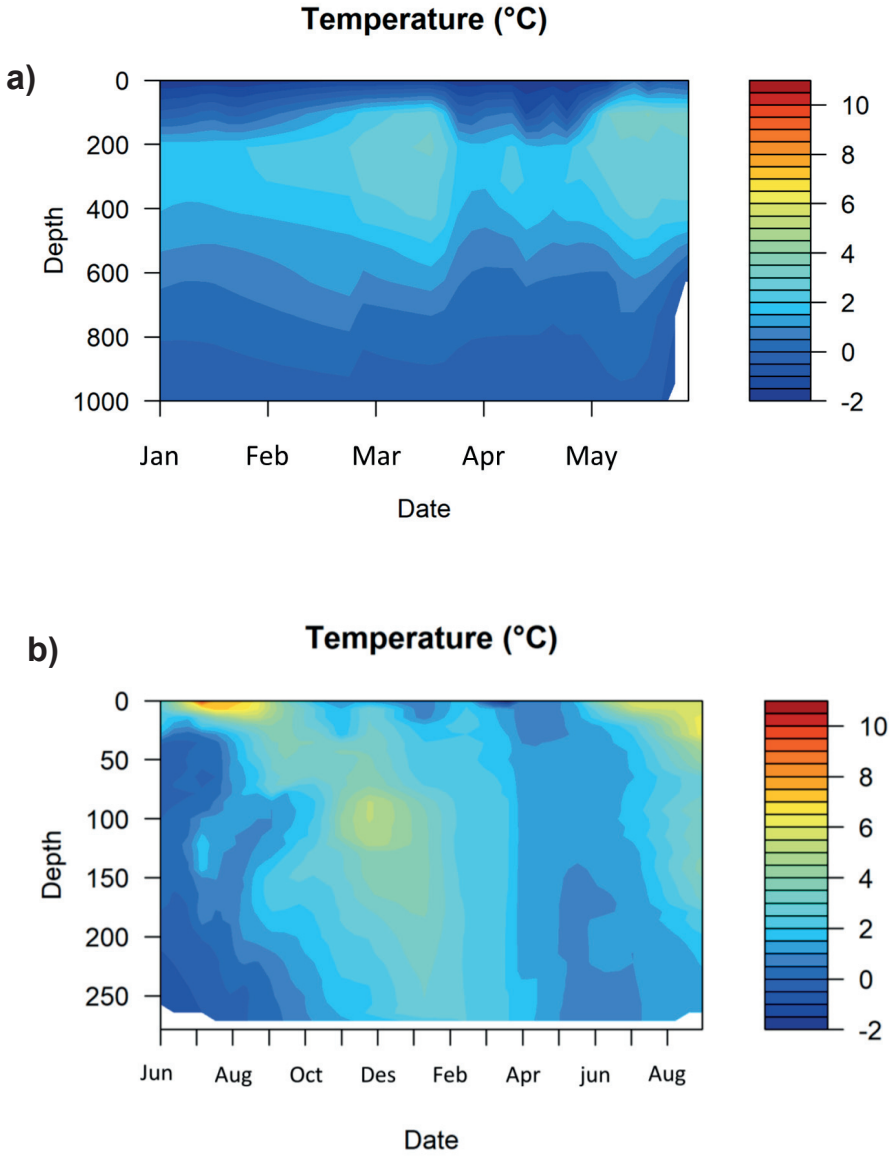
a)
Temperature (°C)
Depth
0
200
400
600
800
1000
Jan
Feb
Mar
Apr
May
Date
10
8
6
4
2
0
-2
b)
Temperature (°C)
Depth
0
50
100
150
200
250
Jun
Aug
Oct
Des
Feb
Apr
jun
Aug
Date
10
8
6
4
2
0
-2

Fig. 2

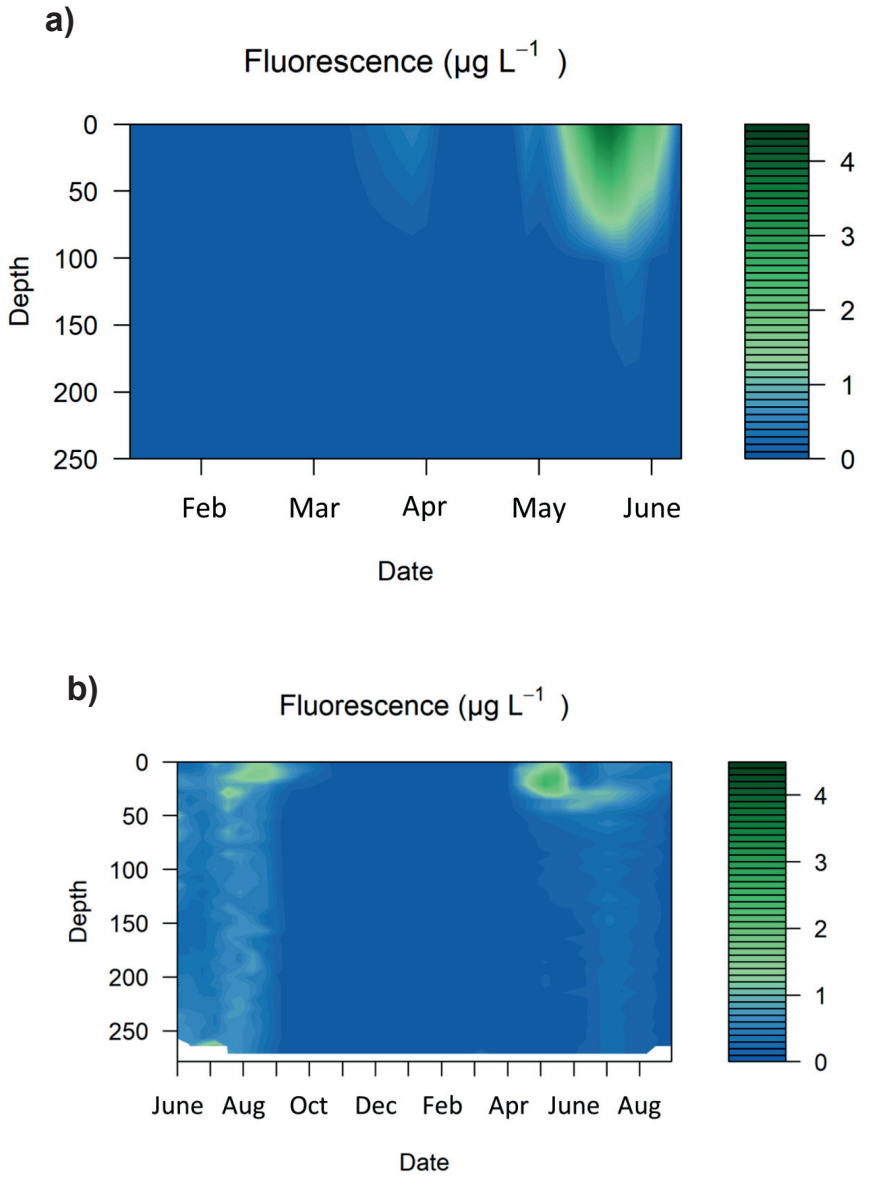
a)
Fluorescence (µg L^{-1})
Depth
0
50
100
150
200
250
Feb
Mar
Apr
May
June
Date
4
3
2
1
0
b)
Fluorescence (µg L^{-1})
Depth
0
50
100
150
200
250
June
Aug
Oct
Dec
Feb
Apr
June
Aug
Date
4
3
2
1
0

Fig. 3

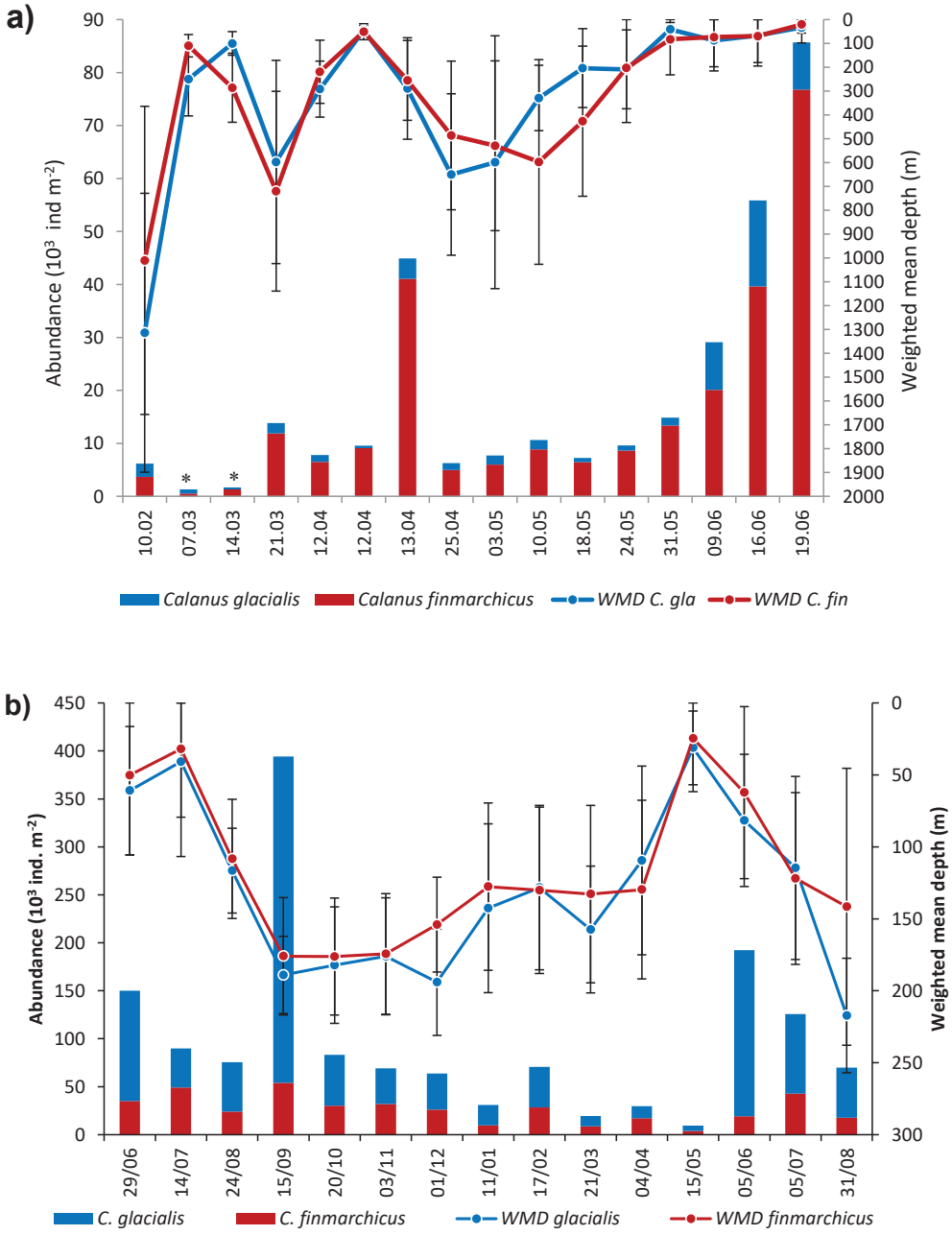
a)
Abundance (10³ ind m⁻²)
Weighted mean depth (m)
0
10
20
30
40
50
60
70
80
90
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
*
*
10.02
07.03
14.03
21.03
12.04
12.04
13.04
25.04
03.05
10.05
18.05
24.05
31.05
09.06
16.06
19.06
Calanus glacialis
Calanus finmarchicus
WMD C. gla
WMD C. fin
b)
Abundance (10³ ind. m⁻²)
Weighted mean depth (m)
0
50
100
150
200
250
300
350
400
450
0
50
100
150
200
250
300
29/06
14/07
24/08
15/09
20/10
03/11
01/12
11/01
17/02
21/03
04/04
15/05
05/06
05/07
31/08
C. glacialis
C. finmarchicus
WMD glacialis
WMD finmarchicus

Fig 4:

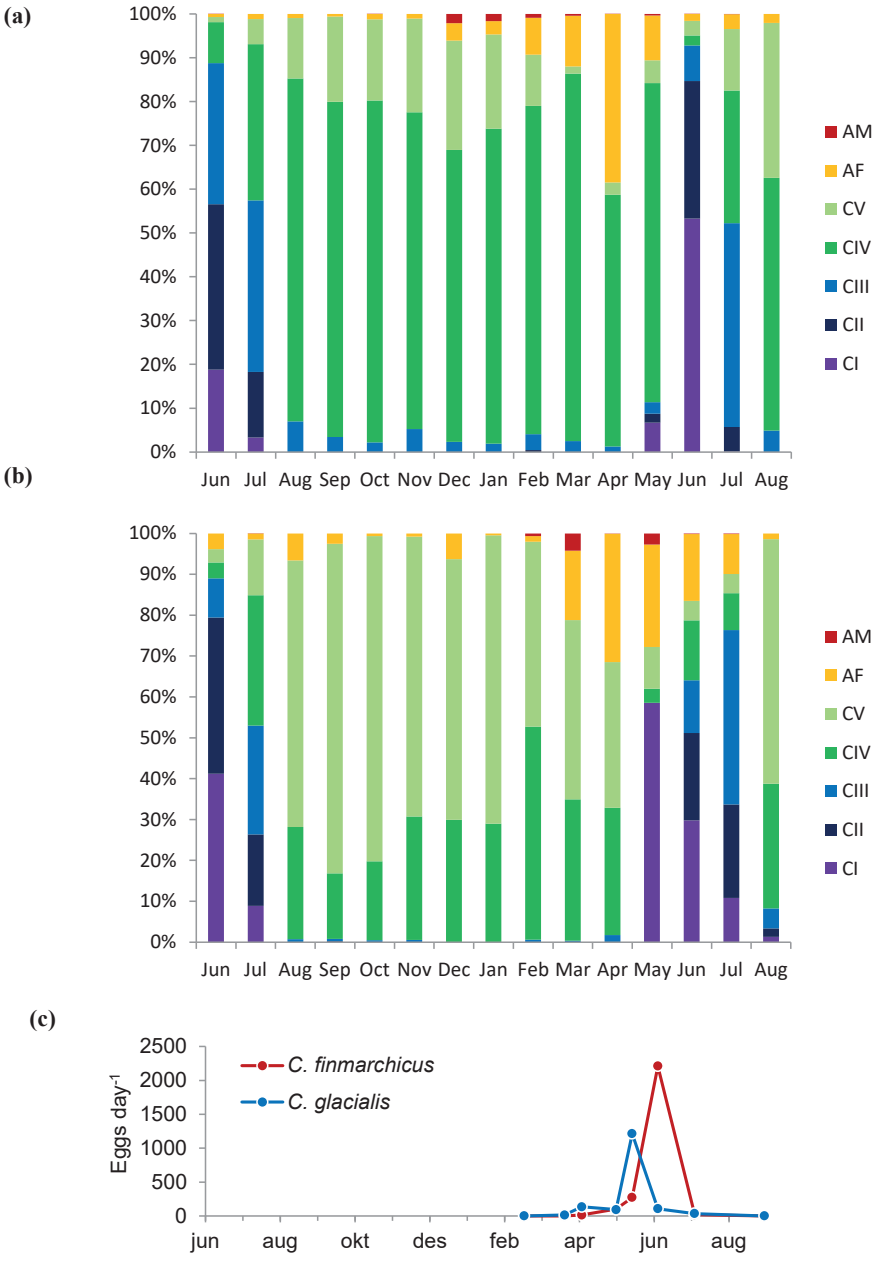
(a)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug
AM
AF
CV
CIV
CIII
CII
CI
(b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug
AM
AF
CV
CIV
CIII
CII
CI
(c)
2500
2000
1500
1000
500
0
Eggs day-1
C. finmarchicus
C. glacialis
jun aug okt des feb apr jun aug

Fig 5

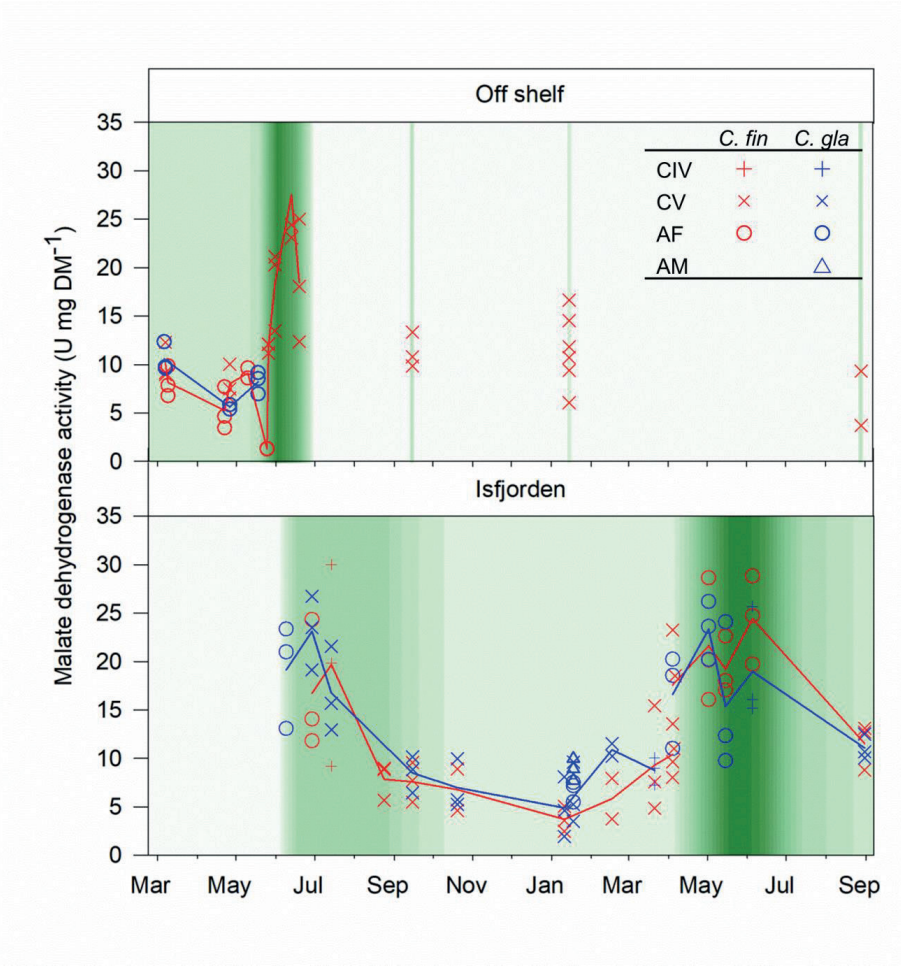
Off shelf
Isfjorden
Malate dehydrogenase activity (U mg DM-1)
C. fin
C. gla
CIV
CV
AF
AM
0
5
10
15
20
25
30
35
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. 6a

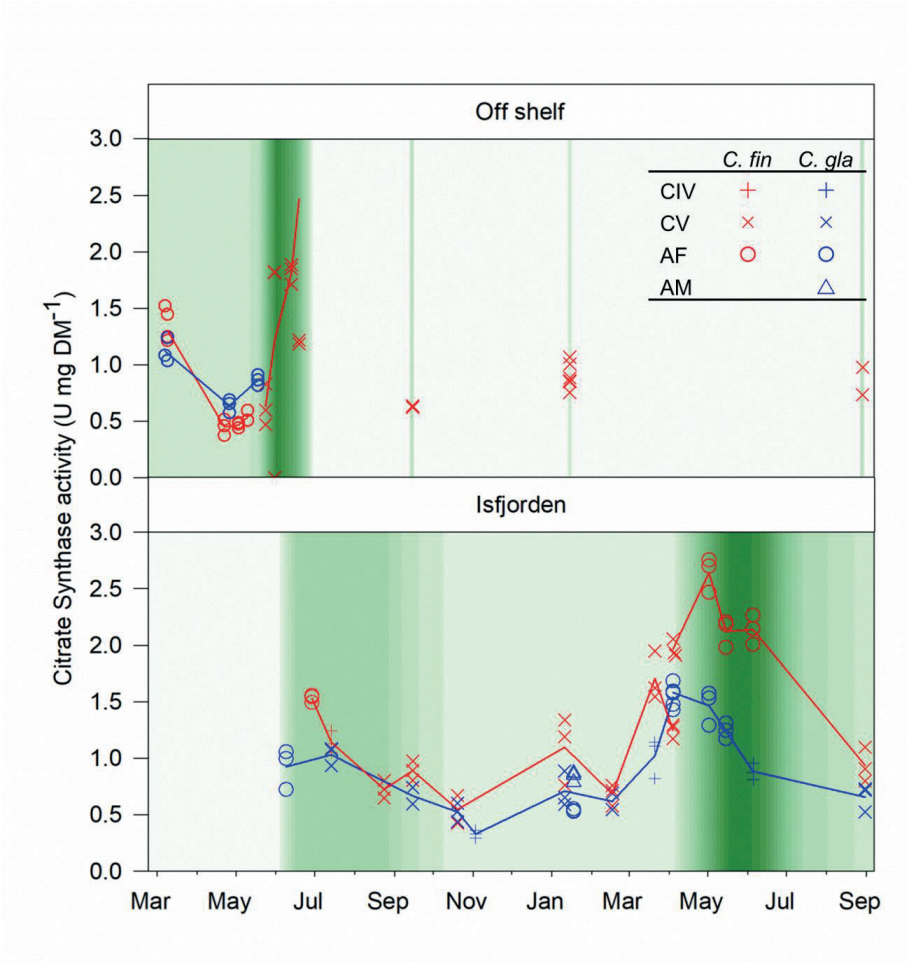
Off shelf
Isfjorden
Citrate Synthase activity (U mg DM-1)
C. fin
C. gla
CIV
CV
AF
AM
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig: 6b

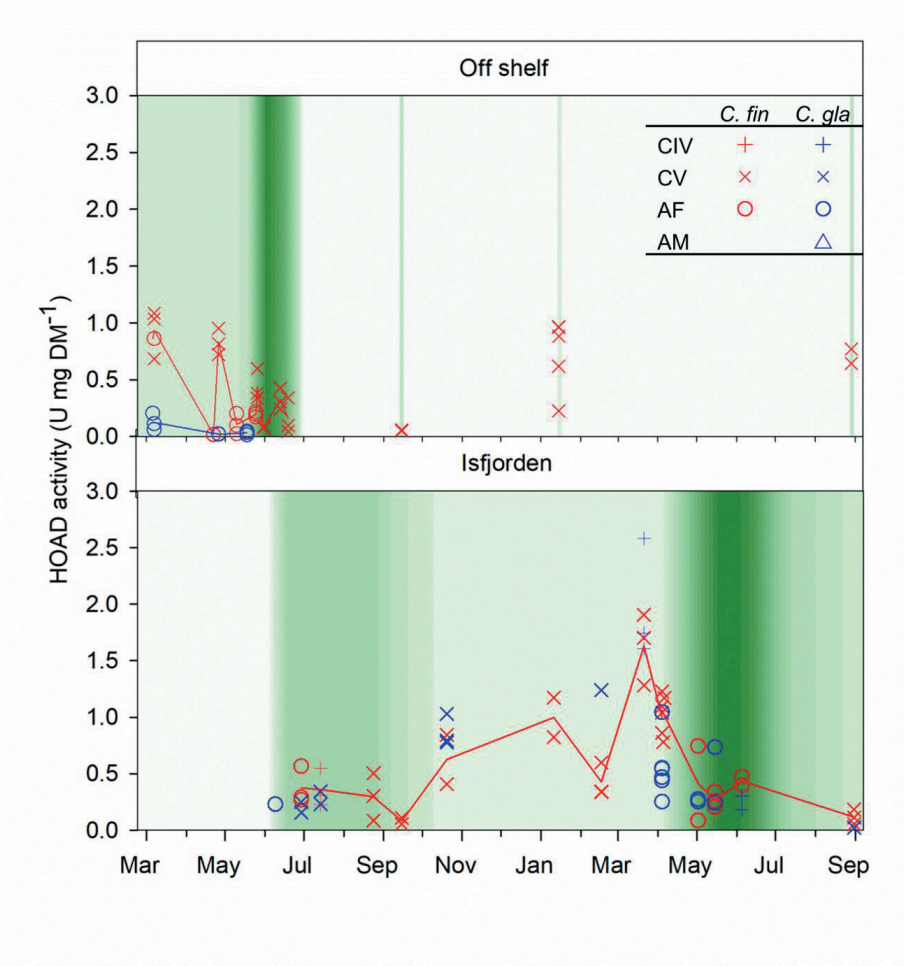
Off shelf
Isfjorden
HOAD activity (U mg DM-1)
C. fin
C. gla
CIV
CV
AF
AM
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. 7

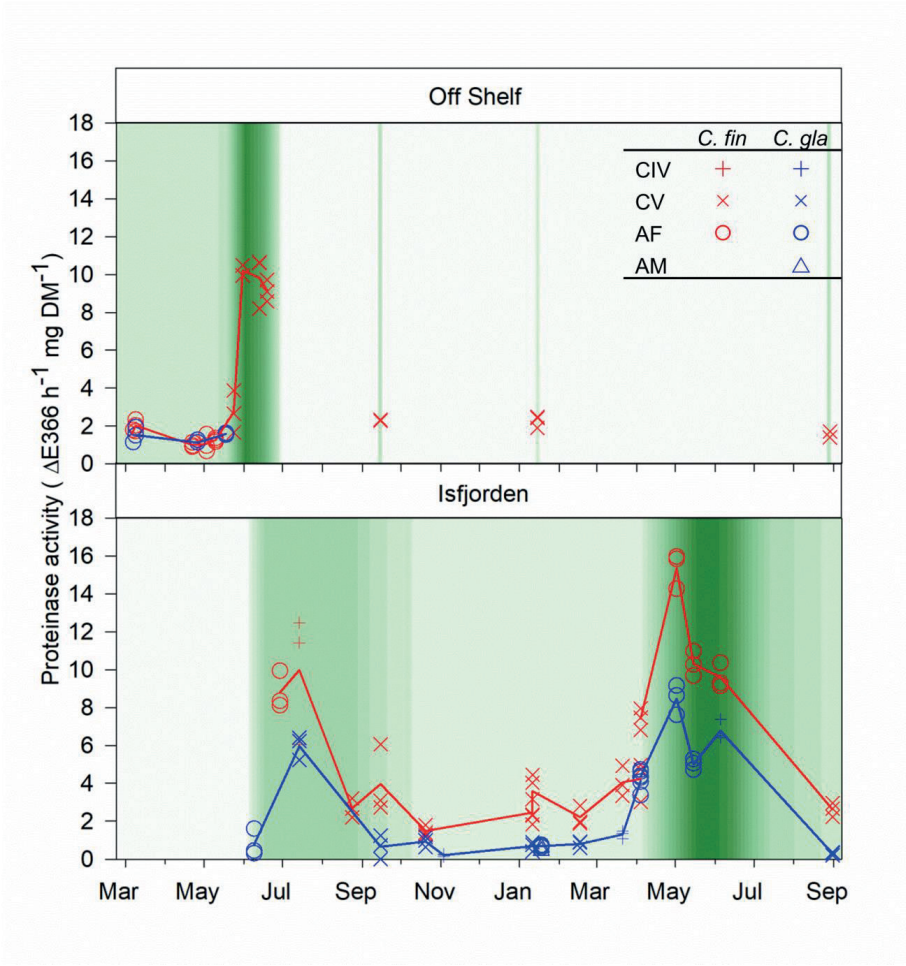
Off Shelf
Isfjorden
C. fin
C. gla
CIV
CV
AF
AM
Proteinase activity (ΔE366 h-1 mg DM-1)
0
2
4
6
8
10
12
14
16
18
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig 8a

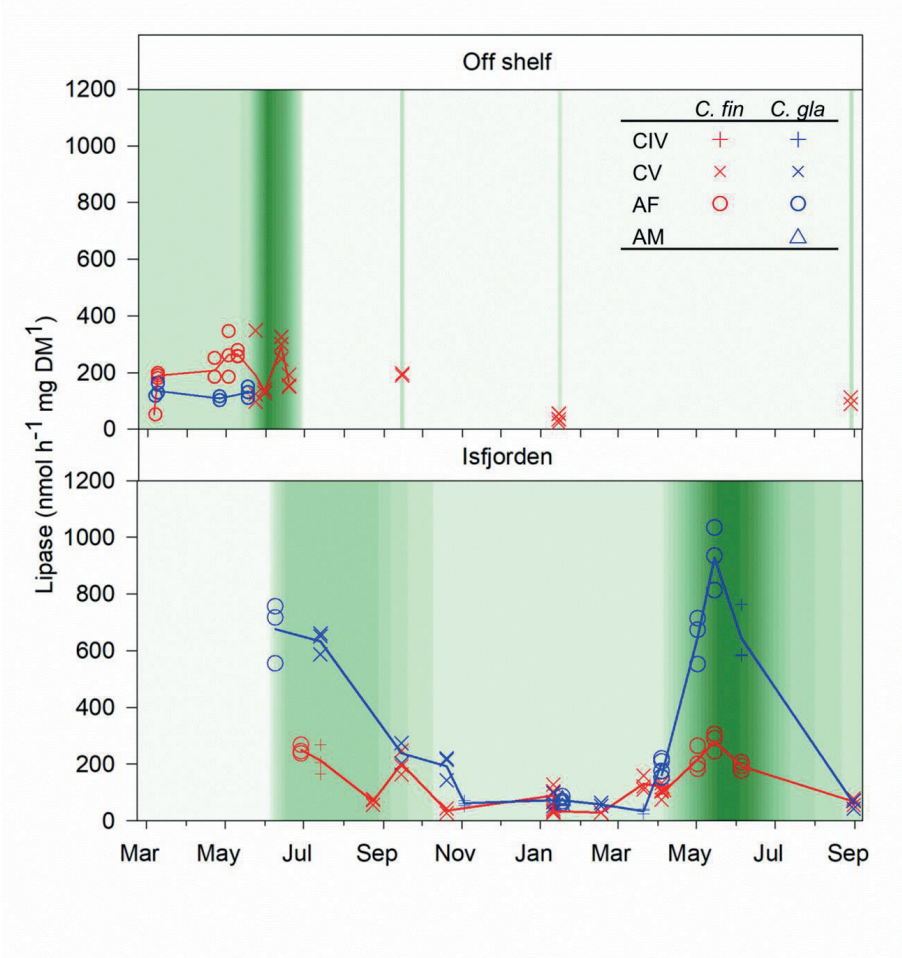
Off shelf
Isfjorden
C. fin
C. gla
CIV
CV
AF
AM
Lipase (nmol h-1 mg DM-1)
0
200
400
600
800
1000
1200
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. 8b

Table 1

		Lipase	CS	MDH	HOAD	Chl a	WMD	WMD temp	Day length
C. finmarchicus	Proteinase	0.785***	0.854***	0.768***	-0.118	0.571***	-0.803***	-0.601***	**0.584*** **
	Lipase		0.638***	0.648***	-0.174	0.647***	-0.652***	-0.545***	**0.668*** **
	CS			0.674***	0.278	0.329*	-0.647***	-0.539***	**0.462****
	MDH				-0.12	0.618***	-0.637***	-0.498**	**0.711*** **
	HOAD					-0.391*	0.177	0.0897	**-0.461****
C. glacialis	Proteinase	0.670***	0.386*	0.714***	-0.419*	0.544***	-0.898***	-0.576***	**0.798*** **
	Lipase		-0.0153	0.656***	-0.254	0.797***	-0.757***	-0.692***	**0.774*** **
	CS			0.202	0.007	**0.304**	-0.491**	-0.186	**0.589*** **
	MDH				-0.304	0.433**	-0.676***	-0.775***	**0.837*** **
	HOAD					-0.103	0.496**	-0.0575	**-0.397***

Table 2:

		Lipase	CS	MDH	HOAD	Chl a	Day length	Temperature
C. finmarchicus	Proteinase	0.0970	0.691***	0.817***	-0.148	0.912***	**-0.075**	0.124
	Lipase		0.231	0.340	-0.687***	0.161	**0.585*** **	-0.246
	CS			0.430*	-0.227	0.537**	**-0.222**	0.172
	MDH				-0.130	0.623***	**0.028**	0.148
	HOAD					-0.446**	**-0.319**	0.275

SUPPLEMENTARY

Fig. S1: Weighted mean depth for Adult females (a) and CV (b) of *C. finmarchicus* (red) and *C. glacialis* (blue) at Karls Krona Deep, Isfjorden from June 2015 to August 2016.

Fig. S2: malate dehydrogenase activity (a) and Citrate synthase activity (b) per individual for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where darker green means higher Chl *a*. White background means no data on Chl *a* max.

Fig. S3: 3-hydroxyacyl-CoA dehydrogenase activity per individual for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where darker green means higher Chl *a*. White background means no data on Chl *a* max.

Fig. S4: Proteinase activity (a) and lipase activity (b) per individual for *C. finmarchicus* (red) and *C. glacialis* (blue). Data from off shelf are shown in the top panels and data from Isfjorden are shown in the bottom panels. The background indicates Chl *a* max, where lightest green is <0.01 µg L^{-1} and the darkest is 8 µg L^{-1}darker green means higher Chl *a*. White background means no data on Chl *a* max.

Table S1: Overview of sampling parameters and depth and the dry weight data for the copepods throughout this study.

Table S2: Pearson correlation between the enzyme activity from January to May and the environmental factors Chlorophyll a max in water column (Chl a), Weighted mean depth of community (WMD), temperature at weighted mean depth (WMD temp) and Day length in Isfjorden, Svalbard. Correlations are calculated for all measurements done for the period of upregulation of enzyme activity, p value indicated as following: *: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001.

Table S3: Pearson correlation between the enzyme activity and Day length in Billefjorden, Svalbard. Correlations are calculated for all measurements done for the period of upregulation of enzyme activity, p value indicated as following: *: p=0.05-0.01, **: p=0.01-0.001, ***: p<0.001.

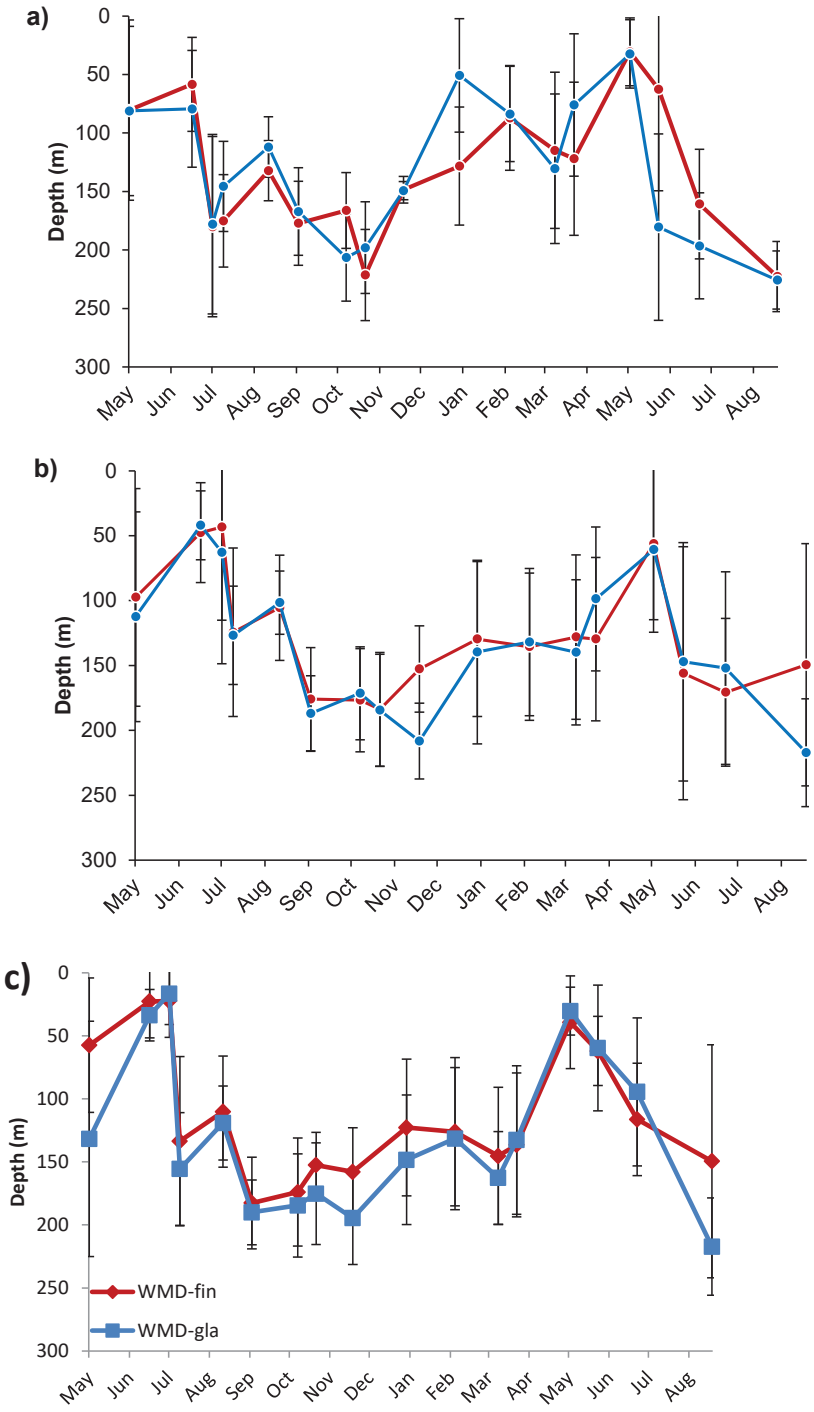
a)
b)
c)
Depth (m)
0
50
100
150
200
250
300
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
WMD-fin
WMD-gla

Fig. S1

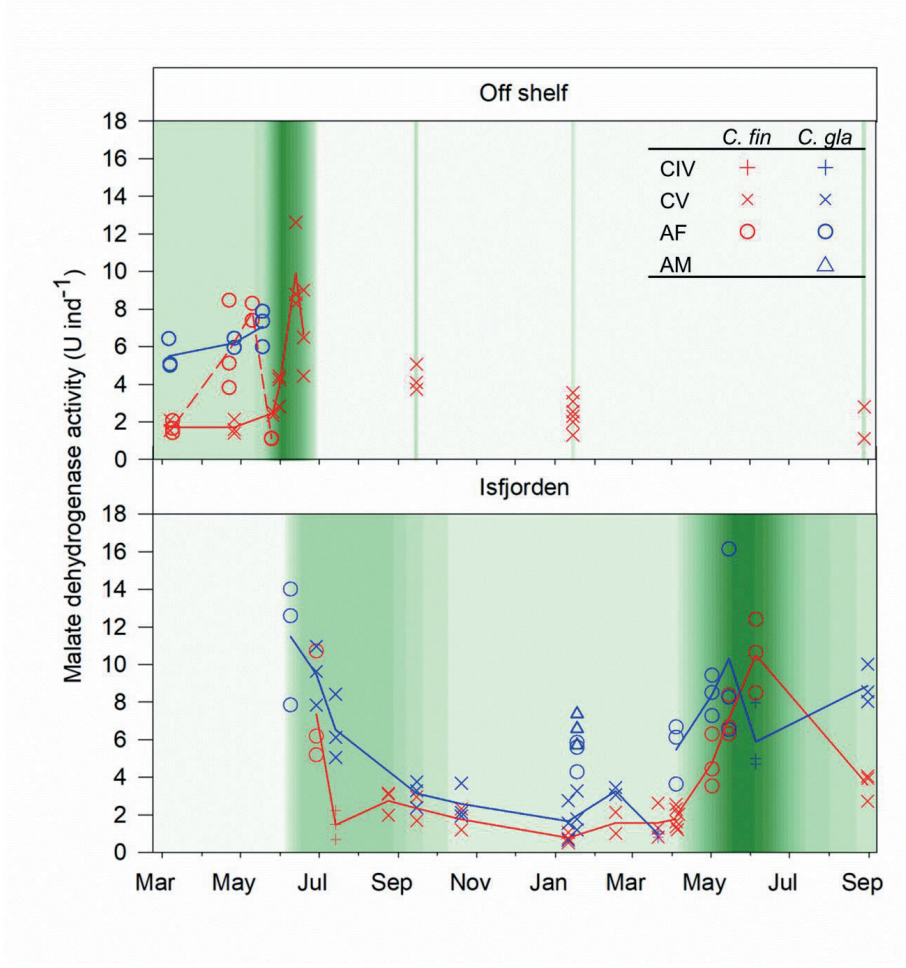
Off shelf
Isfjorden
Malate dehydrogenase activity (U ind^{-1})
C. fin
C. gla
CIV
CV
AF
AM
0
2
4
6
8
10
12
14
16
18
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. S2a

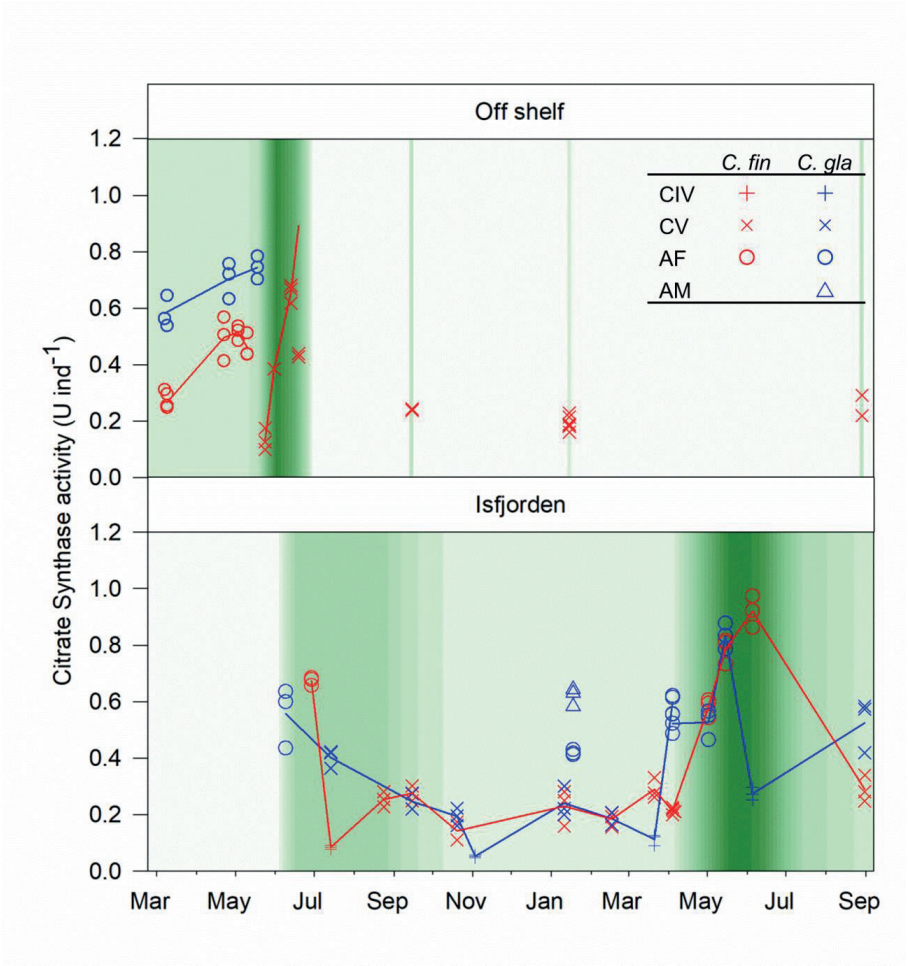
Off shelf
Isfjorden
Citrate Synthase activity (U ind-1)
C. fin
C. gla
CIV
CV
AF
AM
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. S2b

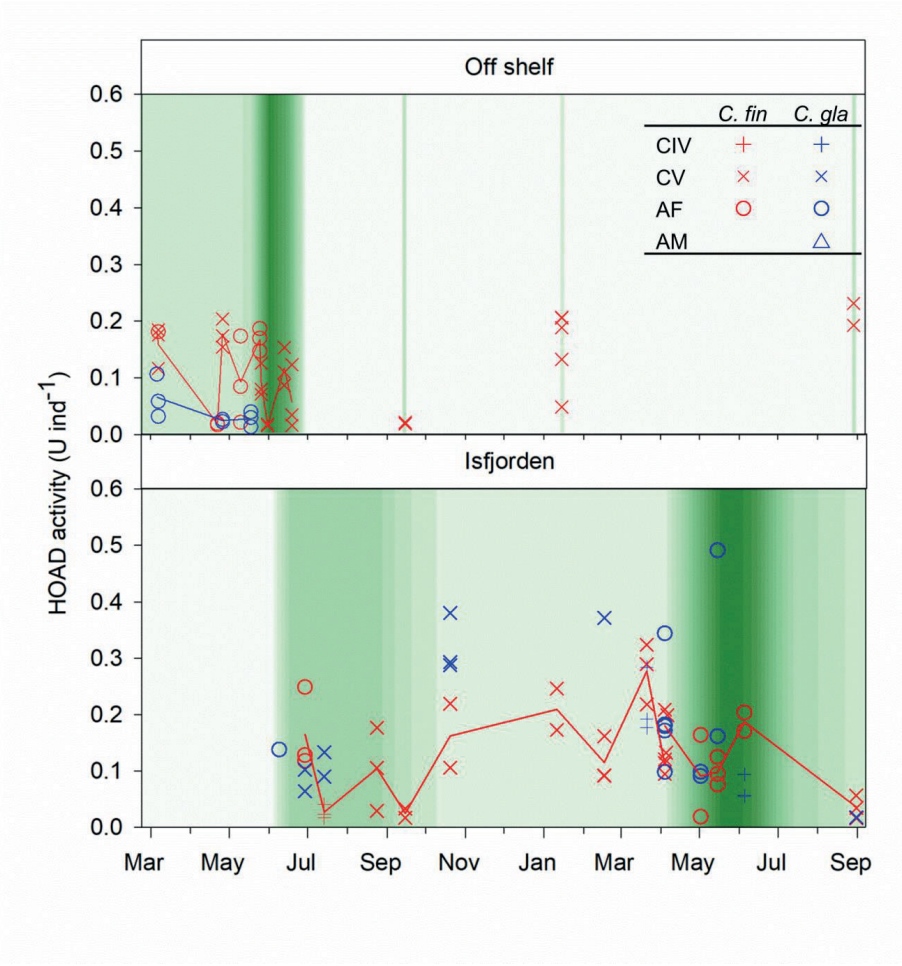
Off shelf
Isfjorden
C. fin
C. gla
CIV
CV
AF
AM
HOAD activity (U ind-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. S3

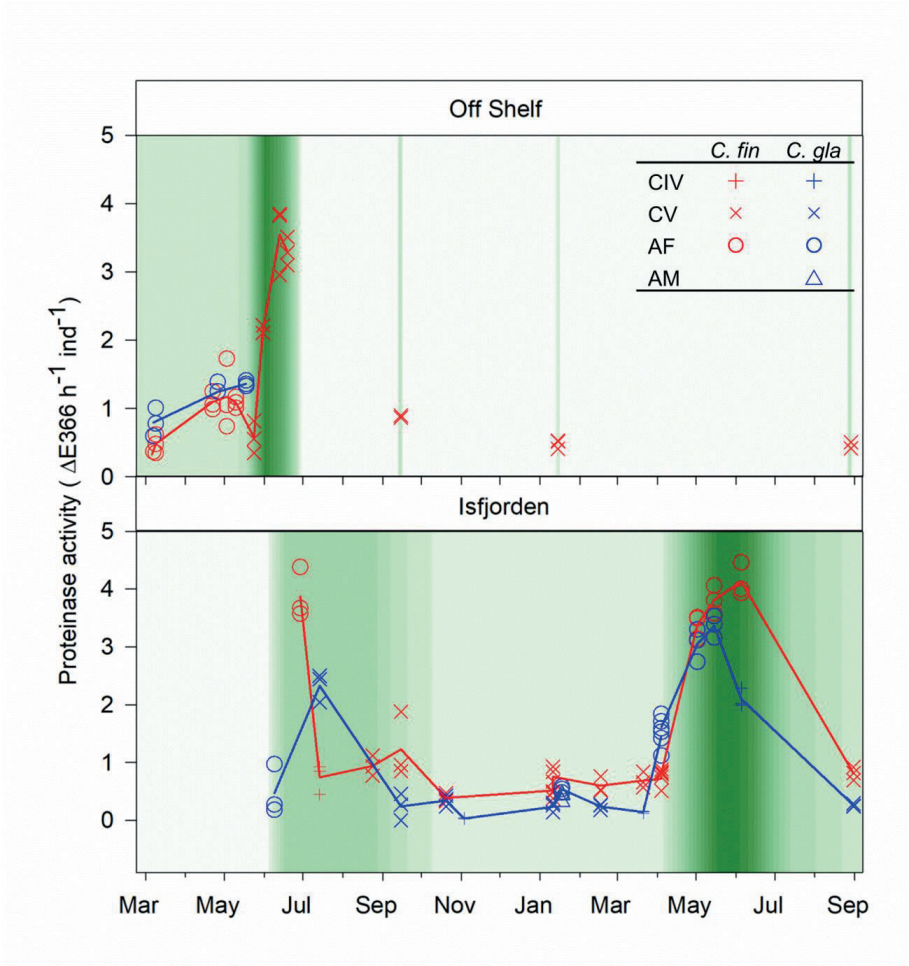
Off Shelf
Isfjorden
Proteinase activity (ΔE366 h-1 ind-1)
C. fin
C. gla
CIV
CV
AF
AM
0
1
2
3
4
5
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. S4a

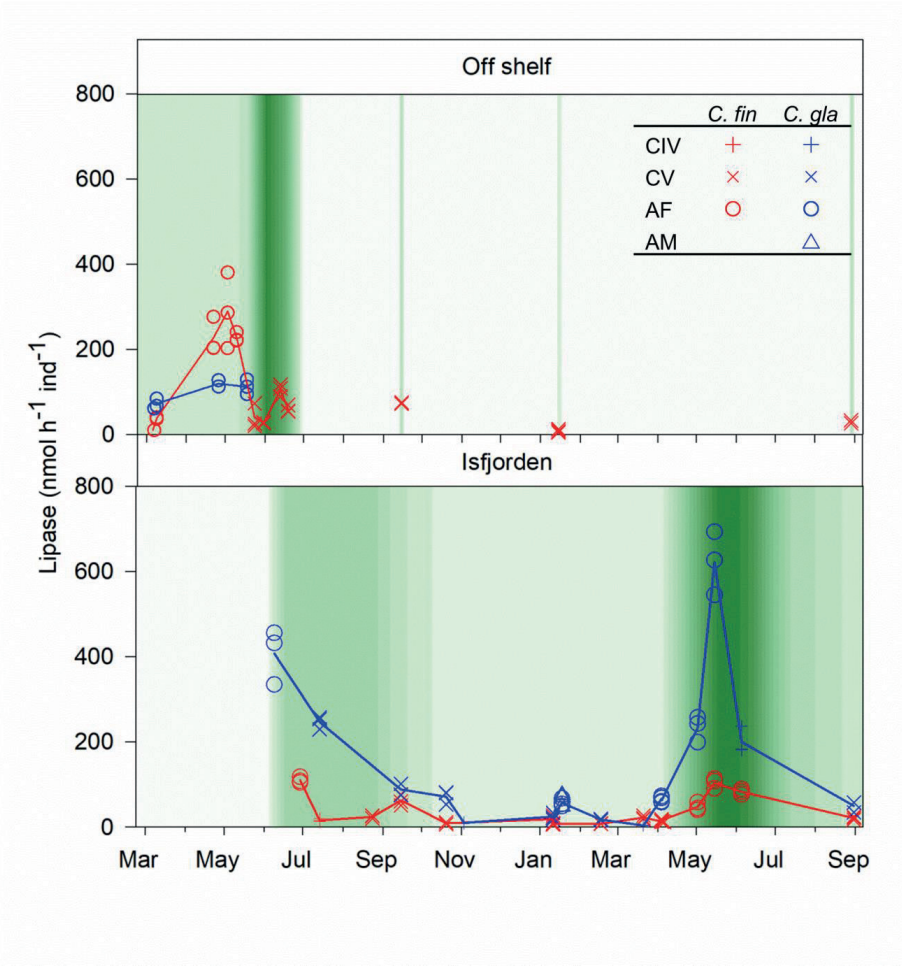
Off shelf
Isfjorden
C. fin
C. gla
CIV
CV
AF
AM
Lipase (nmol h-1 ind-1)
0
200
400
600
800
Mar
May
Jul
Sep
Nov
Jan
Mar
May
Jul
Sep

Fig. S4b

Table S1

					Dry mass (µg)					
					C. finmarchicus			C. glacialis		
Station	Date	Gear	Community samples, depth intervals (m)	Enzyme and dry mass samples, depth (m)	stage	Dry mass (µg)	SD	stage	Dry mass (µg)	SD
IsK	09.06.15	WP2 (200 µm)	255-200-100-50-20-0	150-0						
IsK	29.06.15	MPS (200 µm)	180-150-100-50-20-0	100-0	F	437.9	123.6	F	602.3	133.0
IsK	29.06.15	MPS (200 µm)		100-0				CV	411.7	101.8
IsK	14.07.15	WP2 (200 µm)	260-200-100-50-20-0	50-0				CV	386.7	119.3
IsK	24.08.15	MPS (200 µm)	250-150-100-50-20-0	250-150	CV	347.1	149.5			
IsK	15.09.15	MPS (200 µm)	250-150-100-50-20-0	250-150	CV	305.4	109.7	CV	370.8	109.7
IsK	20.10.15	MPS (200 µm)	262-200-100-50-20-0	262-200	CV	262.1	85.5	CV	370.0	184.3
IsK	02.11.15	MPS (200 µm)	260-200-100-50-20-0	260-100	CV	262.1	85.5	CV	370.0	184.3
IsK	01.12.15	MPS (200 µm)	243-200-100-50-20-0	200-100				CIV	160.5	68.5
IsK	01.12.15	MPS (200 µm)	243-200-100-50-20-0	200-100	CV	259.6	229.3	CV	420.7	195.8
IsK	11.01.16	MPS (200 µm)	258-200-100-50-20-0	258-100	CV	210.3	85.8	CV	335.7	89.3
IsK	18.01.16	MPS (200 µm)						F	777.3	217.0
IsK	18.01.16	MPS (200 µm)						M	739.9	163.3
IsK	17.02.16	WP2 (200 µm)	250-100-50-20-0	250-100	CV	274.2	125.5	CV	298.3	80.1
IsK	21.03.16	WP2 (200 µm)	250-100-50-20-0	250-100	CV	173.3	66.2	CIV	108.8	35.3
IsK	04.04.16	WP2 (200 µm)	260-100-50-20-0	50-0	CV	114.6	43.8	F	325.0	95.2
IsK	04.04.16	WP2 (200 µm)	260-100-50-20-0	260-180	CV	167.5	38.6	F	394.3	186.1
IsK	02.05.16	MPS (200 µm)		50-0	F	217.1	51.5	F	364.6	80.3
IsK	15.05.16	MPS (200 µm)	260-200-100-50-20-0	50-0	F	371.3	84.1	F	670.4	246.9
IsK	05.06.16	MPS (200 µm)	260-200-100-50-20-0	50-0	F	427.7	324.0	CIV	314.6	81.9
IsK	05.07.16	MPS (200 µm)	260-200-100-50-20-0	200-100	CV	214.2	51.6	CV	653.6	243.4
IsK	31.08.16	MPS (200 µm)	260-200-100-50-20-0	200-100	CV	305.4	112.3	CV	795.4	188.0
N-ICE	10.02.15	MPS (200 µm)	2600-600-200-50-20-0	-						
N-ICE	07.03.15	WP2 (200 µm)	1000-600-200-85-0	200-0	CV	170.6	39.3	F	520.4	150.0

N-ICE	07.03.15	WP2 (200 µm)	1000-600-200-85-0	200-0	F	205.0	50.4	F	917.9	242.8
N-ICE	14.03.15	WP2 (200 µm)	1000-600-200-85-0	200-0	CV	257.6	102.3			
N-ICE	14.03.15	WP2 (200 µm)	1000-600-200-85-0	200-0	F	350.0	162.9			
N-ICE	21.03.15	MPS (200 µm)	1500-600-200-50-20-0	-						
N-ICE	12.04.15	MPS (200 µm)	520-200-100-50-20-0	-						
N-ICE	12.04.15	MPS (200 µm)	670-200-100-50-20-0	-						
N-ICE	13.04.15	MPS (200 µm)	1000-600-200-50-20-0	-						
N-ICE	22.04.15	MPS (200 µm)		200-0						
N-ICE	25.04.15	MPS (200 µm)	1300-600-200-50-20-0	-						
N-ICE	26.04.15	MPS (200 µm)		600-100	F	1099.0	386.1			
N-ICE	03.05.15	MPS (200 µm)	1600-1000-600-200-50-0	600-100						
N-ICE	10.05.15	MPS (200 µm)	1700-600-200-50-20-0	100-0	F	860.5	230.9			
N-ICE	18.05.15	MPS (200 µm)	1250-600-200-50-20-0	100-0						
N-ICE	24.05.15	MPS (200 µm)	900-600-200-50-20-0	-						
N-ICE	25.05.15	MPS (200 µm)		50-0						
N-ICE	26.05.15	MPS (200 µm)		50-0						
N-ICE	31.05.15	MPS (200 µm)	730-600-200-50-20-0	100-0	CV	211.7	99.6			
N-ICE	03.06.15	MPS (200 µm)		100-0	CV	296.8	124.1			
N-ICE	03.06.15	MPS (200 µm)		100-0	F	338.0	60.2			
N-ICE	09.06.15	MPS (200 µm)	1900-600-200-50-20-0	-						
N-ICE	13.06.15	MPS (200 µm)		50-0						
N-ICE	16.06.15	MPS (200 µm)	730-600-200-50-20-0	-						
N-ICE	19.06.15	MPS (200 µm)	500-200-100-50-20-0	100-0	CV	361.3	191.2			
N-ICE	19.06.15	MPS (200 µm)		50-0	F	403.3	98.9	CV	380.0	30.0
Off shelf	15.09.15	MPS (200 µm)		680-200	CV	382.3	169.2			
Off shelf	15.01.16	MPS (200 µm)		50-0	CV					
Off shelf	15.01.16	MPS (200 µm)		600-200	CV					
Off shelf	29.08.16	MPS (200 µm)		600-200	CV	299.2	116.2			

Table S2: Correlation between the enzyme activity from January to May and the environmental factors Chlorophyll a max in water column (Chl a), Weighted mean depth of community (WMD), temperature at weighted mean depth (WMD temp) and Day length in Isfjorden, Svalbard. Correlations are calculated for all measurements done for the period of upregulation of enzyme activity, p value indicated by * as described underneath the table.

		Lipase	CS	MDH	HOAD	Chl a	WMD	WMD temp	Day length
C. finmarchicus	Proteinase	0.82***	0.91***	0.812***	-0.423	0.572**	-0.804***	-0.651**	0.819***
	Lipase		0.86***	0.766***	-0.288	0.818***	-0.874***	-0.555**	0.829***
	CS			0.862***	-0.0897	0.496*	-0.586*	-0.689**	0.906***
	MDH				-0.307	0.498*	-0.565*	-0.725**	0.806***
	HOAD					-0.568*	0.547*	0.263	-0.193
C. glacialis	Proteinase	0,738***	0,329	0,854***	-0,248	0,454*	-0,928***	-0,860***	0,878***
	Lipase		-0,105	0,631**	-0,264	0,912***	-0,817***	-0,530*	0,763***
	CS			0,312	0,192	-0,260	-0,370	-0,639**	0,439*
	MDH				-0,0829	0,404	-0,668**	-0,757***	0,784***
	HOAD					-0,0488	0,294	-0,347	0,196

Table S3:

	Lipase	CS	MDH	HOAD	Day length
Proteinase	0.778***	0.856***	0.621*	-0.556*	0.607**
Lipase		0.645**	0.427	-0.541*	0.593**
CS			0.869***	-0.234	0.43
MDH				-0.141	0.508*
HOAD					-0.574*

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