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Cleopatra II

Life history traits of copepods in a changing Arctic - Seasonal patterns in the physiology of *Calanus glacialis*

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List of abbreviations

AARS	Aminoacyl-tRNA synthethase
acetyl-CoA	Acetyl-Coenzyme A
ANOVA	Analysis of Variance
aqua dem.	Distilled water
AWI	Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research
C	Carbon
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
CBB	Commassie brilliant blue
Chl *a*	Chlorophyll *a*
CI - CV	Copepodite stage I - V
CLEOPATRA II	Climate effects on food quality and trophic transfer in the Arctic Marginal ice zone
CS	Citrate synthase
CVIF	Adult females
DM	Dry mass
DMSO	dimethyl sulfoxide
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EC	Enzyme Commission number
EDTA	ethylene-diamineteraacetic acid
FITC	Casein fluorescein isothiocyanate from bovine milk
HCl	Hydrochloric acid
HOAD	3-hydroxyacyl-CoA dehydrogenase
HPTS	8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt
indv.	Individual
K^+	Potassium
KV	Coast guard vessels
Li^+	Lithium
Mg^{2+}	Magnesium
MDH	Malate dehydrogenase
MSA	Methane sulfonic acid
MUF	Methylumbelliferyl

N	Nitrogen
Na^+	Sodium
NADH	nicotinamide adenine dinucleotide
NH_3	Ammonia
NH_4^+	Ammonium
pH_e	Extracellular pH
PPi	Pyrophosphate reagent
POLMAR	Helmholtz Graduate School for Polar and Marine Research
RFU	Relative fluorescence units
RV	Research vessel
SD	Standard deviation
SE	Standard error
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
spp.	Species
SR	Spearman rank order correlation
TAG	Triacylglycerols
TCA	trichloroacetic acid
Tris	Tris(hydroxymethyl)aminomethan
UNIS	The University Centre in Svalbard
WP-2	Working party 2 plankton sampling net
WP-3	Working party 3 plankton sampling net

Summary

In the last few decades, the Arctic has experienced rapid changes in the physical and biological marine environment. The sea ice cover shrinks, sea surface temperatures increase and the ice-free season prolongs, which results in profound changes in the food and light regime. In Arctic shelf seas, zooplankton communities are dominated by the large calanoid copepod *Calanus glacialis*, which links primary production with higher trophic levels. *C. glacialis* accumulates energy reserves in surface waters during the productive season and overwinters in a state referred to as diapause in deep waters. Diapause is characterized by arrested development and metabolic depression. The physiology and metabolism and the factors that determine the duration and timing of diapause in *C. glacialis* are, however, poorly understood. With ongoing environmental changes it is most important to understand the physiology and timing of life cycle events of *C. glacialis* in order to predict the effects of climate change on the pelagic food web. Thus, in a comprehensive approach, this study aims to tackle seasonal patterns in the physiology of *C. glacialis* and elucidate if changes in the metabolic activity are related to external cues, i.e. light, food and temperature, or if they are internally regulated.

Within the framework of the Norwegian research project CLEOPATRA II (Climate effects on food quality and trophic transfer in the Arctic marginal ice zone), *C. glacialis* was sampled monthly in Billefjorden, a high-Arctic sill fjord on the western coast of Svalbard. In order to investigate the influence of different environments on the physiology of *C. glacialis*, the copepods were also collected in Kongsfjorden and Rijpfjorden whenever logistically possible. In a combined field and experimental study, the biochemical composition, digestive and metabolic enzyme activities and pH and ion concentration in the haemolymph of the *C. glacialis* in different phases of diapause were related to depth distribution of the copepods and different food and light conditions.

The present study showed a clear seasonal pattern in digestive and metabolic enzyme activities as well as acid-base regulation and extracellular ion concentrations in *C. glacialis*. The physiological patterns were similar between *C. glacialis* populations from three fjords in the Svalbard archipelago. The timing of diapause, however, differed among the populations and was adjusted to the prevailing environmental conditions, i.e.

food, light and temperature regime. These findings suggest that *C. glacialis* may also be able to adjust its physiology and the timing of life cycle events to future climate driven changes in the physical and biological environment.

During overwintering, *C. glacialis* needs to be neutrally buoyant to save energy and minimize the risk to attract predators. Recently, ion replacement has been suggested as a means to fine-tune buoyancy in Antarctic diapausing copepods. In our study, we found that high-density cations (Na^+, Mg^{2+}, Ca^{2+}) in the haemolymph of *C. glacialis* are exchanged for low-density Li^+ ions during the winter and spring transition. The maximum Li^+ concentration in the copepods exceeded by far the Li^+ concentration in seawater, which suggests that this ion has a biological function and might support upward migration in *C. glacialis*. Our study did not find a correlation between the pH and ion concentration in the haemolymph, however, the pH followed a clear seasonal pattern and was low (pH 5.5) in winter and high (pH 7.9) in summer. Low extracellular pH values have previously been related to metabolic depression in marine organisms.

Besides the low pH in diapausing *C. glacialis*, we found low digestive enzyme activities (proteinase and lipase/esterase) in copepods at depth in winter and high activities during the productive season, when the copepods resided in surface waters. Digestive enzyme activities correlated closely with food availability in copepods from Billefjorden and also in individuals from the incubation experiments at different food and light conditions: the synthesis of enzymes increased with food availability. During the experiments, however, the increase in activity started about five days earlier in copepods which were in the activation phase of diapause compared to the ones which were in the beginning of the diapause period. Thus, the response time of *C. glacialis* to changes in environmental conditions varies depending on the diapause phase.

Metabolic enzyme activities were about 50% lower in diapausing *C. glacialis* as compared to active individuals, while it was the other way around for catabolic enzyme activity. During the activation phase of diapause in late winter, high catabolic enzyme activities were followed by profound changes in the biochemical composition and a drop in lipid content, which was probably related to moulting, gonad maturation and egg production. Thus, metabolic activities of the shelf species *C. glacialis* were relatively high during diapause and in conclusion, our findings suggest that this species

does not overwinter in 'true' diapause. Future studies should investigate intra- and interspecific variability in the physiology of copepods during diapause to provide a comprehensive definition of metabolic and physiological characteristics of diapausing copepods.

Zusammenfassung

In den letzten Jahrzehnten haben sich die physikalischen und biologischen Umweltbedingungen in der Arktis zunehmend verändert. Die Meereisfläche nimmt kontinuierlich ab, die Temperaturen an der Meeresoberfläche steigen an und die Zeiträume, in denen die Arktis gebietsweise eisfrei ist, verlängern sich. Die Zooplanktongemeinschaften der arktischen Schelfmeere werden von der calanoiden Copepodenart *Calanus glacialis* dominiert, die ein Bindeglied zwischen den Primärproduzenten und höheren trophischen Ebenen darstellt. *C. glacialis* akkumuliert Energiereserven in Form von Lipiden in den Oberflächengewässern während der produktiven Jahreszeit und überwintert in so genannter Diapause im Tiefenwasser. Diapause ist durch eine gehemmte Entwicklung der Copepoden und stark reduzierte Stoffwechselprozesse charakterisiert. Die zugrundeliegenden Stoffwechselprozesse und die Faktoren, die den Zeitpunkt und die Dauer der Diapause bestimmen, sind jedoch kaum verstanden. Bei anhaltenden Veränderungen der arktischen Meeresumwelt ist es jedoch wichtig, die Physiologie während der verschiedenen Lebenszyklusereignisse von *C. glacialis* zu verstehen, um die Auswirkungen des Klimawandels auf das pelagische Nahrungsnetz vorhersehen zu können. Aus diesem Grund untersucht die vorliegende Studie saisonale physiologische Veränderungen in *C. glacialis* in einem umfassenden Ansatz und versucht zu erklären, ob Stoffwechselaktivitäten sich aufgrund externer Signale wie Licht, Futter und Temperatur ändern, oder ob diese Prozesse intern geregelt sind.

Im Rahmen des norwegischen Projektes CLEOPATRA II (Climate effects on food quality and trophic transfer in the Arctic marginal ice zone) wurde *C. glacialis* ein Jahr lang monatlich in dem arktischen Schwellenfjord Billefjorden, an der Westküste von Spitzbergen, gesammelt. Um den Einfluss von verschiedenen Habitaten auf die Physiologie von *C. glacialis* zu untersuchen, wurden zudem Proben im Kongsfjord und Rijpfjord genommen, sofern dies logistisch möglich war. Im Zusammenhang mit dem Nahrungsangebot und der Tiefenverteilung der Tiere wurde die biochemische Zusammensetzung, Aktivitäten von Verdauungsenzymen und metabolischen Enzymen sowie der extrazelluläre pH und die Ionenkonzentration in der Hämolymphe der Copepoden bestimmt. In einer kombinierten Feld- und experimentellen Studie wurde die physiologische Reaktion von *C. glacialis* in verschiedenen Diapausephasen auf unterschiedliche Futter- und Lichtbedingungen untersucht.

Im Rahmen der vorliegenden Studie wurden deutliche saisonale Veränderungen in der Aktivität von Verdauungsenzymen und metabolischen Enzymen sowie in der Säure-Base-Regulation und Ionenkonzentration in der Hämolymphe von *C. glacialis* gefunden. Die physiologischen Veränderungen waren ähnlich zwischen den Populationen in den drei Fjorden im Spitzbergen-Archipel. Abhängig von den vorherrschenden Umweltbedingungen unterscheiden sich jedoch der Zeitpunkt und die Dauer der Diapause in den Populationen. Diese Anpassungen an äußere Bedingungen lassen vermuten, dass *C. glacialis* möglicherweise in der Lage sein wird, seinen Lebenszyklus an klimabedingte Änderungen in der physikalischen und biologischen Umwelt anzupassen.

Ein neutraler Auftrieb ermöglicht es den überwinternden Copepoden, möglichst wenige Energiereserven zu verbrauchen und den Fraßdruck zu verringern. Vor Kurzem wurde im Rahmen einer Studie mit antarktischen Copepoden ein Ionenaustausch als möglicher Mechanismus zur Feinregulation des Auftriebs vorgeschlagen. Im Rahmen unserer Studie haben wir herausgefunden, dass während des Überwinterns Li^+-Ionen gegen solche Ionen, die die Dichte erhöhen (Na^+, Mg^{2+}, Ca^{2+}), in der Hämolymphe von *C. glacialis* ausgetauscht werden. Die maximale Li^+-Konzentration in den Copepoden überschritt bei Weitem die Li^+-Konzentration im Meerwasser. Dies lässt Rückschlüsse auf eine mögliche biologische Funktion des Lithiums zu und lässt vermuten, dass es eine unterstützende Funktion beim Aufstieg in die Oberflächengewässer hat. Unsere Studie konnte keine Korrelation zwischen der Ionenkonzentration und dem pH in der Hämolymphe der Copepoden nachweisen, jedoch war ein klarer saisonaler Verlauf im extrazellulären pH zu erkennen. Der pH war niedrig im Winter (pH 5,5) und hoch im Sommer (pH 7,9). Ein niedriger extrazellulärer pH wurde bereits in anderen Studien mit einer verringerten Stoffwechselaktivität in marinen Organismen in Verbindung gebracht.

Neben einem niedrigen extrazellulären pH wurden im Rahmen dieser Studie eine geringe Verdauungsenzymaktivität (Proteinase und Lipase/Esterase) in überwinternden Copepoden und hohe Aktivitäten in Individuen aus Oberflächengewässern im Frühjahr und Sommer gefunden. Die Verdauungsenzymaktivität war eng mit der Nahrungsverfügbarkeit im Billefjord verknüpft, und auch während der Inkubationsexperimente unter verschiedenen Futter- und Lichtbedingungen konnte eine

Zunahme der Aktivität mit vorhandener Nahrung nachgewiesen werden. Die Zunahme der Verdauungsenzymaktivität zeigte sich fünf Tage später in den Copepoden, die sich zur Versuchszeit im Beginn der Diapause befanden, im Vergleich zu den in Copepoden in der Aktivierungsphase. Somit kann die Diapausephase möglicherweise Auswirkungen auf die Reaktionszeit der Copepoden haben.

In überwinternden Copepoden waren die metabolischen Enzymaktivitäten halb so hoch wie in aktiven Tieren, wohingegen die katabolischen Enzymaktivitäten in den Copepoden im Winter höher waren als im Sommer. Während sich die Copepoden in der Aktivierungsphase der Diapause befanden, wurde im Zusammenhang mit hohen katabolischen Enzymaktivitäten eine starke Veränderung in der biochemischen Zusammensetzung und ein Abfall im Lipidgehalt beobachtet, was vermutlich auf Prozesse wie Häutung, Gonadenreife und Eiproduktion zurückzuführen ist. Aus den relativ hohen metabolischen Aktivitäten in überwinternden Individuen lässt sich schlussfolgern, dass die Schelfart *C. glacialis* möglicherweise nicht in einer ‚echten‘ Diapause überwintert. Zukünftige Studien sollten die intra- und interspezifische Variabilität in der Physiologie von überwinternden Copepoden untersuchen, um eine umfassende Definition der physiologischen und metabolischen Anpassungen, die der Diapause zugrunde liegen, zu ermöglichen.

1 Introduction

1.1 Scientific background

Zooplankton communities worldwide are dominated by calanoid copepods (Longhurst 1985, Fransz and Gonzalez 1997) and three *Calanus* species, i.e. *C. finmarchicus*, *C. glacialis* and *C. hyperboreus* are particularly abundant in terms of biomass in Arctic ecosystems (Jaschnov 1970). As mainly herbivores, *Calanus* spp. link primary production with higher trophic levels, such as carnivorous zooplankton and commercially important fish, but also whales and seabirds (Runge 1988, Beaugrand et al. 2003, Wold et al. 2011, Kraft et al. 2013). *Calanus* spp. are important contributors to the energy flux in Arctic ecosystems, since they transform low-energy carbohydrates and proteins from their algae nutrition into energy-rich wax esters (Falk-Petersen et al. 2009).

The life cycles of the three *Calanus* species are well adapted to the strong seasonality in food availability and light regime, which makes them good indicators of environmental changes and the factors that influence the health and productivity of the Arctic ecosystem (Hays et al. 2005, Blachowiak-Samolyk et al. 2008). *Calanus* spp. perform ontogenetic vertical migration to survive food scarcity and avoid predation risk in deep waters during winter and accumulate energy reserves in surface waters during the productive season (Conover 1988, Kaartvedt 2000). The three species differ in their distribution, which is displayed in differences in their life history traits, i.e. life cycle length, reproduction and body mass (Conover & Huntley 1991, Falk-Petersen et al. 2009).

The smallest and boreal species *C. finmarchicus* (Gunnerus) is mainly associated with Atlantic water masses. It is transported northwards by the North Atlantic current into the Arctic Ocean and the Barents Sea. Its center of distribution is the Norwegian Sea and the Labrador Sea (e.g. Fleminger & Hulsemann 1977, Conover 1988, Planque et al. 1997). Depending on the environmental conditions, *C. finmarchicus* has one to three generations per year (Marshall and Orr 1955, Conover 1988). Gonad maturation and reproduction depend strongly on food availability and egg production only occurs at low rates before the phytoplankton bloom (Niehoff et al. 1999, Campbell et al. 2001).

The largest species, *C. hyperboreus* Krøyer is an oceanic species and its distribution extends into the Arctic Basin, the Greenland Sea, the Baffin Bay, the Canadian Archipelago and southwards into the Norwegian Sea and the Gulf of Maine (Grainger 1961, Jaschnov 1970, Conover 1988, Hirche 1991, Hirche and Mumm 1992). The life cycle of *C. hyperboreus* is two to four years (Hirche 1997, Arnkværn et al. 2005). *C. hyperboreus* reproduces based on its internal energy reserves and thus, uncoupled from the phytoplankton bloom (Niehoff et al. 2002).

The medium-sized *C. glacialis* Jaschnov (Fig. 1.1) is a shelf species and endemic to the Arctic. It is transported southwards with the East Greenland Current and also inhabits the northwest coast of North America and the White Sea (Grainger 1961, Conover 1988, Falk-Petersen et al. 2009). Dependent on the temperature and food availability, the life cycle of *C. glacialis* is completed within one or two years (Hirche 1998, Kosobokova 1999). *C. glacialis* has a mixed reproductive strategy: gonad maturation and egg production start based on internal energy reserves and then, females increase spawning frequency by feeding on the icealgae and phytoplankton bloom (Smith 1990, Hirche & Kattner 1993). The offspring then exploits the phytoplankton bloom to grow and develop (Søreide et al. 2010, Box 1). This flexible reproductive strategy of *C. glacialis* makes this species so successful and abundant in Arctic shelf areas. Important predators, such as the little auk (*Alle alle*) and the bowhead whale (*Balaena mysticetus*), rely on the energy-rich *C. glacialis* (Karnovsky et al. 2003, Rogachev et al. 2008). With proceeding changes in sea surface temperature, ice coverage and subsequent changes in food supply (Arrigo et al. 2008, Stroeve et al. 2012), *C. glacialis* might be replaced by the smaller and less energy-rich *C. finmarchicus*, with yet unpredictable consequences for the Arctic ecosystem (Reygondeau & Beaugrand 2011, Wassmann et al. 2011).

Numerous publications have considered the morphology, distribution, life cycle and body composition of the Arctic *Calanus* species (see reviews by Conover & Huntley 1991, Niehoff 2007 and Falk-Petersen et al. 2009). Studies on the physiology and the metabolism of *C. glacialis*, in contrast, are rare. The few available studies investigated respiration rates or feeding activity in spring and summer or early autumn (e.g. Båmstedt 1984, Båmstedt & Tande 1985, Tande 1988, Seuthe et al. 2007), but winter studies on the physiology of *C. glacialis* are completely lacking. Thus, the overwintering strategy and the physiology of *C. glacialis* in a seasonal context are yet

poorly understood. This knowledge, however, is crucial to assess the influence of a changing environment on the survival success of this important Arctic shelf species. This study investigates in a comprehensive approach how *C. glacialis* adjusts its metabolism and physiology during all seasons in a high Arctic fjord.

Fig. 1.1 The Arctic shelf copepod species *Calanus glacialis*

Box 1 Reproductive strategy of *Calanus glacialis* and mismatch-hypothesis after Søreide et al. (2010).

Arctic shelf seas, which are the habitat of *Calanus glacialis*, are characterized by two distinct algal blooms, i.e. the ice algae bloom and the phytoplankton bloom. The ice algae bloom starts in March, when the light returns and the phytoplankton bloom begins with the ice break-up (Hegseth 1998). Timing and magnitude of both blooms are strongly dependent on the prevailing ice conditions (Ji et al. 2013). As climate changes, the sea-ice cover shrinks and ice-free seasons become longer (Gough et al. 2004, Comiso et al. 2008, Stroeve et al. 2012). This will alter the underwater light regime and have severe effects on the timing and intensity of primary production (Arrigo et al. 2008, Kahru et al. 2011).

The reproduction and growth success of *C. glacialis* depends on the quantity and quality of the algae diet. *C. glacialis* females feed on the ice algae and phytoplankton bloom to fuel gonad maturation and spawning. Then, nauplii and copepodites rely on the phytoplankton bloom to grow and develop. With a changing climate in the future, the match between the reproduction of *C. glacialis* and both blooms may be disturbed with yet unpredictable consequences for the entire lipid-driven Arctic ecosystem (Fig. B1.1).

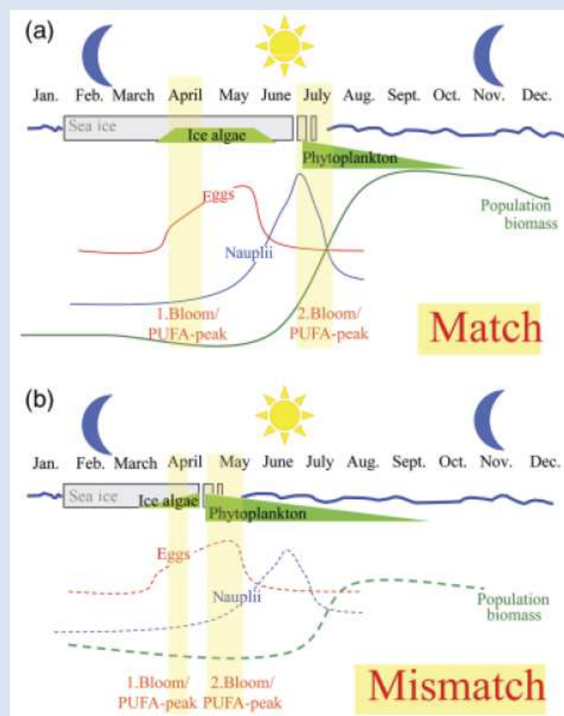

Fig. B1.1 Timing of algal blooms (poly unsaturated fatty acid peaks, PUFA) in Arctic shelf seas. The current time lag (a) between both blooms allows *Calanus glacialis* to start reproduction with the ice algae bloom and the offspring (nauplii and copepodites) to grow with the phytoplankton bloom. With the future scenario of an earlier ice break-up, the growth season for icealgae shortens and the time lag between both peaks becomes shorter (b). This may lead to a decrease in *C. glacialis* population biomass (from Søreide et al. 2010).

1.2 Dormancy

Dormancy is defined as a state of arrested development and growth of an organism (Danks 1987). The range of physiological adjustments in organisms performing dormancy is manifold and shows a broad intra- and interspecific variability due to (i) differences in environmental conditions, (ii) differences in the evolutionary history of taxa and (iii) the necessity to time life cycle events with environmental conditions (Danks 2002). Dormancy occurs in animals from all taxa and latitudes and is a physiological response to various biotic and abiotic environmental stressors, such as temperature, oxygen availability, photoperiod, hypersalinity, predation risk or food scarcity (Dahms 1995). To save energy, the metabolic activity of an organism during dormancy is adjusted. The intensity of this metabolic adjustment varies intra- and interspecific from almost zero to around 80% of the normal resting metabolic rate (Guppy & Withers 1999).

The following forms of dormancy may be distinguished (Mansingh 1971, Danks 1987, Guppy & Withers 1999):

- Quiescence is defined as a direct physiological response to unfavorable environmental conditions. Quiescence is a short-term phenomenon which may occur irregularly whenever adverse environmental conditions arise (Mansingh 1971, Danks 1987). The maximal metabolic depression can occur within hours. In embryos of the brine shrimp *Artemia franciscana*, for example, the concentration of the high-energy molecule adenosine triphosphate (ATP) declines by 80% within a few hours as a response to anoxic conditions (Hand & Podrabsky 2000).

- Diapause: In contrast to quiescence, diapause is controlled endogenously and thus, a compulsory physiological reaction to recurring adverse environmental conditions (Dahms 1995). Diapause is performed by specific ontogenetic stages (Dahms 1995, Guppy & Withers 1999) and it is mainly common in arthropods, especially insects and crustaceans (Elgmork & Nilssen 1978, Dahms 1995).

- Hibernation: Metabolic depression in endotherms is termed hibernation. Hibernation is characterized by reduced heart rates and body temperature in

mammals and birds during winter (review by Guppy & Withers 1999). The top predator of the Arctic food chain, the polar bear (*Ursus maritimus*), is one of the most common examples of an Arctic hibernating mammal (Nelson et al. 1983).

Dormancy in copepods

In copepods, dormancy is distinguished into quiescence and diapause (Dahms 1995, Hirche 1996). It is noteworthy that most studies on copepods do not provide an accurate differentiation into one of the two dormancy forms. In the following, the term dormancy will be used, whenever referring to a study in which a clear definition of quiescence and diapause is not given.

Dormancy is exclusively performed by free-living copepods of the taxa Harpacticoida, Cyclopoida and Calanoida of freshwater and marine habitats (review by Williams-Howze 1997). Ontogenetic stages that can initiate dormancy are resting eggs, nauplii and copepodites (Dahms 1995). Quiescence in copepods is mostly found in the form of dormant eggs in sediments (Uye 1985, Dahms 1995). Dormant nauplii are only described for four harpacticoid and two calanoid species (Coull & Dudley 1976, Uye 1980). The importance of dormancy in later copepodite and adult developmental stages increases generally towards the poles (Watson 1986). For dormant cyclopoid copepodites the term “active diapause” was formed and is defined by arrested development and reproduction, but only minimal reduction in activity (Elgmork 1980, Krylov et al. 1996).

Mansingh (1971) classified diapause in insects into five phases and a few years later the same phases were also described in copepods (Elgmork & Nilssen 1978, Hirche 1996):

- During the preparatory phase, organisms accumulate energy reserves and arrest their development and growth.

- During the induction phase, the metabolic activity of organisms is low and they stop feeding.

- The refractory phase is usually the longest phase of diapause and can last more than half a year. Organisms are torpid and some species are potentially able to

survive anaerobiosis. The metabolic activity reaches the lowest levels during the refractory phase.

- The activation phase usually starts when half of the diapause time is reached. The animals regain their ability to develop and processes like gonadogenesis start.

- During the termination phase, organisms regain their full potential of metabolic activity, growth and development. The termination phase usually takes place in spring.

Depending on habitat, latitude and season, dormancy strategies may vary profoundly among species and even within populations of the same species (Dahms 1995). The calanoid *Labidocera aestiva* is an example for intraspecific variation in dormancy features. It produces resting eggs in a population off the northeast coast of the US, close to Woods Hole, while a population further south does not show this feature (Marcus 1980). Interspecific differences are found in the overwintering strategies of copepods from the Arctic and the Antarctic. Dormant species are found in both hemispheres, however, as the tendency to perform dormancy increases towards the poles, there are less dormant species in the Antarctic.

The calanoid copepod species *Calanoides acutus*, *Calanus propinquus* and *Metridia gerlachei* dominate the Antarctic zooplankton communities in terms of biomass (Schnack-Schiel et al. 1991, Hopkins 1993). All three species perform ontogenetic vertical migration and accumulate lipid reserves during the productive season in spring and summer (Schnack-Schiel & Hagen 1995), however, *C. acutus* seems to be the only species which performs diapause (Atkinson 1998). *C. propinquus* mainly remains in the upper 200 m and *M. gerlachei* is more dispersed over the water column, while *C. acutus* is almost exclusively found in deep waters during winter. Moreover, the former two species accumulate triacylglycerols, while *C. acutus* accumulates wax esters as lipid reserves during the productive season (see 1.3.1 characteristics of lipids, Schnack-Schiel & Hagen 1995).

In the Arctic, three of the largest copepod species, i.e. *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*, are known to perform ontogenetic vertical migration and overwinter in diapause (Hirche 1998). *C. glacialis* is similar to *C. acutus*, as both species are able to start reproduction before the phytoplankton bloom, but their spawning frequency increases with food availability (Smith 1990, Hirche & Kattner 1993). The Arctic counterpart to *M. gerlachei* is *Metridia longa*. Both species are omnivorous and active during winter, but *M. longa* accumulates more wax esters (Hopkins et al. 1984). At both poles, life history traits of the copepods are adapted to a strong seasonality in light and food regime (reviews by Conover and Huntley 1991 and Smith and Schnack-Schiel 1990). However, the hydrography and ice-cover differs between the Arctic and the Antarctic. The Antarctic Circumpolar Current is the dominant circulation that flows clockwise around the Antarctic, while the Arctic experiences a constant inflow of the North Atlantic Current. The light regime in the Arctic is characterized by a longer polar night compared to the Antarctic. Moreover, multiyear ice is more common in the Arctic. These difference in photoperiod and ice-coverage result in a higher primary production in the Antarctic (Codispoti et al. 1991), which may be a reason for the different overwintering strategies of the dominant copepods between the poles (review by Smith and Schnack-Schiel 1990).

All Arctic *Calanus* species perform ontogenetic vertical migration, accumulate wax esters in surface waters during the productive season and rely on these internal energy reserves when they are in deep waters during winter (Conover 1988, Falk-Petersen et al. 2009 and see Fig. 1.2 for *C. glacialis*). Depending on the habitat and especially the food supply, the copepods are able to develop and overwinter in different stages, i.e. mainly CIV, CV and adult females in *C. finmarchicus* and *C. glacialis* (Fig. 1.2), while *C. hyperboreus* may also accumulate enough energy reserves and overwinter as CIII (Hirche 1998). Overwintering *Calanus* spp. were characterized by torpidity (Hirche 1983), low digestive enzyme activity (Head and Conover 1983, Hirche 1983), low respiration rates (Marshall and Orr 1958, Ingvarsdóttir et al. 1999) and high content of polyunsaturated fatty acids in their wax esters (Clark et al. 2012). However, to what extent these characteristics apply to all three *Calanus* species in open ocean and shelf habitats has yet to be clarified. Most of the available knowledge on the overwintering strategy in *Calanus* spp. derives from studies on *C. finmarchicus* (Hirche 1996, Hind et al. 2000). This species has been studied extensively as it is an important food source for

commercially important fish and occurs in logistically easier accessible areas compared to *C. glacialis* and *C. hyperboreus*, which inhabit mostly high Arctic ecosystems.

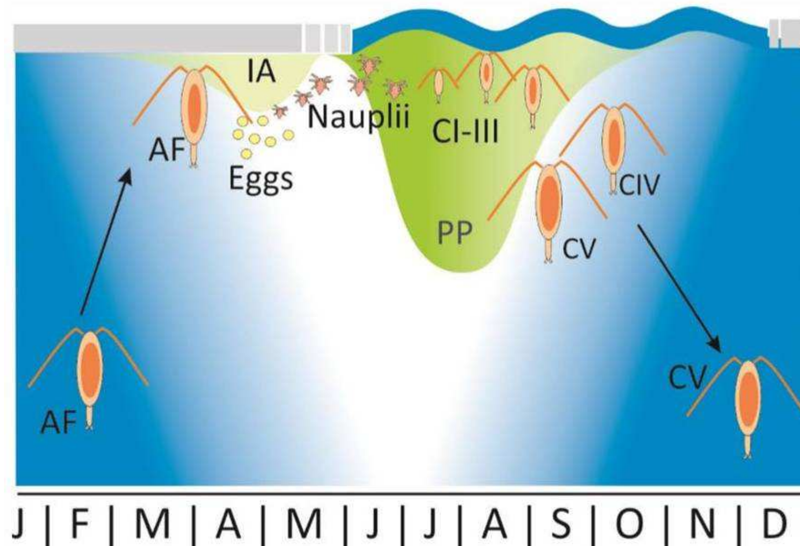

Fig. 1.2 Schematic overview of the life cycle of *Calanus glacialis* in Arctic shelf seas. The system is ice-covered from December to early June. The ice algae (IA) bloom starts in March and the phytoplankton (PP) bloom begins with the ice break-up. Adult females (AF) of *C. glacialis* reproduce in surface waters in spring. *C. glaclialis* develops from nauplii to copepodite stages I to V during spring and summer and descend mainly as stage V in late autumn. Before the population ascends in late winter/ early spring, individuals moult to adult males and females. (Picture credit: Malin Daase)

The factors, which determine the timing and intensity of diapause in *Calanus* spp. are still not identified and an external and internal regulation of the onset and the termination of diapause were suggested. As external cues, sea surface temperature (Kosobokova 1999, Niehoff & Hirche 2005), food availability (Søreide et al. 2008, 2010, Daase et al. 2013) and photoperiod (Miller et al. 1991) were suggested. Possible internal factors that were discussed to regulate diapause in *Calanus* spp. are endogenous clocks (Miller et al. 1991), neurosecretion of hormones (Carlisle & Pitman 1961), lipid content (Irigoien 2004) or a changes in the extracellular pH (Schründer et al. 2013).

1.3 Physiological characteristics of copepods

Copepods have evolved life strategies that are well adapted to the prevailing biotic and abiotic conditions of the environment they inhabit. In the following, characteristic biochemical and physiological adaptations of copepods will be listed with a focus on species from polar regions.

1.3.1 Biochemical composition

The major organic components in copepods are proteins, lipids and carbohydrates. Depending on the seasonality in environmental factors, the composition of these components differs in copepods.

- *Proteins*: Proteins are involved in building up muscle tissue, catalyzing metabolic reactions as enzymes and transporting molecules over membranes. Their amino acid sequence and the resulting three dimensional structure determine the characteristics and functioning of proteins. New proteins are formed during protein synthesis from amino acids, which are obtained either from internal stores or from dietary proteins. Copepods from high latitudes possess relatively lower protein contents compared to species from temperate or tropical regions, i.e. the protein content in copepods per dry mass ranges from approximately 20 to 80% (Båmstedt 1986).

- *Lipids*: Lipids are very suitable to store large amounts of energy on small volumes, because they have a high energy content (39 kJ g^{-1}) compared to proteins (18 kJ g^{-1}). The role of lipids is manifold: they are components of biomembranes (phospholipids), as hormones and antioxidants, they are involved in various physiological processes, they are used as energy stores and they regulate buoyancy (Lee et al. 2006). In copepods, the lipid content per dry mass ranges from 5 to 20% in species from temperate and tropical regions to 75% in species from high latitudes (Båmstedt 1986, Lee et al. 2006). Especially in copepods from polar regions, lipid reserves play a crucial role for surviving several months of food deprivation (Falk-Petersen et al. 2009). Marine zooplankton show four types of storages lipids, i.e. triacylglycerols (TAG), wax esters, phospholipids and diacylglycerol ethers (Lee et al. 2006). In copepods the first two are the main energy stores. Wax esters are especially found in polar

species (Scott et al. 2000), while TAGs are more common in species from temperate regions (Kattner et al. 1981, Kreibich et al. 2008, 2010). Wax esters are less rapidly metabolized than TAGs and thus, are more suitable for long-term deposits over several months during winter (Lee et al. 2006). In contrast, relatively high amounts of TAGs in copepods point to a continuous food supply (Kreibich et al. 2011). The composition of lipids provides information on dietary relationships between phytoplankton and zooplankton (Dalsgaard et al. 2003). This so called trophic biomarker concept is based on studies, which showed that dietary fatty acids are transferred conservatively into lipid stores of the primary consumers (e.g. Graeve et al. 1994, Graeve et al. 2005, Søreide et al. 2008).

- *Carbohydrates*: In terms of quantity, carbohydrates play a minor role in copepods and constitute less than 5% per dry mass (Båmstedt 1986). Carbohydrates mainly occur as polysaccharides in copepods, e.g. as chitin that constitutes the exoskeleton (Ikeda 1972, Båmstedt 1986).

1.3.2 Enzyme activities

Digestive activities

Digestive enzymes are the functional link between food ingestion and assimilation. Mayzaud (1986) divides digestion into three steps, i.e. the mechanical phase, the chemical phase and the intracellular phase. In the mechanical phase food particles are grinded by the mandible blades. During the chemical phase, so called B-and F-cells produce digestive enzymes in the mid-gut. These enzymes hydrolyze organic macromolecules, i.e. proteinases act on peptide bonds; lipases and esterases act on carboxyl ester bonds and glycosidases act on polysaccharides. In the intracellular phase, semi-digested dietary particles are absorbed into gastrodermal cells, where they are further hydrolyzed by intracellular enzymes into basic molecules, i.e. amino acids, fatty acids and hexoses.

Enzyme functioning is influenced by temperature and pH (Feller & Gerday 1997, Freese et al. 2012). Activities increase until a specific temperature and pH optimum and then decrease due to thermal instability and denaturation of the enzyme (Cornish-Bowden 1995). The temperature optimum of enzyme activity in crustaceans is usually

between 30 and 50°C (Vetter 1995, López-López et al. 2003, Solgaard et al. 2007). In a recent study Freese et al. (2012) found thermal adaptation in digestive enzyme activity in *C. glacialis*. In *C. glacialis*, lipase/esterase activities were higher between 0 and 20°C compared to the activities in two boreal species. Cold adaptation is defined as all physiological adjustments that allow the copepods to survive in cold environments (Clarke 1991, Somero 1997). On the enzymatic level, cold adaptation results in low activation energies or a shift of the optimum to low temperatures (Clarke 1991, Feller 2003). The median gut pH in copepods is between pH 6 and 8 (Pond et al. 1995). In this pH range, enzyme classes, which cleave dietary proteins in crustaceans, reach their optimum pH (Saborowski et al. 2004, Solgaard et al. 2007).

Since the early 1930’s, researchers have assessed the feeding activity of copepods by using digestive enzyme activities as proxies (Bond 1934, Hasler 1935). Since then, some authors discovered a positive correlation between digestive enzyme activity and food availability (Mayzaud & Conover 1976, Hirche 1981), whereas others found a negative (Hassett & Landry 1983) or no correlation (Båmstedt 1984). Thus, it has been under debate if enzymes may be used as proxies for the feeding activity in copepods (Oosterhuis & Baars 1985). However, in a seasonal context or compared at different experimental conditions, digestive enzymes can reflect a correlation between the availability and uptake of food (Boucher & Samain 1974, Hassett & Landry 1983).

Metabolic activities

The metabolism of an organism includes anabolic processes, during which macromolecules are synthesized and catabolic processes, during which internal substances are degraded and converted into energy. Metabolic activity scales with temperature and, thus, the metabolic rate of ectothermic animals is severely affected by the temperature of the surrounding environment (Kinne 1963, Somero 1997, Pörtner 2002). Moreover, metabolic activity negatively correlates with body size, i.e. the smaller a copepod, the higher its specific metabolic activity (Hassett 2006). Copepods with high lipid stores per dry mass show low specific metabolic rates, because lipids are metabolic inactive tissue (Hassett 2006).

In crustaceans, the metabolic potential is usually either assessed by measuring respiration rates (Marshall & Orr 1958, Mayzaud 1976, Morata & Søreide 2013) or

enzyme activities (Saborowski & Buchholz 2002, Kreibich et al. 2008, Meyer et al. 2010). The rationale behind the latter is based on the observation that changes in basic metabolic and catabolic pathways are reflected in changes of enzyme activities (Auerswald et al. 2009). In contrast to respiration measurements, the determination of enzyme activities minimizes artifacts, like for example stress, which are caused by handling the animals in the laboratory (Ohman et al. 1998).

The following enzymes are representatives of important metabolic processes in crustaceans and were investigated in this study of *C. glacialis*: Citrate synthase (CS) and malate dehydrogenase (MDH) are an index for the overall metabolic activity; they are both enzymes of the citric acid cycle (Meyer et al. 2002, Kreibich et al. 2008, Teschke et al. 2007). MDH also transports electrons between the cytosol and the mitochondrion and correlates with respiration (Meyer et al. 2010), while CS was correlates with egg production in calanoid copepods (Kreibich et al. 2008). Aminoacyl-tRNA synthethase (AARS) catalyzes the first step of the protein synthesis and has been used as a proxy for growth and state of dormancy in *C. finmarchicus* (Yebra et al. 2006). Three-hydroxyacyl-CoA dehydrogenase (HOAD) is a key enzyme of the β-oxidation of fatty acids and thus, it is a proxy for the lipid catabolism (Auerswald & Gäde 1999, Hassett 2006).

So far, most studies on crustaceans only concern enzymes of one specific metabolic pathway, e.g. citrate synthase as a key enzyme for the citric acid cycle (Clarke & Walsh 1993, Vetter 1995). Studies that measure enzymes of various pathways in a comprehensive approach are rare in the research of copepods, but allow observing switches between metabolic pathways (Auerswald et al. 2009, Hassett 2006).

1.4 Vertical migration - The role of buoyancy

The overwintering success of *C. glacialis* depends on its ability to remain in deep waters without depleting its energy reserves by moving around and attracting predators (Varpe et al. 2007). To stay in a certain water depth, the copepods need to be neutrally buoyant with the surrounding seawater. Low-density lipids have been discussed to play a major role in buoyancy regulation during overwintering (Irigoien 2004, Pond 2012). These lipids are compressed at water depths below 500 m and then, the copepods become heavier and sink until they reach neutral buoyancy (Pond 2012). The lipid

content of the copepods, however, changes during winter because of energy consuming processes such as moulting, gonad maturation and reproduction (Campbell and Dower 2003, Campbell 2004).

Recently, ion replacement has been suggested as another mechanism to fine-tune buoyancy in diapausing copepods (Sartoris et al. 2010, Schründer et al. 2013, 2014). During winter, ions that increase the density of the copepods (e.g. Na^+, Mg^{2+}) are replaced by ions that reduce the density (e.g. NH_3/NH_4^+) in order to prevent the organism from sinking. This ion replacement causes an increase in the concentration of NH_3/NH_4^+ in the haemolymph of copepods. NH_3 is potentially toxic and thus, the copepods need to shift the chemical equilibrium to the less diffusible NH_4^+ by lowering their extracellular pH (Sartoris et al. 2010, Schründer et al. 2013). A seasonal correlation between a low pH and high concentrations of NH_4^+ ions, however, has not yet been shown in Arctic copepods. Beside a potential influence on the NH_3/NH_4^+ equilibrium in the haemolymph (Sartoris et al. 2010, Schründer et al. 2013), a low extracellular pH has been suggested to be associated with metabolic depression in the marine worm *Sipunculus nudus* (Reipschläger and Pörtner 1996). In copepods, however, a seasonal relation between extracellular pH and metabolic depression has not yet been observed.

1.5 Aims and outline of this thesis

Physical and biological conditions in the Arctic are changing with yet unpredictable consequences for marine life. The duration and extent of sea ice cover diminish due to an increased inflow of relatively warm Atlantic water masses and rising sea surface temperatures. This severely affects the timing and intensity of primary production. The consequences of these changes on the physiology of the mainly herbivorous shelf species *C. glacialis* are yet not foreseeable. *Calanus glacialis* is an important contributor to the energy flux in Arctic shelf areas. At present, however, the metabolic and physiological processes underlying the life cycle of *C. glacialis* are not well understood and the mechanisms that induce and terminate diapause are still elusive. Specifically, previous studies did not determine if the overwintering period is regulated by environmental cues or internal processes. Moreover, the timing and intensity of metabolic adjustments during diapause of *C. glacialis* are not yet characterized. Thus, this study aims to tackle seasonal patterns in metabolic and physiological processes in

C. glacialis by combining an extensive field study with experiments at different food and light conditions in the laboratory.

This thesis addresses the following main objectives:

I. Investigating to what extent *C. glacialis* regulates its cation concentration and pH in the haemolymph during activity and diapause (**Manuscript I**)
→ Hypothesis: *C. glacialis* accumulates cations that reduce its density, like e.g. NH_4^+, and has a low extracellular pH during winter and vice versa during summer

II. Relating digestive enzyme activities to the seasonal migration of *C. glacialis* (**Manuscript II**)
→ Hypothesis: Digestive enzyme activities of *C. glacialis* relate solely to food availability

III. Relating the activities of enzymes of key metabolic processes to the seasonal migration of *C. glacialis* (**Manuscript III**)
→ Hypothesis: The metabolic activity of *C. glacialis* is close to zero in individuals that reached the overwintering water depth

IV. Comparing the physiology of *C. glacialis* populations from three fjords in the Svalbard archipelago (**Synopsis chapter 4.1**)
→ Hypothesis: Depending on the environmental conditions the physiological adjustments vary between the *C. glacialis* populations

V. Investigating the physiological response of *C. glacialis* during diapause to different food and light conditions (**Synopsis chapter 4.2**)
→ Hypothesis: The physiological response of diapausing *C. glacialis* will be faster in individuals, which are in the activation phase compared to copepods, which are in the beginning of diapause.

Ultimately, this study aims to elucidate the seasonal patterns in the metabolism and biochemical composition of *C. glacialis*. The study was conducted within the framework of the Norwegian research project CLEOPATRA II (Climate effects on food

quality and trophic transfer in the Arctic marginal ice zone funded by the Research Council of Norway), which is a cooperation project between scientists from the University Centre in Svalbard (UNIS) and the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI) in Bremerhaven. By analyzing *C. glacialis* that was sampled year-round from July 2012 to July 2013, manuscript I to III evaluate physiological and metabolic adjustments in the copepods. Cation concentrations and pH in the haemolymph (**Manuscript I**), quantitative and qualitative digestive enzyme activities (**Manuscript II**) and metabolic enzyme activities and the biochemical composition (**Manuscript III**) of *C. glacialis* are related to water depth and seasons. The synopsis summarizes the major results from manuscript I to III and compares the physiology of *C. glacialis* populations from three fjords of the Svalbard archipelago. Moreover, it presents and discusses the findings from incubation experiments, which tested the effect of different food and light conditions on the physiology of *C. glacialis* and relates them to the seasonal study.

2 Material and methods

This chapter gives a brief overview of the sampling procedure and the analytical methods, which were used in this thesis. Detailed descriptions are given in the respective chapters (**Manuscript I - III**).

2.1 Field work

To compare the impact of environmental conditions on the physiology of *Calanus glacialis*, the copepods were sampled in three fjords of the Svalbard archipelago. Billefjorden (78°N; 16°E) and Kongsfjorden (79°N, 12°E) are both located on the western coast, while Rijpfjorden (80ºN, 22ºE) is on the northern side of Svalbard (Fig. 2.1).

Fig. 2.1 The three fjords (Billefjorden, Kongsfjorden and Rijpfjorden) around the Svalbard archipelago, in which *Calanus glacialis* was sampled. The map was created with TopoSvalbard, Norwegian Polar Institute (http://toposvalbard.npolar.no/)

Billefjorden was chosen as the main sampling area and sampling was conducted monthly from July 2012 to July 2013. Billefjorden is a sill fjord that consists of an outer basin (maximum depth ~230 m) and an inner basin (maximum depth ~190m). Both basins are isolated by a sill from each other and from the outer fjord system (Nilsen et

al. 2008). The two sills restrict the water exchange with the open ocean, which limits zooplankton advection. Thus, we assume to have sampled the same *C. glacialis* population over the year (Grigor et al. 2014). The fjord was ice-covered from February to early June 2013. In the upper 50 m, the water temperatures ranged between -1.7°C during ice-coverage and 5°C in late summer. At depth below 100 m, temperature was around -1°C throughout the year (Fig. 2.2).

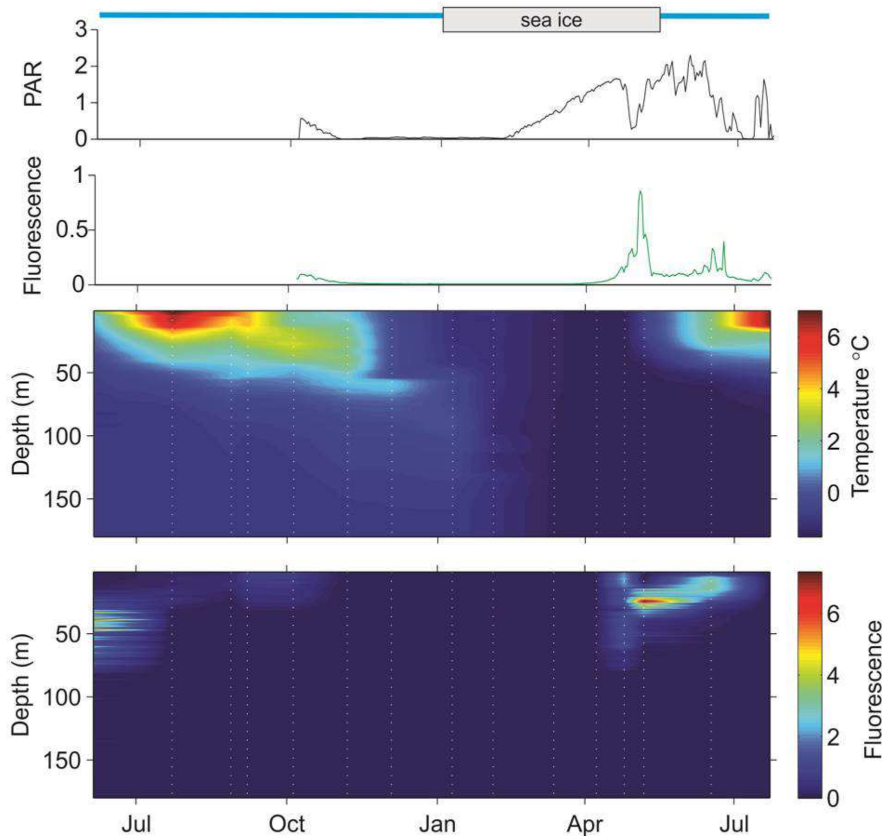

Fig. 2.2 Sea ice-cover (grey bar), photosynthetically active radiation (PAR) and fluorescence in the upper 19 m (upper panels) in Billefjorden from July 2012 to July 2013. The lower panels shows the temperature profile [°C] and fluorescence of the water column. Data were obtained from a mooring equipped with CTDs and miniloggers at 19, 30, 46, 56, 90, 111, 126 and 180 m water depth.

Kongsfjorden is an open fjord with water depths ranging from 400 m in the outer basin to 60 m in the inner basin (Kwasniewski et al. 2003). The fjord opens into the West Spitsbergen Shelf and is highly influenced by the West Spitsbergen Current that transports warm and saline Atlantic water into the fjord. In the fjord, the Atlantic water masses are mixed with Arctic water and local water masses from two glaciers and terrestrial run-off (Cottier et al. 2005, Svendsen et al. 2002).

Rijpfjorden is the northernmost fjord of the Svalbard archipelago and at maximum 240 m deep. It opens towards the deep Polar Basin and is dominated by cold Arctic water masses. Rijpfjorden is ice-covered for six to eight months every year (Wallace et al. 2010).

Depending on the ice-conditions, Billefjorden was reached either by boat (RV *Helmer Hanssen*, KV *Svalbard* or *Farm*), by zodiac or snow mobile. Sampling in Kongsfjorden and Rijpfjorden was conducted whenever possible during research cruises with RV *Helmer Hanssen*, RV *Lance* or KV *Svalbard* (see Table 1 for sampling schedule). The copepods were sampled with a WP-3 or WP-2 plankton net (1000 µm and 200 µm mesh size, respectively) from 180 m to 100 m water depth (from July 2012 to February 2013 and again in July 2013) and from 50 m to surface (in July 2012 and from March to June 2013) in Billefjorden. In Kongsfjorden, *C. glacialis* was sampled in the upper 50 m in July 2012 and below 200 m in January and February 2013. In Rijpfjorden, copepods were captured in the upper 50 m in July 2012, below 150 m from September 2012 to February 2013 and under the ice in May 2013. The sampling depth was chosen according to the highest abundance of the individuals. In July 2012, copepods started to migrate and thus, were distributed throughout the water column. We used the opportunity to sample and compare individuals from both depth layers in Billefjorden.

Table 1 Sampling in the three fjords Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013.

	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Billefjorden	x	x	x	x	x	x	x	x	x	x	x	x	x
Kongsfjorden	x						x	x					
Rijpfjorden	x		x				x	x			x		

Immediately after capture, the animals were transported to the laboratories at the University Centre in Svalbard (UNIS) or sorted onboard a research vessel in a laboratory at ambient temperature. Under a stereo-microscope, *C. glacialis* of copepodite stage IV (CIV), V (CV) and adult females (CVIF) were sorted alive. Individuals were briefly rinsed in demineralized water. Then, they were either snap-frozen in liquid nitrogen for analyses of enzyme activities, water-soluble protein content and lipid content or placed in pre-weighed Sn-cartridges for analyses of dry mass (DM), carbon (C) and nitrogen (N) content or haemolymph was extracted (see Fig. 2.3 for

sample processing). Samples for biochemical analyses were stored at -20°C and samples for enzyme activity analyses were stored at -80°C until processing. In addition, healthy looking CV were sorted alive under the stereo-microscope and placed in barrels for incubation experiments (see chapter 2.3).

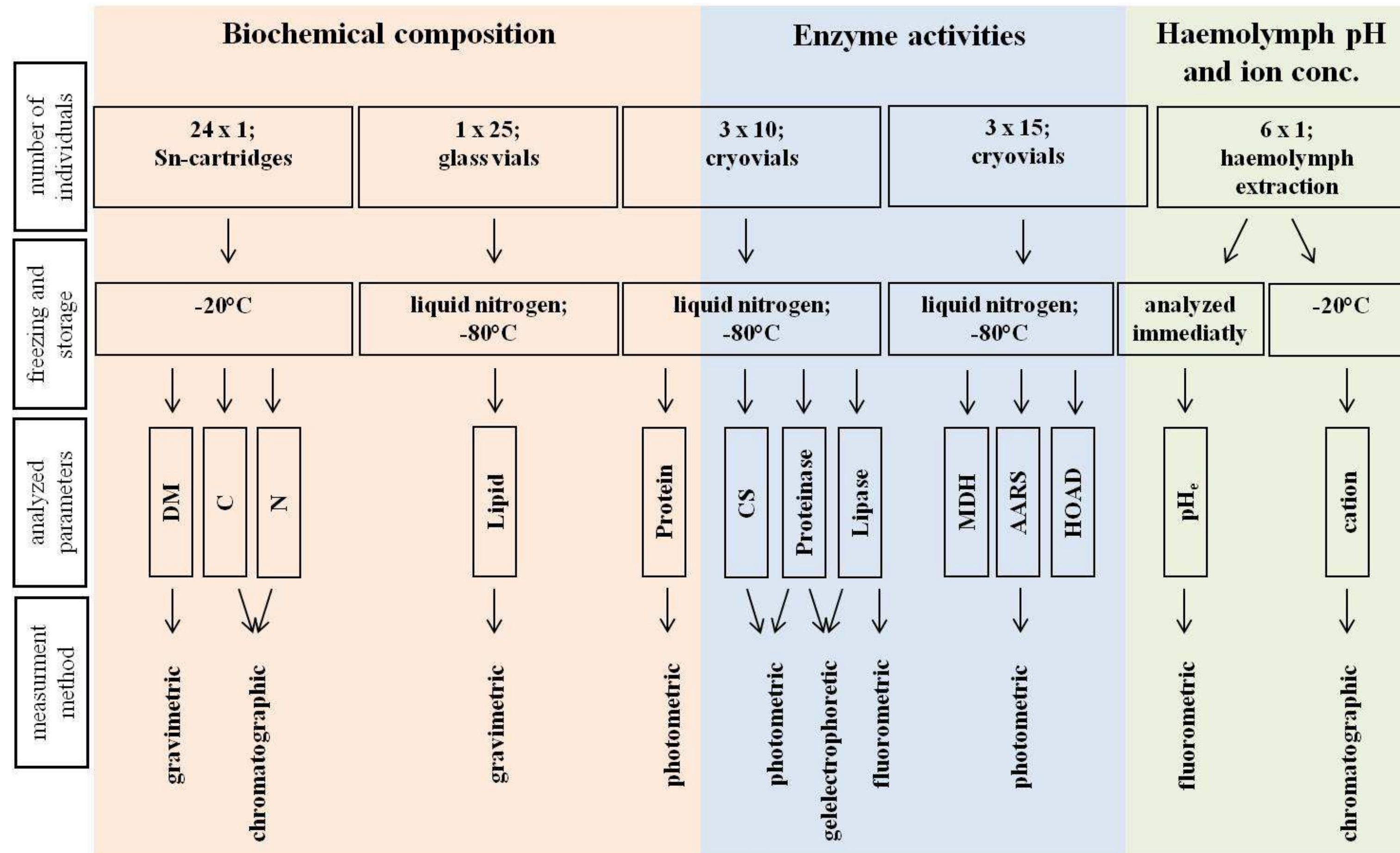

Fig. 2.3 Overview of all physiological and biochemical parameters, which were analyzed for *Calanus glacialis* copepodite IV, V and adult females from Billefjorden (Manuscript I - III), Kongsfjorden and Rijpfjorden (chapter 4.1.2) and CV of an incubation experiment at different food and light conditions (chapter 4.2). Shown are the number of individuals, which were frozen for the different parameters, the method of freezing and the storage temperature and the measurement method for the determination of the biochemical composition, the pH and ion concentration in the haemolymph and enzyme activities of *C. glacialis*. Abbreviations of analyzed parameters: malate dehydrogenase (MDH), aminoacyl-tRNA synthetase (AARS), 3-hydroxyacyl-CoA dehydrogenase (HOAD), citrate synthase (CS), lipase/esterase (lipase), water-soluble protein content (protein), total lipid content (lipid), dry mass (DM), carbon (C) and nitrogen (N) content, extracellular pH (pH_e) and cation concentration (cation).

2.2 Analytical work

In the following, the analytical methods, which were performed to assess the physiology of *C. glacialis* are described (see Fig. 2.3 for an overview of all analyzed parameters).

2.2.1 Biochemical composition

The biochemical composition of *C. glacialis* was determined in terms of DM, C and N content (**Manuscript II, III**), total lipid content (**Manuscript III**) and water-soluble protein content (**Manuscript III**).

To determine DM, individual *C. glacialis* CIV, CV and adult females were dried in Sn-cartridges at 60°C for 48 hours. Then, the pre-weighed cartridges were weighed on an ultra-microbalance. C and N content were determined by combustion with a CHN-Analyzer (EuroVector, EuroEA3000), using acetanilide as standard and the software Callidus Version 2E3. Between the procedures, the samples were kept in an exsiccator to prevent absorption of condensation water.

The total lipid content of *C. glacialis* CV was determined after Bligh & Dyer (1959). The frozen samples were lyophilized for 24 hours. Afterwards, dichloromethane/methanol (2:1, v/v) was added and the lipids were extracted by homogenizing the samples with a Potter-Elvehjem homogenizer. This was followed by two sonication steps. In between the steps, the supernatants were transferred into centrifuge vials and the solvent was added to a final volume of 8 ml. The extracts were cleaned with 0.88% potassiumchlorid solution and the total lipid content was determined gravimetrically after evaporation of the solvent.

Water-soluble protein content was quantified after Bradford (1976) by applying a Bio-Rad protein assay (BIO-RAD 500-0006). Bovine serum albumin was used as a standard (0 to 0.1 mg/ml). The samples were homogenized in Tris/HCl buffer at pH 7.0 (Table 2) and centrifuged at 15,000 g at 4°C for 15 min (Thermo Scientific, Heraeus Fresco 17). The homogenates (1:27 diluted with distilled water) were then added to 250 µl of 1:5 diluted protein assay. After incubating the assay for 15 min at 25°C in a 96-well plate, the absorption of the assay was measured in triplicates at 600 nm and 25°C with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.2.2 Enzyme analyses

The homogenates from all samples for enzyme analyses were gained by homogenizing the copepods in Tris/HCl buffer at pH 7.0 and centrifuging the sample at 15,000 g at 4°C for 15 min. (Thermo Scientific, Heraeus Fresco 17). The homogenates were then transferred into new reaction tubes and kept on ice until further processing.

As part of this thesis, it was aimed to measure as many different enzyme groups from the same sample of copepods as possible. Digestive enzyme activity (qualitative and quantitative), citrate synthase (CS) activity and water-soluble protein content were all measured from the same sample. An aim of this thesis was to also measure malate dehydrogenase (MDH), 3-hydroxyacyl CoA dehydrogenase (HOAD) and aminoacyl-tRNA-synthetase (AARS) from the same sample. Therefore it was necessary to homogenize the copepods for all enzyme measurements in the same buffer. However, Tris/HCl buffer at pH 7.0 proved to be an unsuitable buffer system for MDH, HOAD and AARS, since enzyme activities were either to variable or below the detection limit. The different enzyme classes require buffer systems, which stabilize their activities and diminish an interference with metals or any other compounds (Mayzaud 1986, Table 2).

Table 2 Buffer systems and respective abbreviations, which were used for the enzymes assays of the following enzymes: proteinase, lipase/esterase, malate dehydrogenase (MDH), citrate synthase (CS), aminoacyl-tRNA synthetase (AARS) and 3-hydroxyacyl-CoA dehydrogenase (HOAD).

enzymes	**buffer system**	**abbreviations for buffer systems**
Proteinase	0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) at pH 7.0	Tris/HCl
Lipase	0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) at pH 7.0	Tris/HCl
CS	0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) at pH 7.0	Tris/HCl
MDH	0.1 M potassium phosphate at pH 7.0	PP
AARS	0.1 M Tris/HCl (supplemented with 10 mM CaCl2) at pH 7.8	Tris/HCl
HOAD	107 mM triethanolamine/HCl (supplemented with 5.3 mM EDTA) at pH 7.0	TRA/HCl

Another aim was to reduce the number of copepods, which are necessary for analyses of MDH, HOAD and AARS activity. Thus, the sample volume was decreased from 30 µl to 6.7 µl for MDH and HOAD and from 250 µl to 66.7 µl for AARS activity measurements (Table 3). Accordingly, the total volume of the respective assays was reduced from 900 µl to 200 µ for MDH and HOAD and from 750 µl to 200 µl for AARS activity determination. Thus, instead of micro-cuvettes, 96-well plates were used and subsequently, more measurements could be performed in parallel.

Table 3 Adjustments of the methods for malate dehydrogenase (MDH), 3-hydroxyacyl-CoA dehydrogenase (HOAD) and aminoacyl-tRNA synthetase (AARS) activity measurements. Instead of micro-cuvettes, 96-well plates were used for the photometrical determination of enzyme activities.

	MDH		**HOAD**		**AARS**	
	mirco-cuvette	**96-well plate**	**mirco-cuvette**	**96-well plate**	**mirco-cuvette**	**96-well plate**
number of individuals	5	2	5	3	20	10
sample homogenate (µl)	30	6.7	30	6.7	250	66.7
substrate (µl)	30	6.7	30	6.7	200	53.2
total volume (µl)	900	200	900	200	750	200

2.2.2.1 Digestive enzyme activities **(Manuscript II)**

Total proteinase (EC 3.4.21-24) activity was quantified after Saborowski et al. (2004), modified after Kreibich et al. (2008). Twenty µl of the homogenates or Tris/HCl buffer at pH 7.0 for the controls were pre-incubated on a thermo shaker for 5 min at 30°C. After 5 µl azocasein (1% in aqua dem., Fluka BioChemika, 11615) were added to the homogenates or controls, the assays were incubated for 60 min at 30°C. The reaction was stopped by adding 50 µl trichloroacetic acid (TCA, 8% in aqua dem.) and homogenates and controls were centrifuged at 15,000 g at 4°C for 15 min. The absorbance of the supernatants was measured in an ultra micro-cuvette (Hellma 105.203-QS) with a spectrophotometer (Thermo Scientific, UV1) at 366 nm and recorded with the software VisionLite (Version 2.2).

Lipase/esterase (carboxylic ester hydrolases; EC 3.1.1) activity was determined after Knotz et al. (2006). As substrate 4-methylumbelliferyl butyrate dissolved in dimethyl

sulfoxide (MUF-butyrate, Fluka BioChemika, 19362; DMSO, AppliChem A3608) was used. Ten µl of the substrate were added to 20 µl homogenate or 20 µl Tris/HCl buffer at pH 7.0 for controls and 470 µl Tris/HCl buffer at pH 7.0. Standard curves were determined with 4-methylubelliferone (MUF, Sigma M1381) in DMSO (15.625 to 1000 µmol l^{-1}). Samples and controls were incubated on a thermo shaker for 30 min at 25°C and then, the fluorescence was measured with a NanoDrop 3300 at 450 nm (emission) and recorded with the software ND-3300 V 2.7.0. The autolysis of MUF-butyrate was measured and subtracted from the assay-results.

2.2.2.2 Metabolic enzyme activities **(Manuscript III)**

CS (EC 4.1.3.7) activity was measured after Stitt (1984), modified after Saborowski and Buchholz (2002). To 20 µl of the homogenate or buffer Tris/HCl buffer at pH 7.0 for controls, 20 µl 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma Aldrich, D8130), 20 µl acetyl-CoA (Acetyl-Coenzyme A trilithium salt, Roche Diagnostics, 13893324) and 520 µl Tris/HCl buffer at pH 7.0 were added in a semi-microcuvette. The assays were incubated for 5 min at 25°C (Peltier element, Krüss Optronic) and then, the reaction was started with 20 µl oxalacetic acid (Sigma Aldrich, O4126). The absorbance was measured continuously for 3 min at 25°C and 405 nm. Measurements were recorded with the software VisionLite (Version 2.2).

MDH (EC 1.1.1.37) activity was modified after Teschke et al. (2007). Samples were homogenized in PP buffer at pH 7.0. NADH (Roche Diagnostics 10107735001) in a volume of 6.7 µl was added to 6.7 µl homogenate and 180 µl PP buffer at pH 7.0 in a 96-well plate. The assay was incubated for 5 min at 25°C and then, the reaction was started with 6.7 µl oxalacetic acid (Sigma Aldrich, O4126). The absorbance was measured continuously for 5 min at 25°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

HOAD (EC 1.1.1.35) activity was modified after Auerswald and Gäde (1999). Samples were homogenized in TRA/HCl buffer at pH 7.0. NADH (Roche Diagnostics 10107735001) was added in a volume of 6.7 µl to 6.7 µl homogenate and 180 µl TRA/HCl buffer at pH 7.0 in a 96-well plate. The assay was incubated for 5 min at 25°C and the reaction was started with of 6.7 µl acetoacetyl-CoA (Sigma A-1625). The

absorbance was measured continuously for 5 min at 25°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

AARS (EC 6.1.1.) activity was modified after Chang et al. (1984). Samples were homogenized in Tris/HCl buffer at pH 7.8. 66.7 µl homogenate were added to 80 µl demineralized water and 53.2 µl pyrophosphate reagent (PPi, Sigma, P7275) in a 96-well plate. The assay was incubated for 5 min at room temperature. The absorbance was measured continuously for 10 min at 37°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.2.2.3 Substrate SDS-PAGE (Manuscript II)

Proteins were separated by discontinuous substrate sodium dodecyl sulphate polyacrylamide gel electrophoresis (substrate SDS-PAGE) modified after Laemmli (1970) and Kreibich et al. (2011) for lipase/esterase and after Freese et al. (2012) for proteinase patterns. This method allows to separate proteins according to their size and thus, their molecular weight can be determined. Components of the solutions, which were used to prepare the gels are described in table 4. Mini-gels (8 cm x 10 cm x 0.75 cm) consisted of a running gel and a stacking gel (Table 5). To make proteinase bands visible, FITC (Sigma C0528) was mixed in one running gel.

For SDS-PAGE, the homogenates were diluted 1:2 with sample buffer and applied on mini-gels. Ten µl of homogenate and 5 µl of a molecular marker (Roti®-Mark Standard, Roth T851) were pipetted onto the gels. Two gels were run at the same time for approximately 1 h at maximum 300 V, 30 mA and 2°C in a vertical gel electrophoresis chamber (Hoefer, Mighty Small II SE 250). The gel chamber was filled with electrode buffer. During the run, gels were kept in darkness and temperature was kept constant at 2°C with a thermostat (ThermoHaake, DC 10).

After the run, the gel for the documentation of lipolytic activity pattern was treated as described in Díaz et al. 1999. The gel was first placed in 2.5% Triton X 100 (in phosphate buffer) for 30 min and then washed in phosphate buffer. Afterwards, the gel was placed in a 100 µM MUF-butyrate solution in phosphate buffer for approximately 10 min. Proteolytic activity bands were made visible by placing the gel in 2.5% Triton X 100 (in Tris/HCl buffer at pH 8.0). Then, it was rinsed with demineralized water and

transferred into Tris/HCl buffer at pH 8.0 for 120 min. The lipase gel was stained in a CBB G®-250 solution. The proteinase gel was stained in a CBB R®-250 solution. Images of the gels were taken with a gel documentation system (Gel Doc™ EZ Imager, Bio Rad) under UV light (for lipolytic enzyme patterns) and under transmission light (for proteolytic enzyme patterns and documentation of markers). Analysis was performed with Image Lab 5.0 software (Bio-Rad).

Table 4 Overview of the solutions and buffers, which were used for the preparation of the mini-gels for discontinuous substrate sodium dodecyl sulphate polyacrylamide gel electrophoresis (substrate SDS-PAGE).

solutions	substances
running gel buffer	1.5 M Tris/HCl buffer adjusted to pH 8.8
stacking gel buffer	0.5 M Tris/HCl adjusted to pH 6.8
acrylamide/ bisacrylamide	30% acrylamide, 0.8% bisacrylamide
sodium dodecyl sulfate	10% SDS solution
ammoniumpersulfate	10% APS solution
TEMED	N,N,N´,N´-Tetramethylethylendiamin
sample buffer	25% stacking gel buffer, 20% bromophenol blue, 30% glycerine, 4% SDS, 21% aqua dem.
electrode buffer	0.025 mol l^{-1} Tris/HCl buffer adjusted to pH 8.3 with 0.192 mol l^{-1} Glycine and 0.1% SDS
FITC	0.05% casein fluorescein isothiocyanate from bovine milk
phosphate buffer	50 mM phosphate buffer at pH 8.0
CBB G®-250 solution	0.02% Commassie brilliant blue G®-250 in 5% aluminium sulphate, 10 % ethanol, 2 % phosphoric acid
CBB R®-250 solution	0.05% Commassie brilliant blue R®-250 in 50% methanol and 7% acetic acid

Table 5 Overview of the solutions in ml, which were used for the running and stacking gel of the mini-gels for discontinuous substrate sodium dodecyl sulphate polyacrylamide gel electrophoresis (substrate SDS-PAGE).

solutions	running gel [ml]	stacking gel [ml]
aqua dem.	4.180	2.750
running gel buffer	3.310	-
stacking gel buffer	-	1.120
acrylamide	5.000	0.583
10% SDS	0.125	0.045
10% APS	0.063	0.022
TEMED	0.010	0.010
total volume	12.5	4.5

2.2.3 Extracellular pH and cation concentrations (Manuscript I)

Extracellular pH (pH_e) and cation concentrations (Li^+, NH_4^+, Na^+, Mg^{2+}, K^+, Ca^{2+}) in the haemolymph of *C. glacialis* were analyzed according to Sartoris et al. (2010) and Schründer et al. (2013). On every sampling occasion, haemolymph was extracted from individual copepods, which were placed on a petri dish under a stereo-microscope in a controlled temperature room at ambient temperature. Each individual was dried carefully with tissue paper and the haemolymph was extracted with an ultra-thin borosilicate glass capillary. Immediately after the extraction, 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was added in a final concentration of 1 nM to a minimum of 400 nl haemolyph. The pH_e was measured fluorometrically with a NanoDrop 3300. To measure the cation concentrations, the remaining haemolymph was diluted in 40 µL distilled water. Cation concentrations were measured chromatographically with a Dionex ICS-1500 at 40°C. An IonPac CS 16 column with methane sulfonic acid (MSA, 30 mmol L^{-1}) was used as an eluent at 0.36 ml min^{-1}. The peak area of each cation was referred to a standard (Dionex Six Cation-I Standard, Thermo Scientific, 040187) of known concentration.

2.3 Incubation experiments under different food and light conditions (chapter 4.2)

Alongside the field campaign in Billefjorden, incubation experiments were performed to assess how changes in light and food regime affect the digestive (proteinase and lipase/esterase) and metabolic enzyme (CS) activity and the biochemical composition (DM, C and N content) of *C. glacialis*. The aim was to investigate of copepods respond

differently depending on the time of the year and thus, two experiments were conducted:

- in November/December 2009 (21.11.2009 - 17.12.2009) as part of my Master thesis
- in August/September 2012 (28.08.2012 - 17.09.2012) as part of this PhD project

For both experiments, *C. glacialis* CV were placed either in barrels with only GFF-filtered seawater or in barrels with GFF-filtered seawater, which was enriched with the fast growing diatom *Thalassiosira weissflogii* in a concentration of approximately 4500 cells ml^{-1}. Every second day, approximately 60% of the water in the barrels was removed by inverse filtration and the barrels were refilled with new filtered seawater (0.2 µm pore size). The barrels were either kept under continuous light or in darkness, which resulted in four treatments: with food and light; with food and in darkness; without food and with light; without food and in darkness. Both experiments were performed at the laboratories of the University Centre of Svalbard (UNIS), but the experiment in 2009 was continued at the Alfred Wegener Institute (AWI) in Bremerhaven after 10 days. For the transport, the copepods were placed in 1 L plastic bottles and stored in a cooling container with ice. The experiment lasted for 26 days in November 2009 and for 21 days in August 2012 (for experimental conditions see Table 6).

Table 6 Overview of incubation experiments at different food and light conditions with *Calanus glacialis* CV in November/December 2009 (November experiment) and August/September 2012 (August experiment). The table shows the incubation time in days, the number of sampling events (sampling frequency), the number of replicates, the volume of the incubation barrels and the number of individuals placed in each barrel.

	incubation time [days]	**sampling frequency**	**number of replicates**	**volume of barrels [L]**	**individuals per barrel**
November 2009	26	8 times	1 barrel per treatment sampled in triplicates	35	600
August 2012	21	7 times	2 barrels per treatment sampled in duplicates	20	500

2.4 Statistics

Data were analyzed with the free software R 3.0.1 (**Manuscript I - III**) or SigmaStat 3.5 (Systat Software, Inc.). In tables and figures, data are presented as mean ± standard deviation, unless, the variation was very high, then the standard error was shown for clarity reasons. To test data for normal distribution, a Shapiro-Wilk test was applied. For normally distributed data showing variance homogeneity, an ANOVA was performed and followed by Tukey post-hoc tests or Holm-Sidak post-hoc tests. For non-normally distributed data, a Kruskal-Wallis test was used and followed by Tukey post-hoc tests or pairwise Wilcoxon signed-rank tests. To test for dependencies between parameters, a Spearman Rank Order correlation was applied. For comparison among two groups a Student's t-test was performed. As level of significance, 5% ($\alpha = 0.05$) was determined. Results were regarded as statistically significant and the null hypothesis was rejected, when the p-value was lower than the α-level. The software PRIMER V 6.1.6 was used to examine similarities among biochemical parameters and enzyme activities of *C. glacialis* CV over the year. Non-metric multidimensional scaling (MDS) plots were based on Bray-Curtis similarity analyses on square root transformed data.

3 Manuscripts

In the following an overview of the three manuscripts, which are part of this thesis will be given and the contributions of the authors will be presented.

Manuscript I

Daniela Freese, Barbara Niehoff, Janne E. Søreide, Franz Josef Sartoris

Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*

The study design was done by all authors. The field work was performed by myself and Janne E. Søreide. Ion and extracellular pH measurements were conducted by myself. Data analysis and writing of the manuscript was done by myself in close cooperation with B. Niehoff, F. J. Sartoris and J.E. Søreide.

The manuscript was submitted to Limnology and Oceanography.

Manuscript II

Daniela Freese, Janne E. Søreide, Barbara Niehoff

Digestive enzyme activities in the Arctic copepod *Calanus glacialis* reflect its ontogenetic vertical migration

The study was designed and planned by all authors. The field work was conducted by myself and Janne E. Søreide. The biochemical analyses of the samples were done by myself. I analyzed the data and wrote the manuscript in close cooperation with B. Niehoff and J.E. Søreide.

The manuscript was submitted to PLOS ONE.

Manuscript III

Daniela Freese, Janne E. Søreide, Martin Graeve, Barbara Niehoff

Metabolic enzyme activities and body composition during the ontogenetic vertical migration of the Arctic copepod *Calanus glacialis*

Design and planning of the study was done by all authors. The field work was conducted by myself and Janne E. Søreide. Enzyme analysis and adjustment of methods was performed by myself. The analysis of the data and writing of the manuscript was done by myself in close cooperation with B. Niehoff, J.E. Søreide and M. Graeve.

The manuscript is in preparation for submission to Marine Biology.

Manuscript I

Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*

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Abstract

Arctic shelf zooplankton communities are dominated by the copepod *Calanus glacialis.* This species feeds in surface waters during spring and summer, accumulating large amounts of lipids. Autumn and winter are spent in dormancy in deeper waters. Lipids are believed to play a major role in regulating buoyancy, however, they cannot explain fine-tuning of the depth distribution. To investigate whether ion exchange processes and acid-base regulation support ontogenetic migration as suggested for Antarctic copepods, we sampled *C. glacialis* in monthly intervals for one year in a high-Arctic fjord and determined cation concentrations and the extracellular pH (pH_e) in its haemolymph. During the winter/ spring transition, individuals exchanged Li^+ ions against high-density cations (Na^+, Mg^{2+}, Ca^{2+}), which likely decreased the density of the copepods. At that time, maximum Li^+ concentrations of 197 ± 102 mmol L^{-1} suggest that this cation promotes upward migration in *C. glacialis*, indicating that this element has a biological function. Ion and pH_e regulation in the haemolymph were not directly correlated, but the pH_e revealed a seasonal pattern and was low (5.5) in winter and high (7.9) in summer. Low pH_e during overwintering might be related to metabolic depression and, thus, may support diapause.

1 Introduction

In high latitudes, zooplankton communities are often dominated by large calanoid copepod species of the genus *Calanus* (Jaschnov 1970) and *C. glacialis* is particularly abundant in cold Arctic fjords and trenches of the Svalbard archipelago (Blachowiak-Samolyk et al. 2008, Søreide et al. 2008). These copepods link primary production with higher trophic levels (Falk-Petersen et al. 1990) and are important contributors to the energy flux in the marine realm (review by Falk-Petersen et al. 2009).

Calanus glacialis performs ontogenetic vertical migration and enters a dormancy state referred to as diapause as copepodite stage IV, V or VI to overcome the long periods of food scarcity in polar winter (Conover 1988). Diapause in *Calanus* spp. is characterized by low metabolic activity, arrested development and reliance on internal energy reserves (reviews by Hirche 1996 and Conover and Huntley 1991). It has been discussed that the copepods float motionless in the water column during this time, possibly to save energy, and their large internal lipid stores are considered to play a major role for reaching neutral buoyancy (e.g. Campbell & Dower 2003). Pond (2012) suggests that the copepods swim down to 500 m depth. At that depth, the lipids are compressed and consequently the copepods become heavier and sink until they reach neutral buoyancy. Thus, the content of low-density lipids could determine diapause depth (Irigoien 2004, Pond 2012). In contrast, other studies point out that lipids represent a barrier to descent and rather promote upward migration (Yayanos et al. 1978, Visser and Jónasdóttir 1999). Lipid-based buoyancy is, however, in any case unstable as it is affected by the biochemical composition of the copepods which may change over winter due to gonad maturation and reproduction (Campbell and Dower 2003, Campbell 2004). Moreover, in ecosystems where the copepods overwinter in water depths less than 500 m, lipids do not undergo phase transition and therefore, are easier to metabolize and used more extensively (Jónasdóttir 1999, Pond & Tarling 2011, Clark et al. 2012). Therefore, lipid content and fatty acid composition alone cannot explain fine-tuning of buoyancy on seasonal time scales. A mechanism, which can contribute to changing the density of an organism while it remains iso-osmotic with the surrounding seawater, is ion replacement: To reduce the density and prevent the animal from sinking, e.g. Na^+, Mg^{2+}, SO_4^{2-} are selectively replaced by such ions that lead to a reduced density (e.g. NH_3/NH_4^+). Due to its low density, Li^+ could also be involved in density reduction. In a study on trophic interactions using ions as trophic tracers, high concentrations of Li^+

ions have previously been found in the Arctic copepod *Calanus hyperboreus* (Campbell et al. 2005). These authors did, however, not discuss potential consequences of such high Li^+ concentrations and to date the biological role of Li^+ in crustaceans has not gained much attention. Ion replacement has been known as a mechanism regulating buoyancy for decades from deep-water shrimps (Sanders & Childress 1988) and pelagic deep-water cephalopods (Denton et al. 1969). Also in diapausing Antarctic copepods ion regulation may contribute to buoyancy control as high ammonium concentrations were measured in the haemolymph of *Calanoides acutus* and *Rhincalanus gigas* as representatives of species which can perform diapause, while no ammonium was found in species which remain active in winter (Sartoris et al. 2010, Schründer et al. 2013, Schründer et al. 2014).

NH_4^+ is in a chemical equilibrium with NH_3, which is potentially toxic. Therefore, animals which exhibit NH_4^+ aided buoyancy need to shift the NH_3/NH_4^+ equilibrium towards the less diffusible NH_4^+. Cephalopods and deep-water shrimps have evolved specialized chambers to store NH_4^+ in a low pH environment (Clarke et al. 1979, Sanders and Childress 1988). Copepods, however, do not have such chambers and instead they reduce their pH_e to values as low as 5 to shift the NH_3/ NH_4^+ equilibrium towards the less toxic NH_4^+ (Sartoris et al. 2010, Schründer et al. 2013). However, the relation of pH_e and NH_4^+ over seasonal cycles is yet to be shown in Antarctic copepods and data on Arctic copepods are completely lacking.

As part of the Norwegian research project CLEOPATRA II we sampled the copepod *C. glacialis* in approximately monthly intervals over one year in Billefjorden, a high-Arctic fjord in Svalbard waters. This study seeks to verify the hypothesis of Sartoris et al. (2010) that copepod species, in which activity and diapause alternate, regulate NH_4^+ and pH_e in order to support migration and/or to obtain neutral buoyancy. Based on this, we hypothesize that NH_4^+ concentrations and pH_e change over the year, with high NH_4^+ concentrations and low pH_e found in diapausing Arctic copepods in winter and low NH_4^+ concentrations and high pH_e in active copepods in summer.

2 Material and methods

2.1 Sampling area and sample processing

Calanus glacialis was sampled monthly in Billefjorden (78°40′N; 16°40′E) in Svalbard waters from July 2012 to July 2013. Billefjorden is a high-Arctic sill fjord, which is located at the west-coast of Spitsbergen, Svalbard with a maximum water depth of around 190 m (Nilsen et al. 2008). The shallow sill (40-50 m) restricts exchange of fjord water with the ocean (Nilsen et al. 2008) and we can assume that we are sampling the same zooplankton population throughout the year (Grigor et al. 2014). Locally formed cold water persists in this seasonal ice covered fjord, which is inhabited by a large population of *C. glacialis*. The abundance of the two other *Calanus* species, i.e. *C. finmarchicus* and *C. hyperboreus*, is low in Billefjorden (Arnkværn et al. 2005).

Billefjorden was ice-covered from February to early June 2013 and ice-free during the rest of the year. Temperatures in the upper 50 m ranged from -1.7°C during the ice-covered period and up to 5°C in late summer. Below 100 m, temperature was around -1°C throughout the year. Related to sea-ice conditions the sampling location was either assessed by boat (RV Helmer Hanssen, KV Svalbard, Farm), by zodiac or by snow mobile. Copepods were collected with a WP-3 or WP-2 closing plankton net (1000 µm and 200 µm mesh size, respectively). The closing depth was dependent on the water layer where animals occurred in the highest abundances, which was from 180 to 100 m during winter and from 50 m to the surface during spring and summer. Immediately after capture, plankton samples were transported to the cold rooms of the University Centre in Svalbard (UNIS) or to a controlled temperature room onboard a research vessel. The copepods were then sorted alive under a stereo-microscope close to *in situ* temperatures and haemolymph was extracted from mainly copepodite stage V (CV), but also copepodite stage IV (CIV) and females, whenever these were abundant. We believe that misidentification of *C. glacialis* is unlikely as identification of the live copepods was based on new morphological size distributions and pigmentation characters confirmed by molecular analyses (Gabrielsen et al. 2012, Nielsen et al. 2014).

2.2 Haemolymph extraction and processing

Haemolymph was extracted to fluorometrically measure the extracellular pH (pH_e) and to determine the cation concentrations (for detailed description see Sartoris et al. 2010 and Schründer et al. 2013). In summary, in a controlled temperature room at ambient

temperature copepods were placed on a petri dish under a stereo-microscope and adherent seawater was removed from the animals with tissue. By inserting ultra-thin borosilicate glass capillaries, the haemolymph was extracted from individual copepods and the pH_e was measured immediately after extraction. For measuring cation concentrations, the rest of the haemolymph was diluted in 40 µL distilled water and stored at -20°C until further processing.

2.3 pH_e measurements

pH of the haemolymph was measured according to Schründer et al. (2013). 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was added to a minimum of 400 nl haemolymph as 5% of the sample volume. This resulted in a final HPTS concentration of about 1 nM. Then, the fluorescence was measured with a NanoDrop 3300 at about 511 nm (emission) and recorded with the software ND-3300 V 2.7.0.
pH_e was calculated from the fluorescence ratios (RFU), which were applied to a calibration curve of seawater in a pH range from 5.0 to 8.5 buffered with 50 mM Imidazole (Sigma-Aldrich, I5513).

2.4 Determination of cation concentrations

Cation concentrations (Li^+, NH_4^+, Na^+, Mg^{2+}, K^+, Ca^{2+}) in the haemolymph of the copepods were measured chromatographically with a Dionex ICS-1500 at 40°C, according to Sartoris et al. (2010), using an IonPac CS 16 column with methane sulfonic acid (MSA, 30 mmol L^{-1}) at 0.36 ml min^{-1} as eluent. The ion peak area was measured and referred to a standard (Dionex Six Cation-I Standard, Thermo Scientific, 040187) of known cation concentrations.

Potassium concentrations are not considered to be regulated by copepods and we attribute deviations from the concentration in seawater (10 mmol L^{-1}) to methodological restrictions. K^+ concentration ranged from 3 mmol L^{-1} to 244 mmol L^{-1}. Possible error sources are tissue rapture by inserting the glass capillaries into the copepod with a consequent allocation of K^+ from the intracellular to the extracellular space. We therefore do not present K^+ concentrations.

2.5 Statistical analysis

Statistical analysis was performed using the free software R 3.0.1. Normal distribution was tested with the Shapiro-wilk test. For normally distributed data showing variance homogeneity, one-way ANOVA was used and followed by a Tukey post-hoc test. For non-normally distributed data, a Kruskal-Wallis test was applied and followed by a pairwise Wilcoxon signed-rank test. A spearman rank order correlation (SR) was performed in order to identify dependencies between cations that reduce the density, i.e. Li^+ and NH_4^+; and all other cations that were measured, i.e. Na^+, Mg^{2+}, Ca^{2+}. 5% (α = 0.05) was chosen as level of significance. Results were referred to as statistically significant and the null hypothesis was rejected if the p-value was lower than the α-level.

3 Results

3.1 Haemolymph cation concentrations of *Calanus glacialis* during the year

Over the year, the five cations measured i.e. Li^+, Na^+, NH_4^+, Ca^{2+}, Mg^{2+}, were found in varying concentrations in *C. glacialis* CV (Table 1). NH_4^+ concentrations differed significantly among months but without a clear trend in relation to ontogenetic migration (Fig. 1A). The Li^+ concentrations, in contrast, followed a clear seasonal pattern (Fig. 1 B) and changed significantly over the year. Changes with season were less clear in Na^+ ion concentration, it was, however, correlated negatively to the Li^+ concentration (SR: -0.84, $p < 0.001$; Table 4), suggesting that the low-density Li^+ ions have been exchanged for the high-density Na^+ ions and vice versa.

At the start of our study, *C. glacialis* CV were still found in reasonably high numbers in the upper 50 m of the water column and we were thus able to measure the cation concentrations in the haemolymph before the descent (Fig. 1, Table 1). One month later, in August, most CV resided in water depth >100 m and their cation concentrations, including NH_4^+, Li^+ and Na^+, were in the same range as in July suggesting that the downward migration did not relate directly to measurable ion exchange processes. Also, from September until the end of November 2012, there were no significant changes in the concentration of these cations. No data are available for December, but in January and February 2013 when the population prepared to ascend, the Li^+ concentration in CV had increased from around 70 mmol L^{-1} to >125 mmol L^{-1} (Fig. 1 B). During the following months, from March through May 2013, the Li^+ concentration remained

similarly high in the CV, which at that time resided in the upper 50 m of the water column. In June and July 2013, the Li^+ concentrations had dropped to <10 mmol L^{-1} which overall were the lowest values during the present study.

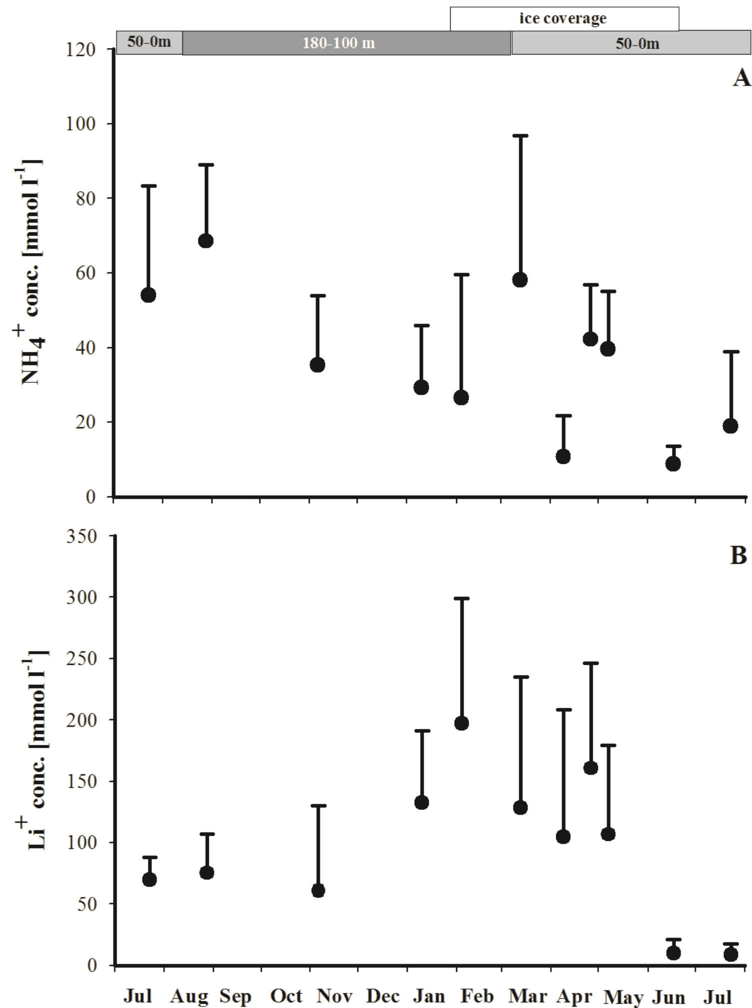

Fig. 1 NH_4^+ (A) and Li^+ (B) concentrations in mmol L^{-1} in *Calanus glacialis* copepodite stage V from July 2012 to July 2013 (mean ± SD, n = see Table 1). Individuals were sampled in the upper 50 m of the water column in July 2012 and from March 2013 to July 2013 and between 180 and 100 m from August 2012 to February 2013. The fjord was ice covered from February to early June 2013.

Table 1 Cation concentrations (Li^+, Na^+, NH_4^+, Mg^{2+} and Ca^{2+}) in mmol L^{-1} in *Calanus glacialis* copepodite stage V from July 2012 to July 2013 (mean ± SD, n = number of individuals).

	Cation concentration [mmol L^{-1}]									
	Li^+	**n**	**Na^+**	**n**	**NH_4^+**	**n**	**Mg^{2+}**	**n**	**Ca^{2+}**	**n**
23.07.2012	69.9 ± 18.4	6	347.5 ± 60.8	6	54.0 ± 29.4	5	23.7 ± 9.7	4	7.2	1
28.08.2012	75.3 ± 31.8	6	322.9 ± 30.9	6	68.6 ± 20.6	6	24.5 ± 7.9	6	9.8	1
06.11.2012	60.9 ± 69.1	6	394.2 ± 62.2	6	35.4 ± 18.6	3	22.8 ± 10.7	6	6.3 ± 1.0	2
10.01.2013	132.2 ± 58.9	6	324.9 ± 73.0	6	29.2 ± 16.9	6	24.7 ± 5.8	6	7.0 ± 3.9	6
04.02.2013	197.2 ± 101.8	6	298.4 ± 106.4	6	26.5 ± 33.1	2	20.3 ± 8.2	6	5.8 ± 1.1	3
13.03.2013	128.5 ± 106.9	6	285.7 ± 145.8	6	58.1 ± 38.8	5	12.5 ± 8.5	6	1.9 ± 1.3	3
09.04.2013	104.5 ± 103.9	6	368.4 ± 101.8	6	10.7 ± 10.9	3	29.9 ± 14.0	6	7.9 ± 1.7	3
26.04.2013	160.7 ± 85.7	6	298.2 ± 103.9	6	42.2 ± 14.6	5	17.9 ± 8.1	6	8.2 ± 2.2	3
07.05.2013	106.8 ± 72.9	6	361.8 ± 71.3	6	39.6 ± 15.4	4	15.5 ± 7.3	4	18.0 ± 2.2	2
18.06.2013	9.9 ± 10.9	2	470.6 ± 13.8	4	8.7 ± 4.9	3	30.8 ± 12.6	4	8.8 ± 1.4	4
23.07.2013	8.8 ± 8.9	6	456.8 ± 36.4	6	18.9 ± 19.9	6	29.3 ± 9.7	6	8.2 ± 2.8	4

In March, April and May 2013, CIV and adult females were more abundant than CV and, thus, we were able to sample these stages in addition to CV. At that time, both stages were found in the upper 50 m of the water column. The general cation composition was similar to that of the CV and there were no significant differences among the cation concentrations in individuals sampled in different months (Table 2 and 3).

Table 2 Cation concentrations (Li^+, Na^+, NH_4^+, Mg^{2+} and Ca^{2+}) in mmol L^{-1} in *Calanus glacialis* copepodite stage IV from March 2013 to May 2013 (mean ± SD, n = number of individuals).

	Cation concentrations [mmol L^{-1}]									
	Li^+	**n**	**Na^+**	**n**	**NH_4^+**	**n**	**Mg^{2+}**	**n**	**Ca^{2+}**	**n**
13.03.2013	37.9 ± 16.2	6	447.2 ± 17.7	6	4.3	1	41.5 ± 8.4	6	5.7 ± 1.9	6
09.04.2013	161.9 ± 86.3	6	309.5 ± 83.6	6	25.7 ± 3.3	5	18.8 ± 10.8	6	5.6	1
26.04.2013	78.0 ± 49.4	6	353.0 ± 60.1	6	26.6 ± 10.3	3	22.2 ± 10.2	6	8.5	1
07.05.2013	49.5 ± 42.6	6	349.8 ± 88.2	6	52.9 ± 16.9	3	26.8 ± 8.8	5	7.3	1

Table 3 Cation concentrations (Li^+, Na^+, NH_4^+, Mg^{2+} and Ca^{2+}) in mmol L^{-1} in *Calanus glacialis* females from March 2013 to April 2013 (mean ± SD, n = number of individuals).

	Cation concentrations [mmol L^{-1}]									
	Li^+	**n**	**Na^+**	**n**	**NH_4^+**	**n**	**Mg^{2+}**	**n**	**Ca^{2+}**	**n**
13.03.2013	103.3 ± 40.0	6	363.8 ± 81.9	6	23.9 ± 11.0	6	21.8 ± 11.3	6	4.5 ± 1.9	6
09.04.2013	67.4 ± 62.2	6	406.5 ± 66.0	6	21.1 ± 15.3	5	25.5 ± 16.7	6	6.3 ± 4.2	5
26.04.2013	22.3	1	449.8 ± 54.9	6	7.9 ± 8.4	2	29.7 ± 16.0	6	8.5 ± 1.0	5

Table 4 Spearman rank order correlation of the concentrations of cations that reduce the density (Li^+ and NH_4^+) with the concentrations of other cations (Na^+, Mg^{2+} and Ca^{2+}) in *Calanus glacialis* copepodite stage V from July 2012 to July 2013.

		Correlation coefficient	**p value**
Li^+	Na^+	-0.836	<0.001
Li^+	Mg^{2+}	-0.427	0.178
Li^+	Ca^{2+}	-0.736	0.008
NH_4^+	Na^+	-0.682	0.019
NH_4^+	Mg^{2+}	-0.609	0.043
NH_4^+	Ca^{2+}	-0.036	0.903

3.2 pH of the haemolymph of *Calanus glacialis*

The pH_e in CV at the surface in July 2012 at the beginning of our study was 7.8 and thus as high as expected for marine crustaceans. In August when the population had descended to depths >100 m, the pH_e in CV decreased to 6.7. In November 2012 and January 2013 the pH_e was even lower at pH 6.2 and 5.7, respectively. The pH_e remained low until late April 2013. In May, the pH_e increased to about 7.0 and in June and July, when CV were found actively feeding in surface waters, pH_e was 7.8 again (one-way ANOVA $p<0.05$, Tukey post hoc test).

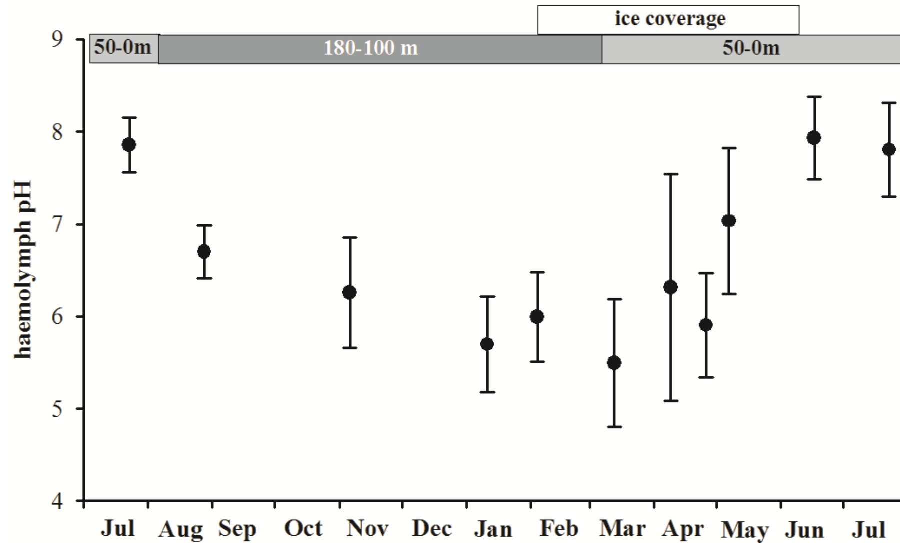

Fig. 2 Extracellular pH values of the haemolymph of *Calanus glacialis* copepodite stage V from July 2012 to July 2013 (mean ± SD, n = 6). Individuals were sampled in the upper 50 m of the water column in July 2012 and from March 2013 to July 2013 and between 180 and 100 m from August 2012 to February 2013. The fjord was ice covered from February to early June 2013.

The pH_e in females, which we were able to measure in March and April 2013, was relatively high compared to the copepodites with a pH_e close to 7 in March and approximately 8 in April (Table 5). The pH_e in CIV was low (5.4) in March while it was significantly higher in April at about 7. Females had almost vanished in May, but the abundance of the CIV was still high enough to allow for measurements and interestingly, at that time it again was as low as 5.9 (Table 5; one-way ANOVA $p<0.05$, Tukey post hoc test).

Table 5 Extracellular pH values of the haemolymph of *Calanus glacialis* copepodite stage IV and adult females (CVIF) from March 2013 to May 2013 and March 2013 to late April 2013, respectively (mean ± SD, n = number of individuals).

	Haemolymph pH			
	CIV	**n**	**CVIF**	**n**
13.03.2013	5.4 ± 0.4	6	6.7 ± 0.8	6
09.04.2013	7.1 ± 0.9	6	8.2 ± 0.6	6
26.04.2013	6.2 ± 0.7	6	7.9 ± 0.6	6
07.05.2013	5.9 ± 0.6	6		

4 Discussion

Until to date, we have limited knowledge about the overwintering physiology of *Calanus* spp. in high latitudes. Logistic challenges make it difficult to reach polar areas in winter and therefore, *in situ* studies are rare. Laboratory studies, on the other hand, have failed to induce the overwintering state *ex situ* (Miller and Grigg 1991). Our study is the first to investigate the pH and ion concentrations in an Arctic copepod species over an entire year, providing the opportunity to test the hypothesis introduced by Sartoris et al. (2010) and Schründer et al. (2013, 2014) that polar diapausing copepod species exchange high-density with low-density ions to fine-tune buoyancy during overwintering. Their studies report much higher concentrations (> 250 mmol L^{-1}) of NH_4^+, a low-density ion, in diapausing Antarctic copepod species, i.e. *Calanoides acutus* and *Rhincalanus gigas* as compared to NH_4^+ concentrations in non-diapausing species (< 20 mmol L^{-1}), i.e. *Paraeuchaeta antarctica* and *Calanus propinquus* (Schründer et al. 2013). This led the authors to the conclusion that the exchange of NH_4^+ for Na^+, Mg^{2+} and Ca^{2+} may be an important mechanism to achieve neutral buoyancy during diapause at greater depths. The high NH_4^+ concentrations in Antarctic diapausing copepods were generally accompanied by low pH_e (Schründer et al. 2013), possibly because the proton concentration must be high to shift the NH_3/NH_4^+ equilibrium from the highly toxic NH_3 towards the less toxic NH_4^+ (Sartoris et al. 2010).

If NH_4^+ and pH_e are regulated in order to support migration and/or obtaining neutral buoyancy in diapausing species, we can hypothesize that NH_4^+ concentration and pH_e change over the year, with high NH_4^+ concentrations and low pH_e in diapausing individuals in winter and low NH_4^+ concentrations and high pH_e in active individuals in summer.

Our study on the Arctic diapausing species *Calanus glacialis* showed that indeed both, NH_4^+ concentrations and pH_e, changed significantly over the year. The NH_4^+ concentration, however, did not follow a seasonal trend nor was it related to copepod development, i.e. to the different stages CIV, CV and females. High and low concentrations alternated rather randomly and we did not discover a relation to ontogenetic migration or overwintering. It also has to be noted that the NH_4^+ concentrations were considerably lower than those measured in the two Antarctic species (Sartoris et al. 2010, Schründer et al. 2013). The NH_4^+ concentrations, which we

found, were however always higher than those generally to be expected in active crustaceans (0.8 mmol L^{-1}, Weihrauch et al. 2004). This suggests that this cation could have a biological function even though our data cannot clearly relate NH_4^+ concentration to seasonal migration. Future studies are thus required to explain differences in the NH_4^+ concentrations in Antarctic and Arctic copepod species and to investigate the general role of ammonium buoyancy and accumulation in diapausing copepods from different ecosystems.

Another striking difference as compared to Antarctic copepods was that Li^+ was found in considerable amounts in the haemolymph of *C. glacialis* and, as shown in CV, its concentrations followed a clear seasonal pattern. The physiological role of Li^+ is not well understood (Leonard et al. 1995), especially in aquatic ecosystems. There are only few studies, which reported high Li^+ concentrations in marine species. Campbell et al. (2005) found Li^+ (89.6 ± 115.6 µg Li^+ gww^{-1}) in *Calanus hyperboreus* samples from the northern Baffin Bay, but this study focused on the trophic transfer of elements through the food web and did not discuss whether Li^+ accumulation could help regulating buoyancy. Another study reported bioaccumulation of Li^+ in marine organisms from different habitats, but did not explain its function (Chassard-Bouchaud et al. 1984). The concentration of Li^+ in seawater is about 0.028 mmol L^{-1} (Riley & Tongudai 1964). With a mean of 100 mmol L^{-1} over the year, the Li^+ concentration in our study exceeded by far the concentrations in seawater. As bioaccumulation of Li^+ through the food web is unlikely due to its low affinity to particles (review by Aral & Vecchio-Sadus 2008), this suggests that *C. glacialis* actively accumulates Li^+ by a transmembrane ion transport, which would depend on energy supply. This in turn, is a strong argument for a biological function of lithium.

Our study suggests that the copepods may benefit from Li^+ accumulation towards the end of the overwintering period as the exchange of Li^+ for Na^+ and other high-density ions reduces the density of the copepods and, thus, could support upward migration. Li^+ is also known to affect circadian rhythms in plant and animal systems by lengthening the circadian period (Östgaard et al. 1982) and its accumulation is a predator-defense mechanism in some plants and fresh water fish (Ralphs 1997, Creson et al. 2003). However, we cannot evaluate in our study if these functions could also be effective in

C. glacialis. It also remains an open question why Arctic copepods contain Li^+ in high concentrations while there was no Li^+ detected in Antarctic species.

The pH_e followed a clear seasonal pattern. In CV, it was close to seawater in July 2012 and then decreased throughout autumn to minimum values lower than pH 6 in winter (January to March 2013). When individuals were actively feeding in surface waters, the pH_e was again almost 8 in June and July 2013. However, low pH_e did not relate to high NH_4^+ concentrations and, thus, compensation for NH_4^+ cannot explain the low pH_e in *C. glacialis* as suggested for Antarctic copepods (Sartoris et al. 2010, Schründer et al. 2013). Low pH_e has previously been related to a reduction in metabolic activity (metabolic depression) in *Sipunculus nudus*, the peanut worm (Reipschläger and Pörtner 1996). It is thus possible that low pH_e in *C. glacialis* is an indication of diapause rather than of buoyancy regulation by means of ion exchange. This matches the relatively high pH_e (6.7 - 8.2) in females, as early gonad development in *C. glacialis* is fueled by internal reserves (for review see Niehoff 2007) and starts prior to their ascend. The pH_e of CIV, however, was always low (5.4-7.1), even in the beginning of May, when the copepods should have been active. This could suggest that CIV become active and start feeding later compared to females in spring. The very distinct seasonal development of the pH_e indicates that it could have a biological function for, or is a result of metabolic processes during overwintering. It could thus potentially be used to determine the grade of diapause in *C. glacialis* and possibly other copepod species.

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Manuscript II

Digestive enzyme activities in the Arctic copepod *Calanus glacialis* reflect its ontogenetic vertical migration

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Abstract

Zooplankton communities in Arctic shelf regions are dominated by the large calanoid copepod *Calanus glacialis*. This species overwinters in deeper waters with low metabolism. In late winter, it migrates to the surface where it feeds on ice algae and phytoplankton to reproduce, grow and accumulate lipid reserves for overwintering. To date, it is not fully understood what regulates the activity of the copepods and how it coincides with food availability. We therefore sampled *C. glacialis* in a high Arctic fjord in monthly intervals for one year and determined proteinase and lipase/esterase activities in relation to food availability and depth distribution of the copepods. We also tackled changes in specific isoforms of the enzymes by substrate SDS-PAGE (sodium dodecylsulfate polyacrylamide gel electrophoresis). In both enzyme classes, we found a clear seasonal activity pattern; activities of individuals in deep waters in winter were only half of those found in copepods actively feeding in surface waters during the productive summer season. SDS-PAGE showed a high heterogeneity of lipolytic enzymes in *C. glacialis*, which reflects the extensive accumulation and metabolization of internal lipid reserves in this species. One band of proteolytic activity was found and it intensified with the onset of the ice-algae and phytoplankton bloom. High digestive enzyme activities were closely correlated to food availability. Our results suggest that enzyme synthesis in *C. glacialis* is largely determined by the nutritional feeding condition, rather than being internally regulated. Thus, *C. glacialis* should be capable to adjust its digestive enzyme activities in accordance to climate driven changes in the algal food regime due to changes in the duration and extension of the Arctic sea ice coverage.

1 Introduction

Copepods of the genus *Calanus* dominate zooplankton communities in northern high latitudes (Jaschnov 1970). In Svalbard waters, *C. glacialis* is a major contributor to the zooplankton biomass and as a link between primary production and higher trophic levels, it plays an important role for the energy flux in the ecosystem (Falk-Petersen et al. 1990, Blachowiak-Samolyk et al. 2008, Søreide et al. 2008). During its one to two year life cycle, *C. glacialis* performs ontogenetic vertical migration and overwinters in greater water depths (Smith & Schnack-Schiel 1990, Kosobokova 1999). The overwintering state is commonly referred to as diapause (Conover 1988). During diapause, copepods arrest their development (Hirche 1991, Scott et al. 2000), they do not feed but rely on internal energy reserves, and fuel gonad maturation from lipid reserves which they accumulated during the previous productive season in spring and summer (reviews by Hirche 1996 and by Conover and Huntley 1991). The length of the life cycle and also the timing of diapause differ among populations from different ecosystems, and have been related to water temperatures (Kosobokova 1999, Niehoff & Hirche 2005) or food availability (Søreide et al. 2008, 2010, Daase et al. 2013). In Svalbard waters, *C. glacialis* usually enters diapause in late July/ early August (Søreide et al. 2010, Daase et al. 2013).

Digestive enzymes link food uptake with the biochemical transformation and assimilation of the ingested components (Mayzaud 1986). Different enzyme classes catalyse the hydrolysis of alimental components, i.e. proteinases hydrolyze peptide bonds (Mayzaud 1986) and lipases/esterases cleave the ester bonds of carboxylic acids (Luppa & Andrä 1983). At present, the methods allow to measuring only maximum potential activities, but not the actual *in situ* activity. Moreover, enzyme activities do not always respond linearly to food supply but may be influenced by other factors, e.g. metabolic requirements and feeding history (e.g. Hasset & Landry 1983, Roche-Mayzaud et al. 1991). It is thus difficult to use digestive enzyme activities as a proxy for feeding activity in copepods (Oosterhuis & Baars 1985). However, when digestive enzymes are studied in a seasonal context or compared at different experimental conditions, they can provide detailed insight on the relation between food occurrence and uptake by the copepods (Boucher & Samain 1974, Hassett & Landry 1983). Accordingly, several laboratory studies, in which either food quantity or quality was manipulated, showed how distinctly enzyme activity can change with dietary conditions

(e.g. Harris et al. 1986, Kreibich et al. 2008, 2011, Freese et al. 2012). Studies addressing seasonal aspects of digestive enzyme activities in copepods and especially in *Calanus* spp. are rare, but indicate less activity during winter than during summer (Hirche 1981, Hirche 1983, Hassett & Landry 1990). Hallberg & Hirche (1980) have shown in overwintering non-feeding *C. finmarchicus* and *C. helgolandicus* that cells in the gut epithelium, which are believed to produce digestive enzymes, were reduced in winter, thus limiting the ability to digest dietary components even if food were available. However, in order to effectively exploit the short productive season for reproduction and growth, it is important that the copepods can respond immediately when ice or pelagic algae become available in spring (Kosobokova 1990, Niehoff et al. 2002, Søreide et al. 2010). After winter, the copepods thus need to regain a certain level of digestive enzyme activity. In *C. hyperboreus*, it has been shown that digestive enzyme activity increased already in late winter and this allows the copepods to assimilate dietary components immediately when food becomes available and feeding starts in spring (Head & Conover 1983). Such information are lacking for other calanoid copepods, including *C. glacialis*. It is however, important to understand how *C. glacialis* regulates the digestive activity in order to predict its ability to adjust to climate driven changes in the algal food regime.

Most enzyme studies measured net enzyme activities only. This approach, however, may mask shifts between iso-enzymes while the total activity does not change (Kreibich et al. 2011). As the capability to synthesize different enzymes may reflect physiological capacities of a species to respond to food of varying quality, we combined total activity measurements with substrate SDS-PAGE (sodium dodecyl sulphate polyacrylamide). With SDS-PAGE, enzymes are separated according to their molecular weight by gelelectrophoresis (Laemmli 1970). This method shows that the enzyme patterns, i.e. the number and location of bands of active proteolytic or lipolytic enzymes on the gels, can change considerably with different feeding conditions (Kreibich et al. 2011, Freese et al. 2012) and over one day as related to the feeding cycle (Guérin and Kerambrun, 1982, Kerambrun and Champalbert, 1993).

To investigate how digestive enzyme activities in an Arctic copepod species change with season and depth distribution, we conducted the first year-round study on *C. glacialis*. Samples were taken monthly in Billefjorden, a high-Arctic sill fjord in

Svalbard waters, from July 2012 to July 2013. Relating the enzyme activities to feeding conditions and depth, we discuss whether enzyme synthesis is solely related to food supply or whether the activities decrease prior to the descent and/or increase prior the ascent, which would suggest internal regulation of enzyme synthesis. We also analysed the enzyme patterns in copepods captured at different seasons. Our study thus aimed at physiological aspects of the feeding biology of *C. glacialis* to enhance our understanding of how this key species of the Arctic shelf ecosystem may respond to a changing food regime, i.e. possible shifts in the timing and composition of algal blooms associated with receding ice-cover.

2 Material and methods

2.1 Ethics Statement

The present study on Arctic copepods does not include protected or endangered species and the use of the species *Calanus glacialis* for research purposes does not require any specific authorization, neither in Norway, where individuals were sampled, nor in Germany were samples were analyzed.

2.2 Sampling area and sample processing

From July 2012 to July 2013, *C. glacialis* was sampled monthly in Billefjorden (78°40′N; 16°40′E) in Svalbard waters. Depending on the sea-ice conditions the sampling location was either assessed by boat (RV Helmer Hanssen, KV Svalbard, Farm), by zodiac or by snow mobile. Billefjorden is a high-Arctic sill fjord, which is located on the west-coast of Svalbard (Nilsen et al. 2008). In the sampling year, the fjord was ice-covered from February until June 2013 with water temperatures ranging from -1.7 to 5.0°C in the surface. Below 100 depth, the temperature was around -1°C year-round. Copepods were collected with a WP-3 or WP-2 plankton net (1000 µm and 200 µm mesh size, respectively) from 180 m to 100 m in autumn and winter and from 50 m to the surface in spring and summer, depending on where animals occurred in the highest abundances. Immediately after capture, the samples were either processed in a controlled temperature room onboard a research vessel or transported to the laboratories of the University Centre in Svalbard (UNIS). Copepods of copepodite stage IV (CIV), V (CV) and adult females (CVIF) were sorted alive under a stereo-microscope at ambient temperature and snap-frozen in liquid nitrogen. Samples were stored at -80°C until further analysis.

2.3 Analyses of enzyme activity

Digestive enzyme activities, i.e. proteinase and lipase/esterase activities, were determined in triplicates in each sample, which contained ten individuals each. All samples were homogenized in 200 µl ice-cold 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0. Homogenates were centrifuged at 15,000 g at 4°C for 15 min (Thermo Scientific, Heraeus Fresco 17).

2.3.1 Proteinase activity

Total proteinase activity (EC 3.4.21-24) was measured after Saborowski et al. (2004), modified after Kreibich et al. (2008). Twenty µl of sample or 20 µl buffer for the controls were pre-incubated on a thermo shaker for 5 min at 30°C. Subsequently, 5 µl azocasein (1% in aqua dem., Fluka BioChemika, 11615) were added to the reaction tubes, which were incubated for another 60 min at 30°C. Fifty-microliter trichloroacetic acid (TCA, 8% in aqua dem.) were added to stop the reaction and samples/controls were centrifuged at 15,000 g at 4°C for 15 min to obtain supernatants, which then were transferred into an ultra micro-cuvette (Hellma 105.203-QS). The absorbance of the supernatants was measured with a spectrophotometer (Thermo Scientific, UV1) at 366 nm and recorded with the software VisionLite (Version 2.2).

2.3.2 Lipase/esterase activity

Lipase/esterase (carboxylic ester hydrolases; EC 3.1.1) activities were measured after Knotz et al. (2006). As substrate, 10 µl 4-methylumbelliferyl butyrate dissolved in dimethyl sulfoxide (MUF-butyrate, Fluka BioChemika, 19362; DMSO, AppliChem A3608) was added to 20 µl sample or 20 µl buffer (controls) and 470 µl 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0. Standard curves were made with 4-methylubelliferone (MUF, Sigma M1381) in DMSO (15.625 to 1000 µmol l^{-1}). Samples and controls were incubated in the dark on a thermo shaker at 25°C for 30 min. Enzyme activities were calculated from the fluorescence, which was determined with the NanoDrop 3300 at 450 nm (emission) and recorded with a software ND-3300 V 2.7.0. Autolysis of MUF-butyrate was measured and subtracted from the assay-results.

2.3.3 SDS-PAGE

To reveal proteinase and lipase/esterase enzyme activity bands, proteins were separated by discontinuous substrate sodium dodecyl sulphate polyacrylamide gel electrophoresis

(substrate SDS-PAGE) modified after Laemmli (1970) and Kreibich et al. (2011) for lipase/esterase and after Freese et al. (2012) for proteinase patterns. Sample homogenates were diluted 1:2 with sample buffer (25% 0.5 Tris/HCl buffer at pH 6.8, 0.02% bromophenol blue, 30% glycerine, 4% SDS) and were applied on Mini-gels, which consisted of a running gel (1.5 M Tris/HCl buffer at pH 8.8) and a stacking gel (0.5 Tris/HCl buffer at pH 6.8). Before the run, 0.05% casein fluorescein isothiocyanate from bovine milk (FITC-casein, Sigma C0528) was pipetted into the running gel to make proteinase bands visible. Ten µL of sample and 5 µL of a molecular marker (Roti®-Mark Standard, Roth T851) were applied onto the gels. The running conditions for two (proteinase and lipase/esterase) gels were 300 V, 30 mA and 2°C for approximately 1 h in a vertical gel electrophoresis chamber (Hoefer, Mighty Small II SE 250), which was filled with electrode buffer (25 mM Tris/HCl buffer with 0.192 M glycine and 0.1% SDS, pH 8.3).

After the run, the gel for the documentation of the lipolytic activity patterns was first placed in 2.5% Triton X 100 (in 50 mM phosphate buffer at pH 8.0) for 30 min and then washed in 50 mM phosphate buffer at pH 8.0. Thereafter, the gel was incubated in a 100 µM MUF-butyrate solution in 50 mM phosphate buffer at pH 8.0 for approximately 10 min (Díaz et al. 1999). The proteinase gel was washed in 2.5% Triton X 100 (in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) pH 8.0), rinsed with with aqua dem. and placed in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) pH 8.0 for 120 min. Then, the lipase gel was stained in a Commassie brilliant blue (CBB) G®-250 solution (5% aluminium sulphate, 10% ethanol, 2% phosphoric acid) and the proteinase gel was stained in a CBB R®-250 solution (50% methanol, 7% acetic acid). Gel images were taken with a gel documentation system (Gel Doc™ EZ Imager, Bio Rad) under UV light (for lipolytic enzyme patterns) and under transmission light (for proteolytic enzyme patterns and documentation of markers). Analysis was performed with Image Lab 5.0 software (Bio-Rad).

2.4 Statistical analysis

Statistical analysis was performed using the free software R 3.0.1. The Shapiro-Wilk test was applied to test data for normal distribution. For normally distributed data, which showed variance homogeneity, one-way ANOVA was applied and followed by Tukey post-hoc tests. For non-normally distributed data, Kruskal-Wallis tests were used

and followed by Tukey post-hoc tests. Spearman Rank Order Correlation was performed in order to identify dependencies between digestive enzymes and Chlorophyll *a*. Five % ($\alpha = 0.05$) was determined as level of significance. Results were regarded as statistically significant and the null hypothesis was rejected, if the p-value was lower than the α-level.

3 Results

3.1 Digestive enzyme activity over one year

In July 2012, when our study started, the copepods began to migrate from the surface to deeper water layers. They were therefore found in relatively high abundances throughout the water column at that time. We used this opportunity to compare individuals from the upper 50 m and from deep waters and found that the proteinase activity was high in CV from surface waters (5.6 ± 0.3 dE_{366} h^{-1} mg DM^{-1}) and low in CV captured at depth >100 m (1.3 ± 0.3 dE_{366} h^{-1} mg DM^{-1}). For the rest of the year, until December 2012, most CV were found below 100 m and their proteinase activities remained low. Also in CIV, sampled in October 2012 during a cruise with RV Helmar Hanssen, the specific enzyme activity was low (Fig. 1). In January and February 2013, before the copepods began their ascent to the surface, the specific proteinase activities in CV were slightly, but not significantly, higher as compared to those in December (Fig. 1A). The individual activity (Fig. 1B), however had not changed, and thus the increase can be attributed to a loss in body carbon which we observed at that time. In January and February, also females were abundant at depth, possibly due to moulting of the CV. Their activities were as low as that of the CV (Fig. 1 A, B). From March to May 2013, the numbers of CV were too low for measuring enzyme activities. We therefore focused on CIV and CVIF, which then were the most abundant stages. From January to March, the proteinase activities of both developmental stages were low (specific enzyme activity below 1.5 dE_{366} h^{-1} mg DM^{-1}). Ice algae developed at the end of March and later, in April, the chlorophyll *a* concentrations in the water column increased. Coincidently, the proteinase activities had increased significantly in April (one-way ANOVA $p<0.05$, Tukey post hoc test). At that time, the copepods had migrated upwards and were found mainly above 50 m. Females almost vanished during April while CIV were still abundant at the end of April and in May 2013 and could thus be analyzed. In both months, the specific activities of the CIV were higher as compared to all other stages. In June and July 2013, CV again reached high abundances. They

concentrated in the upper water column and in June their specific proteinase activity was significantly higher as compared to all other sampling days during the rest of the year (one-way ANOVA p<0.05, Tukey post hoc test). In July 2013, most of the CV had already descended and at depths >100 m their proteinase activity was as low as that at of the overwintering CV of the previous generation.

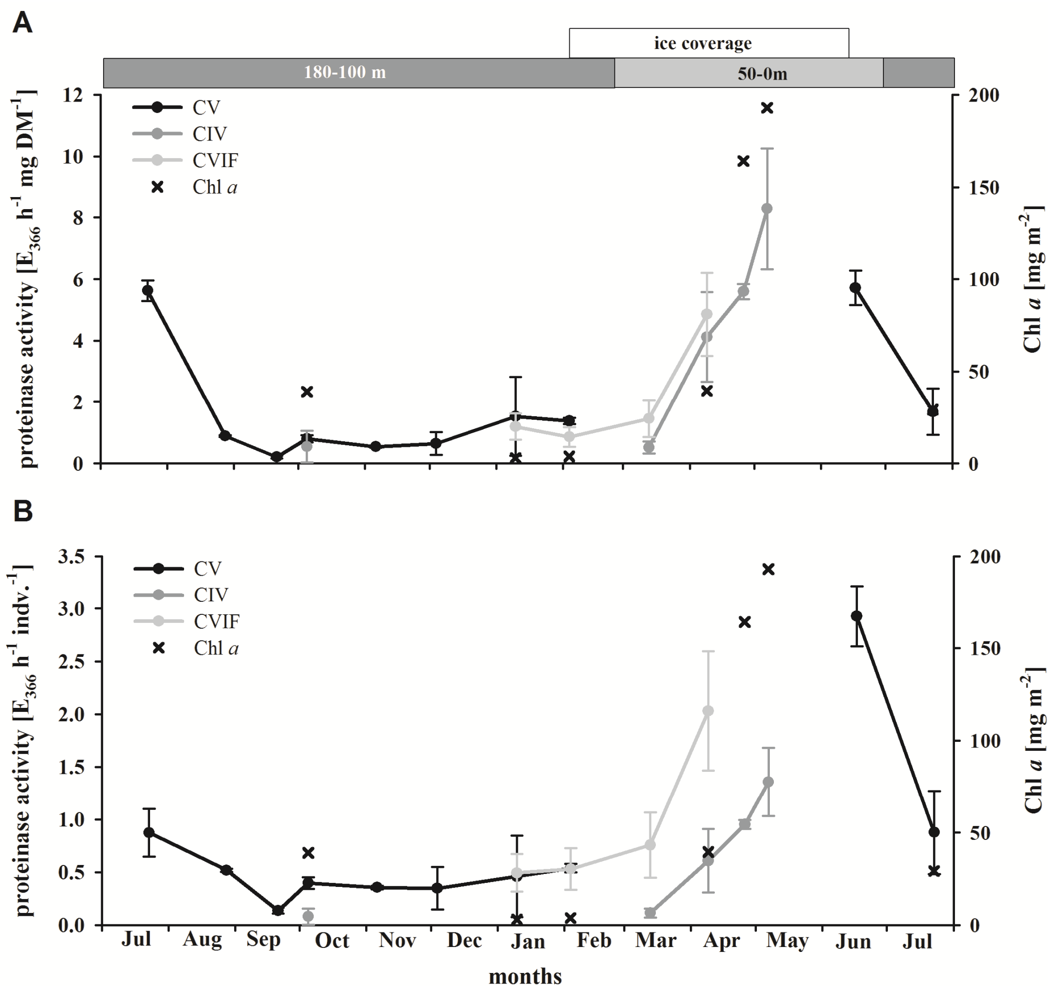

Fig. 1 Specific proteinase activity (A; dE_{366} h^{-1} DM^{-1}) and activity per individual (B; dE_{366} h^{-1} $indv.^{-1}$) of *Calanus glacialis* copepodite stages IV, V and adult females from July 2012 to July 2013 (n = 3 x 10 individuals, mean ± SD, except for CIV begin April n = 2). In July 2012 and from March 2013 to July 2013, *C. glacialis* was sampled in the upper 50 m and from August 2012 to February 2013 from 180 to 100 m water depth. From February to early June, the fjord was covered by ice. Chlorophyll *a* (Chl *a*, mg m^{-2}) was integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface). Ice algae were present from mid-March to end of April/beginning of May.

In general, the lipase/esterase activities followed a similar pattern and accordingly, proteinase and lipase/esterase activities correlated significantly in all three stages (CIV: Spearman Rank Order Correlation (SR): 0.90, p < 0.001; CV: SR: 0.83, p < 0.001; CVIF: SR of 0.92 p < 0.001). In July 2012, the lipase/esterase activity was relatively

high (221.3 ± 43.2 nmol h^{-1} mg DM^{-1}) in CV captured at the surface, while it was low in those, which had already descended to depth >100 m (178.4 ± 38.8 nmol h^{-1} mg DM^{-1}). During the rest of the year, the lipase/esterase activities were low at about 120 nmol h^{-1} mg D^{-1} in CV as was the activity in the CIV captured in October 2012 at depth >100 m (Fig. 2). In January and February, when the specific proteinase activity had increased, also the specific lipase/esterase activities in the CV increased (one-way ANOVA $p<0.05$, Tukey post hoc test). The activities in females at that time were in a similar range (around 150 nmol h^{-1} mg DM^{-1}). In March, when only females and CIV were present in high numbers, the specific lipase/esterase activity of the CIV was less than half of that of the females and the CV in January and February. In early April the activities had increased significantly in both females and CIV (one-way ANOVA $p<0.05$, Tukey post hoc test), and in CIV they reached maximum values in May (351.5 ± 25.4 nmol h^{-1} mg DM^{-1}). Overall maximum activities, however, were found in the CV from the upper 50 m in June 2013 (458.4 ± 29.7 nmol h^{-1} mg DM^{-1}). In July, when most of the CV had migrated to below 100 m depth, the activities were significantly lower than in June (one-way ANOVA $p<0.05$, Tukey post hoc test), but still higher than during autumn and winter 2012.

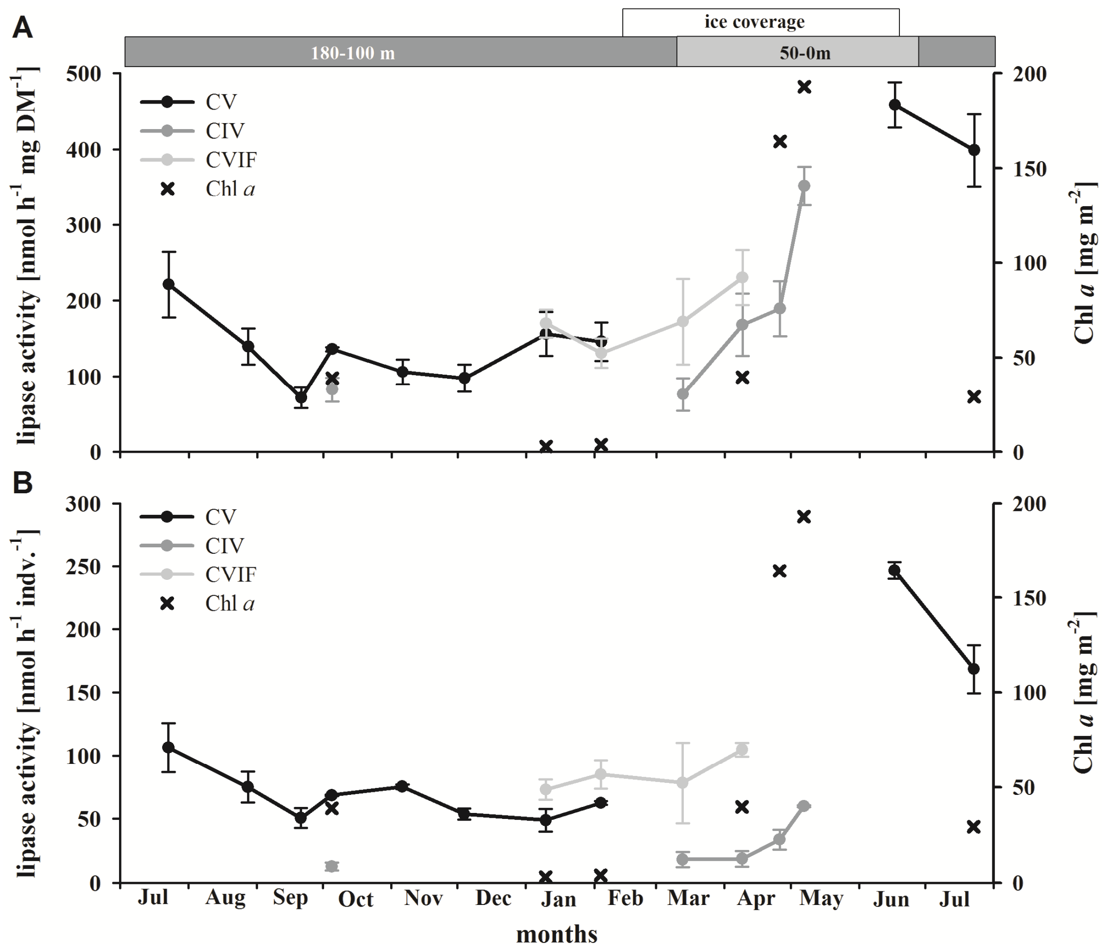

Fig. 2 Specific lipase/esterase activity (A; nmol h^{-1} DM^{-1}) and activity per individual (B; nmol h^{-1} $indv.^{-1}$) of *Calanus glacialis* copepodite stages IV, V and adult females from July 2012 to July 2013 (n = 3 x 10 individuals, mean ± SD, except for CIV begin April n = 2). In July 2012 and from March 2013 to July 2013, C. glacialis was sampled in the upper 50 m and from August 2012 to February 2013 from 180 to 100 m water depth. From February to early June, the fjord was covered by ice. Chlorophyll *a* (Chl *a*, mg m^{-2}) was integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface). Ice algae were present from mid-March to end of April/beginning of May.

3.2 Enzyme pattern unraveled by substrate SDS-PAGE

Substrate SDS-PAGE was applied to reveal if the composition of iso-enzymes was different among the different stages of *C. glacialis* or seasons. We sampled the different stages whenever abundant, and thus we managed to cover all seasons, i.e. late summer (August, CV), autumn (October, CV), winter (January, CV and females), spring (March CIV and May, CIV) and again summer (June, CV). Most samples did not show any proteolytic bands and the variety of iso-enzymes was poor as we found only one band at 23 kDa. This band was found in CV in June (Fig. 3 a) and CIV (Fig. 4 b) in May, when they resided in the upper 50 m. Interestingly, the proteolytic band appeared in females already in January when they still inhabited deeper waters (Fig. 4 a).

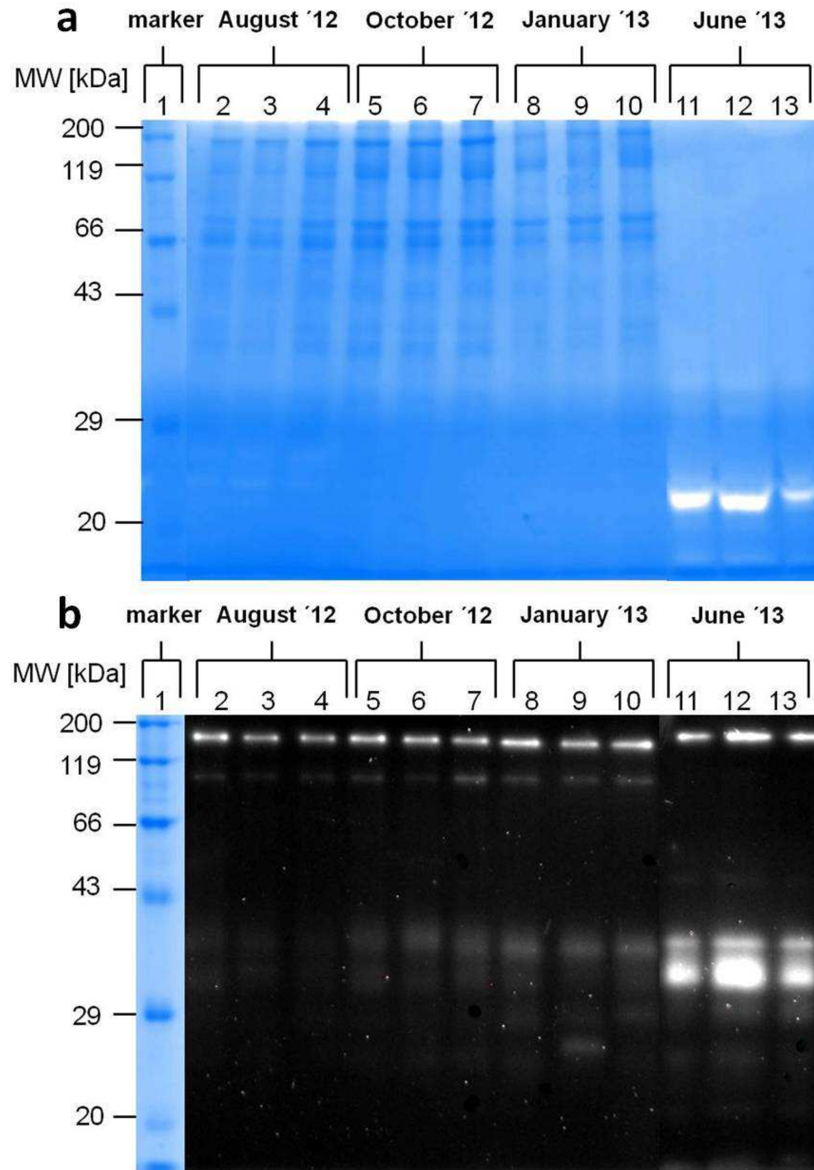

Fig. 3 Proteolytic activity bands (a) and lipolytic activity bands (b) in *Calanus glacialis* copepodite stage V at different times of the year (August 2012: lane 2-4, October 2012: lane 5-7, January 2013: lane 8-10, June 2013: lane 11-13) and the molecular marker (lane 1, 20-200 kDa).

In contrast to proteolytic activity, lipolytic activity was found in all samples, and the intensity of the bands varied among the CV from the different seasons (Fig. 3 b). When the CV resided in surface waters, activity was the highest in June and numerous bands were found between 30 and 169 kDa. In August, when CV were captured below 100 m, there was only one major band of activity at 169 kDa. Minor bands were found at 117 kDa and between 30 and 40 kDa. In October and January, all bands were more intense, suggesting higher lipolytic activity, which was also reflected in net enzyme activities (Fig. 2). Like in CV, lipolytic activity bands in females were visible between 30 and

169 kDa in January and intensified in early April (Fig 5 a). In CIV in March, the lipolytic activity was reflected by only one major band of lipolytic activity at 169 kDa, while in May, there were major activity bands between 30 and 169 kDa (Fig. 5 b).

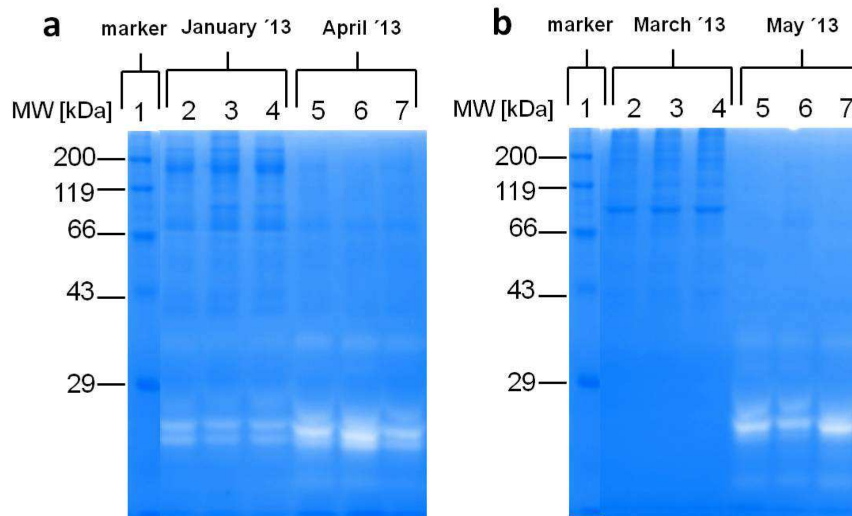

Fig. 4 Proteolytic activity bands in *Calanus glacialis* adult females (CVIF) (a) and copepodite stage IV (b). (a): lane 1: molecular marker (20-200 kDa), lane 2-4: CIV from January 2013, lane: 5-7: CIV from begin of April 2013. (b): lane 1: molecular marker (20-200 kDa), lane 2-4: CVIF from March 2013, lane: 5-7: CVIF from May.

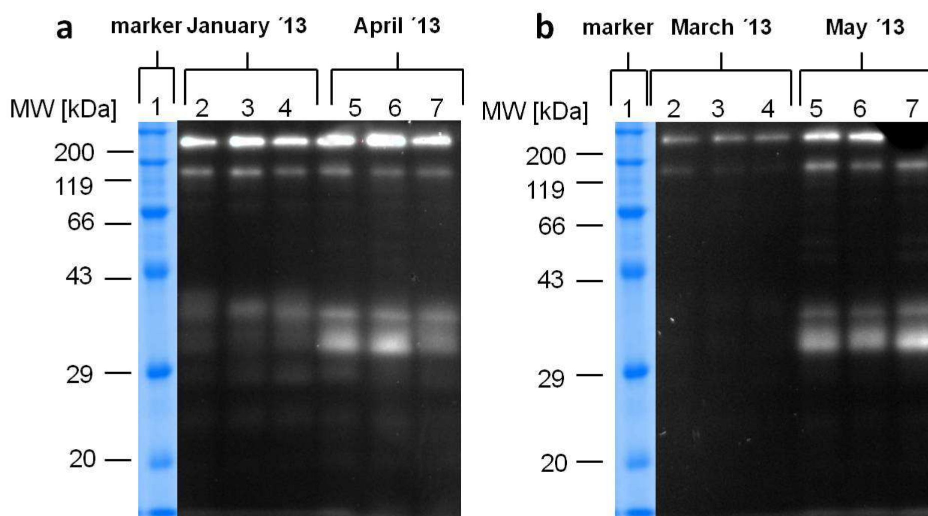

Fig. 5 Lipolytic activity bands in *Calanus glacialis* adult females (CVIF) (a) and copepodite stage IV (b). (a): lane 1: molecular marker (20-200 kDa), lane 2-4: CIV from January 2013, lane: 5-7: CIV from begin of April 2013. (b): lane 1: molecular marker (20-200 kDa), lane 2-4: CVIF from March 2013, lane: 5-7: CVIF from May.

4 Discussion

On the shelf around Svalbard, *C. glacialis* dominates the mesozooplankton communities and, as in the sibling species *C. finmarchicus* and *C. hyperboreus*, its life cycle is characterized by profound changes between activity and dormancy (review by Hirche 1996). Similar to *C. finmarchicus* and *C. hyperboreus*, *C. glacialis* performs ontogenetic vertical migration and spends autumn and winter at greater water depth (reviews by Conover 1988 and Hirche 1996). Due to its concentration on the shelf, however, overwintering depth is usually much lower than that of the two sibling species (Kaartvedt 1996, Dale et al. 1999). Knowledge of the overwintering physiology of *C. finmarchicus* and *C. hyperboreus* may thus not be simply applied to *C. glacialis*.

Seasonal data on *C. glacialis* are scarce and our field study is the first, which investigated the physiology over an entire year. As the inner Billefjorden is separated from the outer water masses due to its sill of 45 m depth advection is negligible, providing the unique opportunity to follow one population over the year. Food availability in the fjord changed, as expected, from high in spring and early summer to low in autumn and winter. Our study aimed at relating the digestive activity to depth and feeding conditions to test whether it is solely related to food supply or whether the activities decrease prior to the descent and/or increase prior the ascent, which would suggest internal regulation of enzyme synthesis. As a proxy for digestive activity, we have measured the activity of proteinases and lipases. The usefulness of enzymes as proxies for the feeding activity in copepods has been under debate (Oosterhuis & Baars 1985) as some authors found positive correlations between digestive enzyme activity and food availability (Mayzaud & Conover 1976, Hirche 1981), whereas others discovered negative (Hassett & Landry 1983) or no correlations (Båmstedt 1984). In a seasonal context or comparing different experimental conditions, however, digestive enzymes well reflect the relation between the availability and intake of food (Boucher & Samain 1974, Hassett & Landry 1983).

The digestive activity of *C. glacialis* followed a clear seasonal pattern with high values when the copepods were feeding in surface waters and low values when they resided at overwintering depth. In *C. finmarchicus* and *C. helgolandicus,* the numbers of so called B-cells, which are located in the gut epithelium and which are responsible for the production of digestive enzymes, are reduced during winter (Hallberg & Hirche 1980).

These species would thus not be capable to efficiently digest food even if algae were available. Other authors, in contrast, suggested that *C. finmarchicus* feeds on microzooplankton and detritus during winter (Marshall & Orr 1958). Their study, however, focused on a population in the Clyde Sea, where food availability does not cease completely. In the Arctic, winter-feeding of the mainly herbivorous *C. glacialis* is not likely for the population that overwinters in deep waters and minimum lipase and proteinase activities in our study should reflect the basic digestive potential without feeding. Low digestive enzyme activity at starvation has previously been shown in other copepod species and was explained as a metabolic adjustment to save energy (Hassett & Landry 1983). In *Temora longicornis* from the Southern North Sea, for example, the proteinase activity in females starving for only three days had decreased to 25% of that of feeding females (Kreibich et al. 2008). As compared to this small copepods species, which lives in a habitat with continuous food supply, *C. glacialis* kept relatively high lipolytic and proteolytic potentials over the entire winter with approximately 25% and 10%, respectively, of the maximum activities. According to Hassett & Landry (1983) such strategy might be advantageous for copepods that live in environments with strong variations in food supply. This should also be true for *C. finmarchicus* and *C. helgolandicus*, which overwinter the winter season also at greater depth without food. Hirche (1983), however, found trypsin and amylase activities close to zero in overwintering individuals and up to 20-fold higher values in feeding individuals. It has to be kept in mind that Hiche (1983) measured activities of specific enzyme classes while we studied the total proteolytic and lipolytic activities as not to exclude potential enzymes. It is thus possible that the comparably high proteolytic activities, which we measured, reflect the potential of proteinases other than trypsin. In *C. glacialis*, SDS-PAGE revealed a proteolytic activity band at 23 kDa. This molecular weight is typical for isoforms of both trypsin and chemotrypsin and both cleave dietary proteins (Saborowski et al. 2004). To compare digestive activities among species in different overwintering habitats, it would thus be helpful to measure the total enzyme potential rather than measuring specific enzyme classes.

In comparison to other crustaceans, like e.g. the crabs *Cancer pagurus* and *Maja brachydactyla*, which showed several proteolytic activity bands in the range from 20 to 70 kDa (Saborowski et al. 2004, Andrés et al. 2010), *C. glacialis* exhibits little variety in proteolytic isoenzymes since SDS-PAGE shows only one band at 23 kDa (Freese et

al. 2012, this study). The variety of lipolytic activity bands was larger in comparison to the proteolytic bands and also in comparison to *Temora longicornis* (Kreibich et al. 2011). Lipases are distinguished in digestive lipases, which cleave nutritive lipids (Vogt 2002) and intracellular lipases, which cleave triacylglycerides in tissue lipids (Vihervaara & Puig 2008, Rivera-Pérez et al. 2010). Thus, the heterogeneity of lipolytic enzymes and the relatively high lipolytic potential during winter in *C. glacialis* might reflect the importance of the lipid metabolisms in this species.

The mechanisms that control timing and duration of dormancy in winter and activity during the productive Arctic season are controversially discussed. Some authors suggest that internal cues, i.e. threshold levels of total lipid content or hormones (e.g. Irigoien, 2004, Clark et al. 2013) regulate onset and end of overwintering while others discuss environmental conditions such as temperature and light (Kosobokova 1990, Miller et al.1991, Hirche 1996, Niehoff & Hirche 2005, Varpe et al. 2007). Since the seasonal cycle in the food regime is most prominent in Arctic ecosystems, also food availability has often been suggested to have a major influence on the timing of diapause (Mayzaud & Poulet 1978, Hirche 1981, Søreide et al. 2010). In our study, the digestive activities increased in female and CIV of *C. glacialis* in late March/early April, when ice algae developed, and high digestive enzyme activities corresponded to the phytoplankton bloom. We thus did not find evidence for the internal regulation of enzyme synthesis prior to food supply as has been shown in *C. hyperboreus* (Head & Conover 1983). Similarly, enzyme activities in the CV at the surface, in July 2012 were relatively high indicating that the copepods were still actively feeding, while the activity of the CV at depth was only half of that of individuals from the surface. These results suggest that also the decrease in digestive enzyme activity is the result of starvation in *C. glacialis* at depth rather than that of internal regulation. It has to be noted that the copepods migrated downward and upward, respectively, in only a few weeks while our sampling interval was approximately one month. As we covered an entire year, sampling at higher frequency was not possible due to logistical constraints and to closely follow the changes in digestive activity during these transition phases in July/August and March/April would be essential.

In conclusion, our study showed a clear seasonal pattern in digestion of *C. glacialis*, with high digestive activities in individuals in surface waters during the productive

season and low activities in diapausing individuals in deep waters. Digestive enzyme synthesis was related to food availability, rather than being regulated internally or being triggered by the vertical distribution of the copepods in the water column. The copepods ascended well before algae developed and, as probably feeding initiated digestive activity, C. glacialis should be capable to exploit earlier phytoplankton blooms. The descent started, however, before food supply had ceased in surface waters suggesting that the copepods would not benefit from phytoplankton late in the season.

Acknowledgements

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Manuscript III

Metabolic enzyme activities and body composition during the ontogenetic vertical migration of the Arctic copepod *Calanus glacialis*

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Abstract

In polar seas, zooplankton have evolved special adaptations to survive long periods of continuous darkness with insufficient food supply. Accumulation of large lipid reserves in spring and summer are followed by a profound reduction in metabolism in autumn and winter. In large calanoid copepods of the genus *Calanus* spp. the metabolic adjustments during winter are referred to as diapause. These lipid-rich copepods comprise up to 80% of the mesozooplankton biomass in Arctic seas, and play a key role in Arctic marine food webs. They overwinter in deep waters for >6 months a year, but their physiology during this time is poorly understood. The Arctic is experiencing rapid changes in climate and more knowledge on the physiology of *Calanus* spp. is crucial to predict potential effects on this species. In this study we aimed to determine to what extent the Arctic shelf species *Calanus glacialis* adjusts its key metabolic pathways during overwintering. We followed metabolic and catabolic enzyme activities and the biochemical composition of this species monthly from July 2012 to July 2013 in Billefjorden, a high-Arctic sill fjord. During overwintering, metabolic enzyme activities were only reduced by half compared to activity levels we observed in spring and summer. The reduction in metabolic enzyme activities were most likely a response to starvation, since enzyme activities decreased gradually over the time the copepods were at their overwintering depth. From the descent in July to December, we found little changes in the biochemical composition of the copepods. Then, a steep drop in lipid reserves was observed, which coincided with high catabolic enzyme activities. The increase in lipid catabolism suggests moulting, gonad maturation and egg production in females. The relatively high metabolic activity in *C. glacialis* during winter suggests that older developmental stages (>CIV) of this shelf species are not entering a ‘real’ diapause.

1 Introduction

Dormancy is a key behavioral adjustment to adverse environmental conditions in copepod taxa from various habitats. In higher latitudes, dormancy can last for more than half a year in large calanoid copepods as a response to the strong seasonality. Copepods of the genus *Calanus* spp. comprise up to 80% of the mesozooplankton biomass in Arctic and sub-Arctic seas and are important contributor to the energy flux in high-latitude marine ecosystems (Falk-Petersen et al. 1990, Blachowiak-Samolyk et al. 2008, Søreide et al. 2008). They perform extensive ontogenetic vertical migrations every year and are in deep waters during winter in a state known as diapause. The metabolic processes of this dormant state are, however, poorly understood. Reasons for this are that so far it has been impossible to induce diapause in *Calanus* spp. in the laboratory and it is logistically very challenging to conduct seasonal studies in polar environments (Miller & Grigg 1991). Moreover, the variability in the onset and termination of diapause differs among species and regions, like e.g. open ocean systems versus shallow fjord systems, and this makes a clear definition of diapause in *Calanus* spp. difficult (Johnson et al. 2008, Clark et al. 2012). In general, diapause is characterized by an accumulation of internal energy reserves during the productive season and metabolic depression during winter (Atkinson et al. 2002). During overwintering, copepods are believed to stop feeding and fuel their basal metabolism by using internal lipid and protein reserves (reviews by Hirche 1996 and Conover & Huntley 1991). Moreover, internal energy reserves are consumed to fuel the ascent, gonad maturation and reproduction in early spring (Jønasdottir 1999, review by Falk-Peterson et al. 2009).

The switch from feeding during the productive season and starvation during overwintering requires metabolic adjustments of basic metabolic pathways, which are reflected in changes of enzyme activities (Ohman et al. 1998, Auerswald et al. 2009). This has already been shown in insects, which are believed to be closely related to crustaceans with regard to their metabolic adjustments during adverse environmental conditions (Elgmork & Nilssen 1978, Auerswald & Gäde 1999). However, seasonal studies that relate metabolic enzyme activities to the physiological state in marine crustaceans are rare. A recent study on the Antarctic krill *Euphausia superba* found low metabolic enzyme activity, but high catabolic enzyme activity and a metabolization of lipid reserves in overwintering krill (Meyer et al. 2010). A seasonal study on *C. finmarchicus* in Loch Etive, a sill fjord on the west coast of Scotland, investigated the

lipid composition in the copepods in relation to their state of physiological activity and found a pronounced catabolism of polyunsaturated wax esters during winter (Clark et al. 2012). A key enzyme of the β-oxidation of fatty acids is 3-hydroxyacyl-CoA dehydrogenase (HOAD) and thus, it is used as an indicator for the catabolism of body lipids (Auerswald & Gäde 1999, Hassett 2006). To correlate changes in the biochemical composition with metabolic/ catabolic enzyme activities, provides information on the metabolic status of an organism and reveals shifts in the utilization of energy reserves. This approach, however, is barely performed in copepod research, although, it provides information on the extent and timing of diapause in copepods. For instance a study on the North Atlantic *C. finmarchicus* showed that aminoacyl-tRNA synthethase (AARS), which is an enzyme related to protein synthesis, can be used as a proxy for growth and state of dormancy (Yebra et al. 2006). During dormancy, *C. finmarchicus* built-up less structural body proteins and thus, showed low AARS activities (Yebra et al. 2006).

Two enzymes which were shown to be reliable proxies of the overall metabolic activity in crustaceans are citrate synthase (CS) and malate dehydrogenase (MDH) (Meyer et al. 2002, Kreibich et al. 2008, Teschke et al. 2007). Both enzymes catalyze a reaction in the citric acid cycle and thus, can be used as an index for the aerobic potential of an animal (Torres & Somero 1988). The MDH also shuttles electrons between the cytosol and the mitochondrion. CS was shown to correlate with egg production in calanoid copepods (Kreibich et al. 2008), while MDH is strongly correlated to respiration (Meyer et al. 2010). To assess the metabolic potential of a copepod by measuring specific metabolic enzyme activities is of advantage compared to determine the metabolic activity by incubation methods, since artifacts caused by the handling of the animals are reduced to a minimum (Ohman et al. 1998).

In the shelf regions around Svalbard, zooplankton communities are dominated by *C. glacialis* (Jaschnov 1970). During its one to two year life cycle *C. glacialis* descends to deeper water layers in late July/ early August (Smith & Schnack-Schiel 1990, Søreide et al. 2010, Daase et al. 2013). It overwinters mainly as copepodite stages IV and V (reviews by Conover 1988 and Hirche 1998). The strong seasonality in the Arctic requires metabolic adjustments of *C. glacialis*, which determine the survival and overwintering success of the population and thus, of the productivity of the Arctic marine ecosystem (Varpe et al. 2009). We focused sampling on the main overwintering

stage CV and sampled monthly from July 2012 to July 2013 in Billefjorden, a high-Arctic sill fjord on the west coast of Svalbard. We aimed to better understand to what extent the metabolism of *C. glacialis* is reduced during winter and if the metabolic adjustments happen prior or after the descent and ascent. By relating activities of key metabolic and catabolic enzymes with the biochemical composition, we aimed to first elucidate if *C. glacialis* adjust their metabolic activity seasonally, and second if they switch between protein and lipid catabolism during winter.

2 Material and methods

2.1 Sampling area and sample processing

The calanoid copepod *Calanus glacialis* was collected monthly in Billefjorden, a high-Arctic sill fjord (78°40′N; 16°40′E), on the west-coast of Svalbard, for one year from July 2012 to July 2013. The maximum water depth of Billefjorden is around 190 m and the sill depth is between 40 to 50 m, which restricts the water exchange in the fjord (Nilsen et al. 2008). We can therefore assume that we sampled the same *C. glacialis* population over the year (Grigor et al. 2014). Since we presumably sampled the same population, we can exclude strong changes in structural body weight of the copepods and thus, enzyme activities and lipid and protein content were calculated per individual. Billefjorden was ice-covered from February to early June 2013. Water temperatures in the upper 50 m ranged from -1.7°C during ice-coverage to 5°C in late summer. The water temperature below 100 m was always around -1°C. Depending on the sea-ice conditions the sampling location was either assessed by boat (RV Helmer Hanssen, KV Svalbard, Farm), by the zodiac or snow mobile. Copepods were sampled with a WP-3 or WP-2 closing plankton net (1000 µm and 200 µm mesh size, respectively). Individuals were either sampled between 180 m to 100 m (July 2012 to February 2013 and July 2013) or between 50 m to surface (March to June 2013). The depth range was chosen according to where individuals occurred in the highest abundances. Immediately after capture, the plankton samples were transported to the laboratories of the University Centre in Svalbard (UNIS), where they were sorted alive under a stereo-microscope in a temperature controlled room at close to *in situ* temperature. We believe that misidentification of *C. glacialis* and confusion with *C. finmarchicus* is unlikely, since we based our identification on a size and pigmentation categorization that was affirmed by molecular analyses (Gabrielsen et al. 2012, Nielsen et al. 2014). Samples were snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

2.2 Analyses of enzyme activity

Activities of four different metabolic enzymes were measured in order to assess metabolic activities of metabolic and catabolic pathways. Activities of all enzymes were determined in triplicates in each sample. Samples were homogenized in the respective buffer and centrifuged at 15,000 g at 4°C for 15 min (Thermo Scientific, Heraeus Fresco 17).

2.2.1 Citrate synthase activity (CS)

CS (EC 4.1.3.7) activity was determined after Stitt (1984), modified after Saborowski and Buchholz (2002). Enzyme activity was determined by adding 20 µl 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma Aldrich, D8130), 20 µl acetyl-CoA (Acetyl-Coenzyme A trilithium salt, Roche Diagnostics, 13893324) and 20 µl sample or 20 µl buffer as controls to 520 µl 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0 in a semi-microcuvette. Samples were incubated for 5 min at 25°C and then 20 µl oxalacetic acid (Sigma Aldrich, O4126) was added to start the reaction. Temperature in the spectrophotometer was kept constant with a Peltier element (Krüss Optronic). The absorbance was measured continuously for 3 min at 25°C and 405 nm. Measurements were recorded with the software VisionLite (Version 2.2).

2.2.2 Malat Dehydrogenase (MDH)

Measurement of MDH (EC 1.1.1.37) activity was modified after Teschke et al. (2007). Samples were homogenized in 0.1 M potassium phosphate buffer at pH 7.0. 6.7 µl NADH (Roche Diagnostics 10107735001), and 6.7 µl sample were added to 180 µl 0.1 M potassium phosphate buffer at pH 7.0 in a 96-well plate. After 5 min of incubation at 25°C, the reaction was started by adding 6.7 µl oxalacetic acid (Sigma Aldrich, O4126). The absorbance was measured for 5 min at 25°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.2.3 Three-hydroxyacyl CoA dehydrogenase (HOAD)

HOAD (EC 1.1.1.35) activity was determined modified after Auerswald and Gäde (1999). Samples were homogenized in 180 µl 107 mM triethanolamine/ HCl (supplemented with 5.3 mM EDTA) buffer at pH 7.0. 6.7 µl NADH (Roche Diagnostics 10107735001) and 6.7 µl sample were added to 180 µl 107 mM triethanolamine/ HCl (supplemented with 5.3 mM EDTA) buffer at pH 7.0 in a 96-well plate. After 5 min of

incubation at 25°C, the reaction was started by adding of 6.7 µl acetoacetyl-CoA (Sigma A-1625). The change in absorbance was recorded for 5 min at 25°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.2.4 Aminoacyl- tRNA-synthetase (AARS)

AARS (EC 6.1.1.) activity was measured modified after Chang et al. (1984). Samples were homogenized in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.8. 66.7 µl sample were added to 80 µl Milli-Q and 53.2 µl pyrophosphate reagent (PPi, Sigma, P7275) in a 96-well plate. The reaction mixture was kept at room temperature for 5 min and afterwards, the decrease in absorbance was measured continuously for 10 min at 37°C and 340 nm with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.3 Water-soluble protein content

Water-soluble protein content was determined after Bradford (1976) using a Bio-Rad protein assay (BIO-RAD 500-0006). As a standard, bovine serum albumin was used (0 to 0.1 mg/ml). Samples were homogenized in 0.1 M Tris/HCl (supplemented with 10 mM $CaCl_2$) buffer at pH 7.0. Homogenates were centrifuged at 15,000 g at 4°C for 15 min (Thermo Scientific, Heraeus Fresco 17). Samples were diluted 1:27 in distilled water and 50 µl diluted sample was added to 250 µl protein assay, which was diluted 1:5 in distilled water. The assay was incubated for 15 min at 25°C in a 96-well plate. The absorbance of standard and samples was measured in triplicates at 600 nm and 25°C with a Synergy HTX Multi-Mode Reader and the software KC4 3.4 Rev. 21.

2.4 Lipid content

Lipid extraction was modified after Bligh & Dyer (1959). Frozen samples of *C. glacialis* copepodite stage V were lyophilized for 24 hours and then, lipids were extracted with dichloromethane/methanol (2:1, v/v) using a Potter-Elvehjem homogenizer. Extracted lipids were cleaned up with 0.88% Potassiumchlorid solution. Total lipid content was determined gravimetrically after evaporation of the solvent.

2.5 Statistical analysis

Statistical analysis was done with the free software R 3.0.1. To test for normal distribution, the Shapiro-Wilk test was used. For data, which were normally distributed and showed variance homogeneity, one-way ANOVA was used and followed by Tukey post-hoc tests. For non-normally distributed data, a Kruskal-Wallis test was applied and followed by Tukey post-hoc tests. When the p-value was lower than the α-level ($\alpha = 0.05$), the results were regarded as statistically significant and the null hypothesis was rejected.

3 Results

In the following, enzyme activities are presented per individual, except if stages or different generations, i.e. CV from July 2012 to February 2013 versus CV from June and July 2013 or deep-living versus surface-living individuals, are compared, then they are given per dry mass (DM).

3.1 Metabolic enzyme activities

3.1.1 Malate dehydrogenase (MDH) and citrate synthase (CS)

In our study we used CS and MDH as proxies of the overall metabolic activity in *C. glacialis*. Our sampling started in July 2012, which coincided with the descent of the *C. glacialis* population in Billefjorden. Both, CS and MDH, followed a similar decreasing activity-pattern in CV from the middle of July to early November 2012 and December 2012 (one-way ANOVA $p<0.05$, Tukey post hoc test, Fig. 1 and 2). In July 2012, part of the population remained in surface waters, while most individuals of the population were already down in deep waters. To assess if metabolic activities were adjusted prior or after the descent, we sampled CV from the surface (50-0 m) and compared their specific CS activity (10.7 ± 0.3 units mg DM^{-1}) with CV from depths below 100 m (7.3 ± 1.1 units mg DM^{-1}).

In October 2012, we had the opportunity to also sample CIV. When specific activities are considered, the specific CS activity was around 9 units mg DM^{-1} in both CIV and CV in October. MDH activities in CIV and CV in October were 32.4 ± 6.5 units mg DM^{-1} and 36.9 ± 8.9 units mg DM^{-1}. During winter, from December 2012 to February 2013, there were no significant changes in MDH and CS activities in CV (Fig. 1 and 2). Due to moulting to adults, the abundance of CV from March to May 2013 was too low

for measuring enzyme activities. However, from January onwards, we found adult females in high abundances in the water column. In females, MDH activities were relatively low and did not change significantly from January to March 2013. CS activities in females increased significantly from January to April. In April 2013, the ice algae bloom started in Billefjorden and thus, we sampled twice per month. In April/ May, only CIV were sampled in sufficient numbers. In this developmental stage, we found an increase in MDH activity from approximately 2.5 units indv.$^{-1}$ in April to 4.3 ± 1.2 units indv.$^{-1}$ in May, when a phytoplankton bloom was observed. CS activity of CIV increased continuously from 0.1 ± 0.0 units indv.$^{-1}$ in March to 0.3 ± 0.0 units indv.$^{-1}$ in May 2013 (one-way ANOVA $p<0.05$, Tukey post hoc test). In June and July 2013, we again sampled CV, newly moulted from CIV. The low abundances of CIV and females in June did not allow us to sample them for analyses. In these last two sampling months, both metabolic enzymes showed specific activities similar to the year before. Specific MDH activity was around 30 units mg DM^{-1} in June and July 2013 (July 2012: 45.6 ± 10.8 units mg DM^{-1}) and specific CS activity in CV was around 10 units mg DM^{-1} in July 2012 and June/ July 2013.

3.1.2 Aminoacyl- tRNA-synthetase (AARS)

The AARS activity, which is related to protein synthesis, was relatively high and stable (around 8 nmol PPi h^{-1} indv.$^{-1}$) in *C. glacialis* CV from July to November 2012 (Fig. 4). Only thereafter, it decreased significantly and reached the lowest values in February 2013 (4.2 ± 1.1 nmol PPi h^{-1} indv.$^{-1}$), after the population had been at overwintering depth for more than half a year (one-way ANOVA $p<0.05$, Tukey post hoc test). In October 2012, the specific AARS activity was around 0.3 nmol PPi h^{-1} mg DM^{-1} in CIV and CV. Females appeared in January 2013 and their AARS activity decreased until March, however, not significantly. In CIV from surface waters, AARS activity was still low, around 2.5 nmol PPi h^{-1} indv.$^{-1}$ from March to late April 2013. In CV, however, which were again abundant in surface waters June 2013, AARS activities were high (12.7 ± 2.5 nmol PPi h^{-1} indv.$^{-1}$) compared to July 2013, when individuals were again found at depths below 100 m.

3.2 Catabolic enzyme activities

3.2.1 Three-hydroxyacyl CoA dehydrogenase (HOAD)

The HOAD activity, which is an indicator for the catabolism of body lipids, showed a development, which was opposite to that of the metabolic enzymes. In July 2012, when most individuals of the *C. glacialis* population in Billefjorden were below 100 m water depth, the HOAD activity was low in CV (0.4 ± 0.2 units $indv.^{-1}$; Fig. 3). During the following months, the activity increased to approximately 1.0 units $indv.^{-1}$ in November and remained between 0.6 to 1.0 units $indv^{-1}$ until February 2013. Females appeared in January and their HOAD activities ranged between 0.65 to 0.85 units $indv.^{-1}$ in March. The CIV, inhabiting the surface layer, in April and May 2013, showed a continuous and significant decrease in HOAD activity (one-way ANOVA $p<0.05$, Tukey post hoc test). In June 2013, CV were again present in sufficient numbers and their HOAD activities were low (0.1 ± 0.1 units $indv.^{-1}$). In July 2013, however the HOAD activity in CV was again as high as 0.8 ± 0.3 units $indv.^{-1}$.

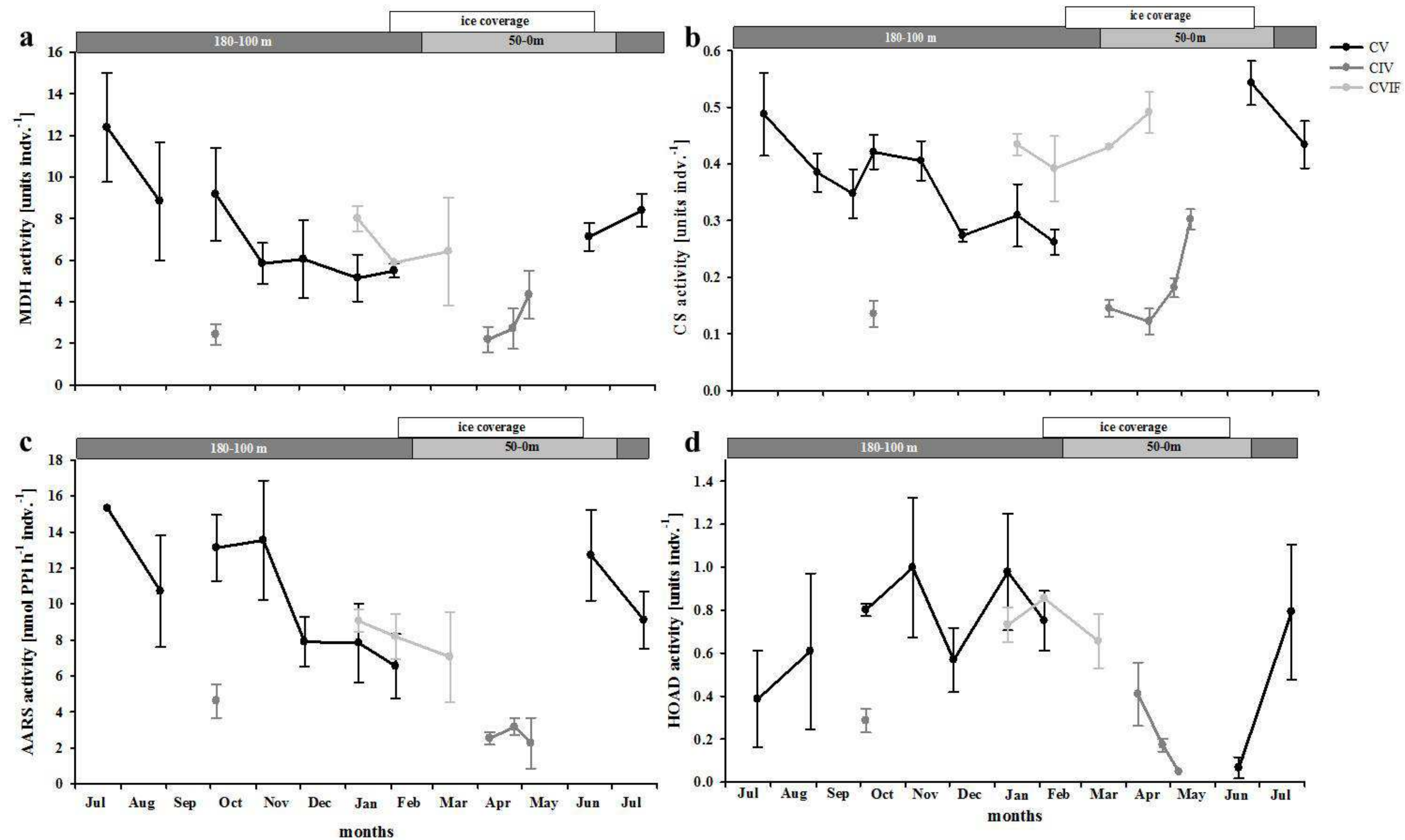

Fig. 1 Metabolic enzyme activities, i.e. malate dehydrogenase activity (MDH; a), citrate synthase activity (CS; b), aminoacyl-tRNA synthetase activity (AARS; c) and catabolic enzyme activity, i.e. 3-hydroxyacyl-CoA dehydrogenase activity (HOAD; d) in *Calanus glacialis* copepodite stages IV, V and adult females (CVIF) from July 2012 to July 2013 (n = 3, mean ± SD, except for MDH: CVIF in February n = 2; AARS: July 2012 n = 2; HOAD: CVFI in February and CIV in May n =2). From July 2012 to February 2013 and in July 2013, *C. glacialis* was sampled from 180 to 100 m water depth and from March 2013 to June 2013, it was collected in the upper 50 m. From February to early June 2013, the fjord was ice-covered.

3.3 Water-soluble protein and total lipid content

The water-soluble protein content in CV was around 75 µg indv.$^{-1}$ from July 2012 to February 2013 and thus, did not exhibit significant changes (Fig. 6). In June/July 2013, when the next generation of CV appeared it was, however, significantly lower (45 µg indv.$^{-1}$) compared to the year before. The female protein content decreased from 91.9 ± 26.1 µg indv.$^{-1}$ in January to 35.4 ± 2.1 µg indv.$^{-1}$ in April, however, the decrease was not significant. In CIV, the water-soluble protein content was high (72.2 ± 16.4 µg indv.$^{-1}$) in March and low in May 2013 (27.8 ± 1.8 µg indv.$^{-1}$).

Due to limited man-power and, in winter, limited number of individuals, total lipid content was determined only in CV and only from July 2012 to April 2013. As expected, the total lipid content of the CV was high between July and October 2012 (around 210 µg indv.$^{-1}$; Fig. 5). From October to December 2012, however, the total lipid content dropped drastically to around 70 µg indv.$^{-1}$ and remained at that level until April 2013.

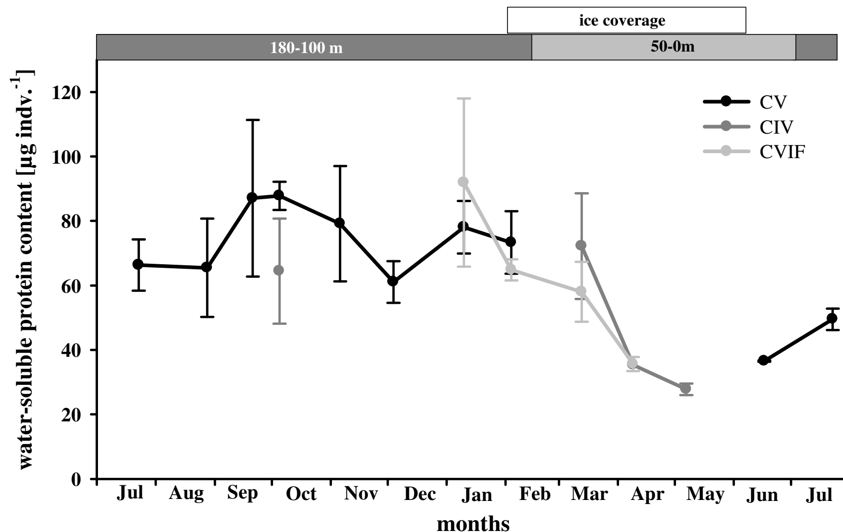

Fig. 2 Water-soluble protein content in *Calanus glacialis* copepodite stages IV, V and adult females (CVIF) from July 2012 to July 2013 (n = 3, mean ± SD). From July 2012 to February 2013 and in July 2013, *C. glacialis* was samples from 180 to 100 m water depth and from March 2013 to June 2013, it was collected in the upper 50 m. From February to early June 2013, the fjord was ice-covered.

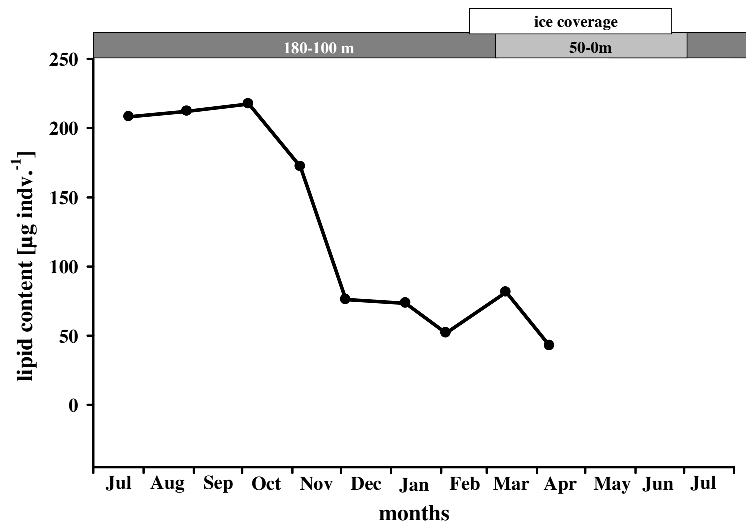

Fig. 3 Total lipid content in *Calanus glacialis* copepodite stages V from July 2012 to April 2013 (mean of 25 individuals). From July 2012 to February 2013, individuals were sampled from 180 to 100 m water depth and in March and April 2013, they were collected in the upper 50 m. From February 2013 on, the fjord was ice-covered.

4 Discussion

Calanoid copepod species of the genus *Calanus* have developed metabolic adjustments to survive long periods of food scarcity in diapause. The interactions of factors that initiate or terminate diapause are still not understood. Some authors suggest that environmental cues like food, temperature and light affect the timing of diapause (Kosobokova 1990, Miller et al.1991, Hirche 1996, Niehoff & Hirche 2005), while others believe that internal cues, like the amount of storage lipids or hormones determine diapause duration (e.g. Irigoien, 2004, Clark et al. 2013). Beside the factors that influence the timing of diapause, the intensity or level of changes in activity during diapause in calanoid copepods is not yet understood. The term diapause is commonly used in research concerned with the physiology of overwintering copepods (review by Dahms 1995 and Hirche 1996). However, the definition of true diapause is based on the physiological changes observed in insect during harsh environmental conditions. It is defined as arrested development, no movement and metabolic depression (Elgmork & Nilssen 1978). In contrast to this definition, we observed that *C. glacialis* moved around as soon as it was brought up to surface waters during winter and handled in the laboratory. Such short-term mobility has also been observed in Antarctic krill during winter and was explained by the metabolization of readily usable glycogen stores (Auerswald et al. 2009). This short-term mobility suggests that the copepods are not in a true state of diapause, which would imply total torpidity (Elgmork & Nilssen 1978).

The larvae and pupae of insects in true diapause show a metabolism that is depressed to values close to zero compared to activity (Guppy & Withers 1999). With only a threefold reduction of the respiration rate in *C. glacialis*, which was found in a study by Morata and Soreide (2013) in Billefjorden in 2009, the metabolic depression in copepods seems not to be as drastic as in insects. Another study on *C. finmarchicus* found 2.6 times lower AARS activities, an enzyme which is involved in protein synthesis, in dormant compared to pre-dormant individuals (Yebra et al. 2006).

In this study, we chose key enzymes of specific metabolic pathways to assess the overall metabolic activity in *C. glacialis*. The value of enzyme activities as proxies for basic metabolic processes has also been shown for crustacean in other studies (Meyer et al. 2002, Kreibich et al. 2008, Auerswald et al. 2009). Citrate synthase and malate dehydrogenase are both enzymes, which are connected to the tricarboxylic acid cycle and thus, give a good idea of the overall metabolic activity of an organism. During winter, the activity in both enzymes was still about half of the peak activity in *C. glacialis* during summer.

The intensity of diapause is affected by the water depth in which the copepods overwinter. Copepods that overwinter in shallow fjord areas are believed to depress their metabolism less compared to individuals that overwinter in the open ocean. *Calanus* spp. which overwinter in the open ocean, at depth below 500 m have low rates of lipid utilization (Jønasdøttir 1999), while individuals, which overwinter in shallow coastal habitats, have higher lipid turn-over rates (Clark et al. 2012). *C. glacialis* can be assigned to the latter category, as it is a shelf species, which predominantly inhabits the shelf and fjord systems in the Svalbard archipelago (Jaschnov 1970, Søreide et al. 2008). Since *C. glacialis* lives in shallow fjord environments, it can be assumed that its lipid reserves are steadily exploited during winter. However, from July to October no change in lipid content was seen. From October on the lipid content decreased significantly The initial lipid content (around 200 µg indv.$^{-1}$) of *C. glacialis* CV from Billefjorden dropped for more than 35%. An explanation for that might be moulting of the large CV to adult females and males (Jønasdottir 1999, Falk-Peterson et al. 2009). The drop in lipid reserves coincided with high HOAD activities, a key enzyme for the catabolism of fatty acids in the β-oxidation. This suggests that individuals were producing energy for metabolic processes by internal lipid utilization.

In general, it can be assumed that when the copepods use one internal reserve more than the other, i.e. lipid versus protein reserves, the enzyme activities of the respective metabolic pathways should either decline or increase (Auerswald et al. 2009). We found that the lipid catabolism, i.e. HOAD activities, was high between October and January, which coincided with the lowest values of AARS activity and thus, with the protein metabolism. This would suggest a switch from protein to lipid utilization. However, we found no significant changes in water-soluble protein content during winter. The protein content decreased during late winter/ early spring, which is when gonad maturation and spawning in females occurs (Niehoff et al. 2002).

Investigating metabolic enzyme activities does not only allow to follow switches from one metabolic pathway to another, it also gives insight into the timing of the metabolic adjustment. To date it is not understood if the copepods slow down their metabolism while they are still in surface waters or when they are already at their overwintering depth. We found that the activities of CS and MDH decreased continuously while *C. glacialis* was already at its overwintering depth and the lowest activities were found from December to February. This suggests that individuals adjust their metabolism after the descent. In a study on *C. hyperboreus*, Head and Harris (1985) did also not find a metabolic adjustment in the copepods before the population descended and concluded that this must happen when the individuals are already at their overwintering depth. The reduction in metabolic activity may thus be a result of starvation of the copepods during winter (Hassett 2006). Low CS activities were already connected with feeding cessation in individuals of the small temperate calanoid copepod *Temora longicornis* (Kreibich et al. 2008). Also in other crustaceans, CS activity decreased with a degradation of the nutritional state (Clarke and Walsh 1993, Meyer et al. 2002, Saborowski & Buchholz 2002). Reduced metabolic activities allow the animals to survive on a minimum energy demand during the time of food deprivation.

In conclusion, the metabolic activity of *C. glacialis* during winter is approximately half of the activity in individuals during the productive season. This adjustment, however, took several weeks and happened after the population had descended to their overwintering depth. By using metabolic enzyme activities as a proxy, our study revealed that *C. glacialis* might not overwinter in what is called true diapause, but rather

overwinter in a milder form of diapause, which allows it to move around in case of external disturbance.

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4 Results and synoptic discussion

In the past decade, the Arctic has faced rapid changes in sea ice thickness and extension (Gough et al. 2004, Comiso et al. 2008, Stroeve et al. 2012). These alterations cause changes in the underwater light regime and thus, severely affect timing and magnitude of primary production (Arrigo et al. 2008, Kahru et al. 2011). The ice conditions determine the timing and intensity of two distinct algal blooms, i.e. the ice algae and the phytoplankton bloom (Ji et al. 2013). The onset of the ice algae bloom is coupled to the return of light in March, and the phytoplankton bloom starts with the ice break-up (Hegseth 1998). *Calanus glacialis* spawns at reduced rates based on internal energy reserves, however, it needs algal food from the ice algae and phytoplankton bloom to reproduce at high rates (Smith 1990, Hirche and Kattner 1993, Niehoff et al. 2002). The offspring then grows and develops based on the phytoplankton bloom (Søreide et al. 2010). The synchronization of its life cycle with both blooms makes *C. glacialis* a successful and important contributor to the energy flux in Arctic shelf areas, and it most important to understand the effects of climate change on the survival success of this pelagic copepod.

This thesis focuses on the physiology of *C. glacialis* and assesses its capacity to adjust to changing environmental conditions. In the following a multidimensional analysis of all data (**Manuscript I - III**) characterizing the physiological state of *C. glacialis* during all seasons in a high-Arctic fjord will be presented. To assess the influence of environmental conditions, the biochemical composition and enzyme activities of *C. glacialis* from three fjords and from individuals, which were experimentally exposed to different food and light conditions, will be compared.

4.1 Physiological and biochemical adaptations during activity and diapause

The core of this thesis is a seasonal case study in Billefjorden, a high-Arctic fjord on the western coast of Svalbard. The following chapter presents physiological characteristics of active and diapausing copepods from Billefjorden (chapter 4.1.1). In a comprehensive approach, the physiology of *C. glacialis* from Kongsfjorden, which is influenced by Atlantic water, and Rijpfjorden, which is influenced by Arctic water, is described (chapter 4.1.2).

4.1.1 Seasonal study on the physiology of *Calanus glacialis* in Billefjorden

The life cycle of *C. glacialis* is well adapted to the strong seasonality in the high Arctic. The copepods accumulate energy reserves in surface waters during the productive season and spend the winter in diapause in deep waters (Conover 1988). The life strategy is reflected in the physiology of the copepods. In the present study, we found clear seasonal enzyme activity patterns: digestive and metabolic enzyme activities were high during spring and summer, when individuals were in surface waters, and low in autumn and winter, when individuals were in deep waters. For the catabolic enzymes, the activity patterns were vice versa. In the following, the seasonal patterns in enzyme activities, the biochemical composition and the extracellular pH and cation composition of *C. glacialis* are described in relation to the vertical migration of the copepods (Fig. 4.1 and Fig 4.2). Finally, the attempt is made to assign the physiological changes in *C. glacialis* to the diapause phases defined by Hirche (1996) in relation to a multi-dimensional scaling analysis (Fig. 4.3 and Fig. 4.4).

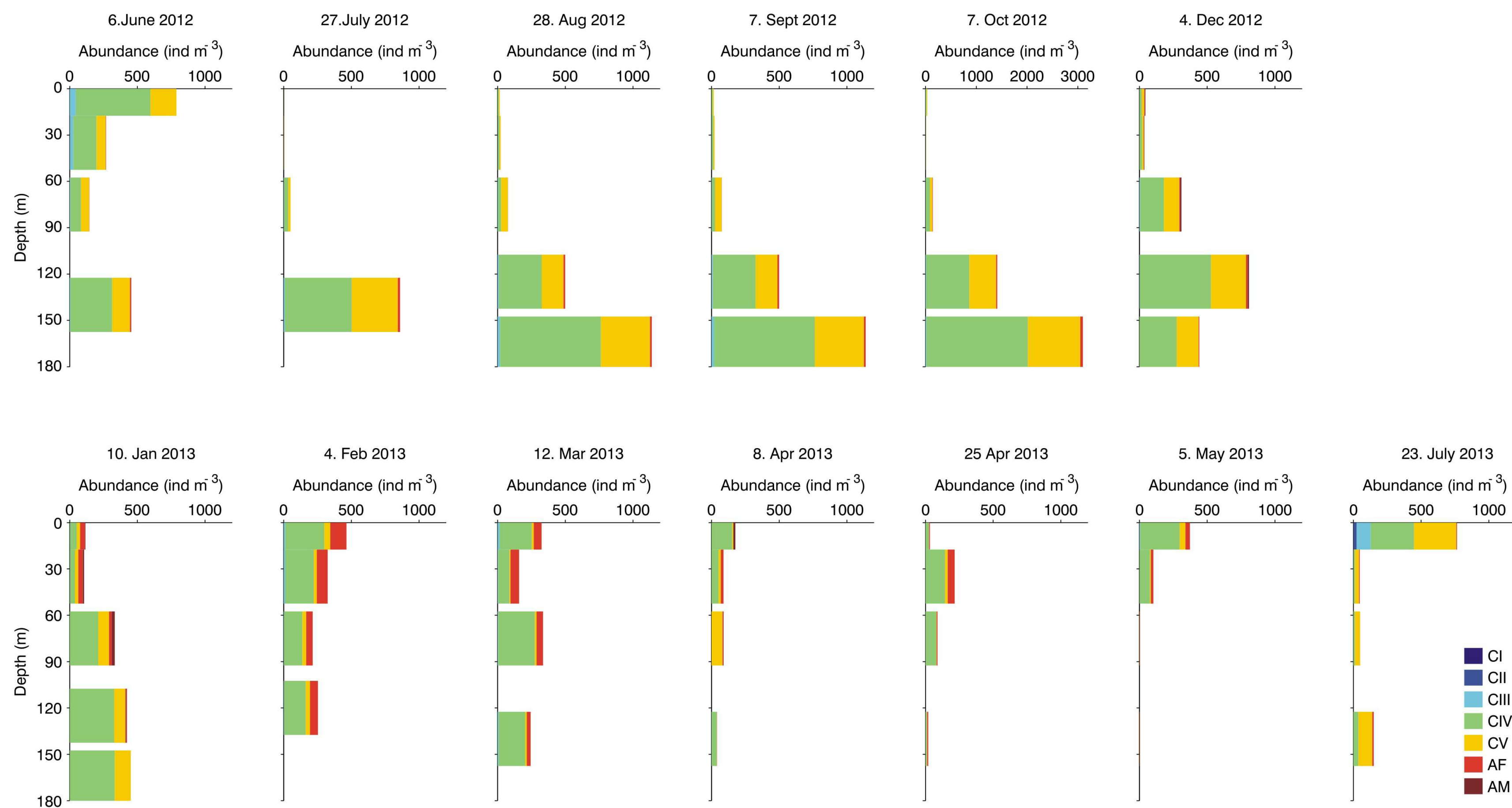

Fig. 4.1 Abundance and stage composition of *Calanus glacialis* from 180 m water depth to surface in Billefjorden from June 2012 to July 2013.

In Billefjorden, *C. glacialis* descended in late July and early August (Fig. 4.1; Søreide et al. 2010, Daase et al. 2013), before algal food is absent, probably to minimize the risk of predation (Fiksen & Carlotti 1998, Kaartvedt 2000). Interestingly, the descent coincided with an increase in sea surface temperature (Fig. 2.2) and indeed, sea surface temperature was suggested as an external trigger for the timing of diapause in *C. glacialis* (Kosobokova 1999, Niehoff & Hirche 2005).

In July 2012, the beginning of the overwintering period, dry mass (~600 µg $indv.^{-1}$) and lipid content (~200 µg $indv.^{-1}$) of *C. glacialis* CV were high as compared to the rest of the year (Fig. 4.2). This is a general pattern which enables the copepods to survive several months of food deprivation (Scott et al. 2000, Falk-Petersen et al. 2009). The high amount of lipid stores was also reflected in high carbon to nitrogen ratios (C:N) (Fig. 4.2). *C. glacialis* CV had a C:N ratio of approximatly eight during overwintering, which is twice as high as in copepods from medium and low latitudes (Båmstedt 1986).

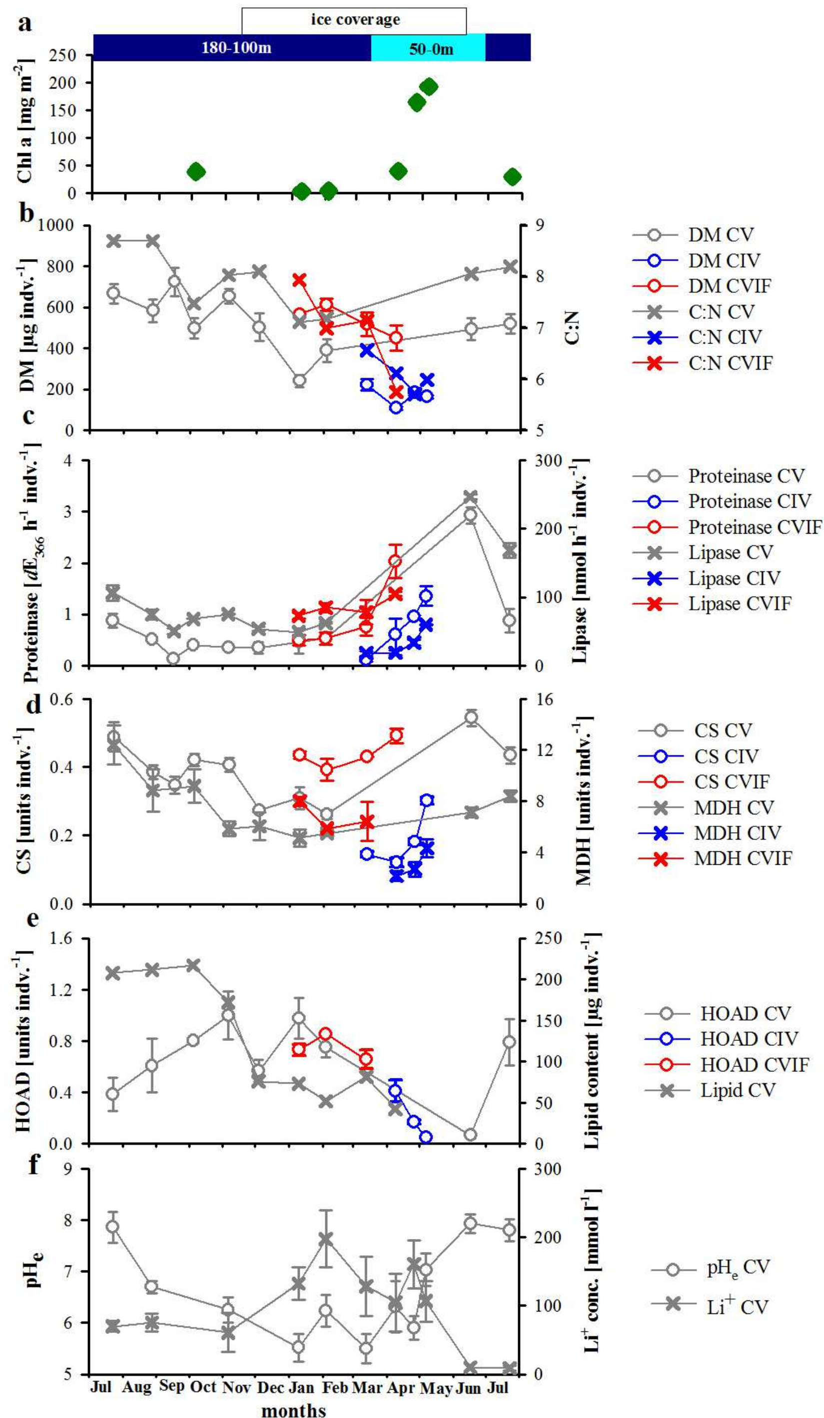

Fig. 4.2 Overview of the environmental conditions in Billefjorden and the physiology of *Calanus glacialis* copepodite stages IV (CIV), V (CV) and adult females (CVIF) from July 2012 to July 2013. The fjord was ice-covered from February to early June 2013. Chlorophyll *a* (Chl *a*) was integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface) (a). The following biochemical and physiological parameters are shown: dry mass (DM) and carbon to nitrogen ratio (C:N) (b); digestive enzyme activities (proteinase and lipase/esterase (lipase)) (c); metabolic enzyme activities (citrate synthase (CS) and malate dehydrogenase (MDH)) (d); catabolic enzyme activity (3-hydroxyacyl-CoA dehydrogenase (HOAD)) and lipid content (e); pH (pH_e) and lithium concentration (Li^+ conc.) of the haemolymph (f) (mean ± SE, for numbers of replicates see Table A 1 - 3 in the appendix). Individuals were sampled in the upper 50 m of the water column from March to July 2013 and below 150 m from July 2012 to February 2013, except for individuals for pH_e and Li^+ measurements, which were sampled in the upper 50 m in July 2012.

Shortly after *C. glacialis* reached its overwintering depth, digestive enzyme activities were low (Fig. 4.2, **Manuscript II**), which suggests that *C. glacialis* stopped feeding and entered diapause. During winter, autotrophic organisms which are the main food source of *C. glacialis* are not available and thus, the copepods are most likely starving during overwintering (Fleming 1939, Hirche 1983). Previous studies showed that starving calanoid copepods have low digestive enzyme activities (Hassett & Landry 1983, Kreibich et al. 2008) and B- and F-cells, which are located in the gut epithelium, synthesize fewer enzymes (Hallberg & Hirche 1980, Mayzaud 1986). Other studies revealed low respiration rates as a consequence of starvation (Ikeda 1977, Morata and Søreide 2013). In this study, we measured the activity of a key metabolic enzyme, malate dehydrogenase (MDH), which shuttles electrons between the cytosol and the mitochondrion and is closely correlated to the respiration rates of organisms (Meyer et al. 2010). MDH activity decreased continuously in overwintering *C. glacialis* from July to December 2012 (Fig. 4.2, **Manuscript III**). In addition to MDH activity, we assessed the metabolic activity in *C. glacialis* by means of citrate synthase (CS) activity measurements. Both enzymes are key enzymes of the tricarboxylic acid cycle and thus are a proxy for the overall metabolic activity of an organism. MDH and CS activity were only half as high in overwintering *C. glacialis* as compared to active individuals. A low metabolic activity enables the copepods to save energy in times of food scarcity (Meyer et al. 2002, Saborowski & Buchholz 2002, Kreibich et al. 2008).

Metabolic processes in general are related to the acid-base status of an organism (Reipschläger & Pörtner 1996) and enzyme activities are pH-dependent (Feller & Gerday 1997, Freese et al. 2012). In overwintering *C. glacialis* low enzyme activities were accompanied by a low extracellular pH (pH_e) (Fig. 4.2). The pH_e decreased continuously from pH 8 in July 2012 to less than pH 6 in January 2013 (**Manuscript I**). A change in the intracellular as well as extracellular pH has previously been related to metabolic depression in embryos of the brine shrimp (*Artemia salina*) and the peanut worm *Sipunculus nudus* (Busa & Crowe 1983, Reipschläger & Pörtner 1996). Recently, Schründer et al. (2013) suggested that pH_e may be related to metabolic depression also in Antarctic copepods, however, so far no seasonal study investigated this correlation in copepods. The distinct seasonal pattern of pH_e we found may thus be correlated to metabolic adjustments in *C. glacialis* and the low values during winter may indicate metabolic depression.

During metabolic depression the energy requirements of an organism are minimal. Copepods use only a small proportion of internal lipid reserves to fuel the basal metabolism. Instead, lipid reserves are depleted to a larger extent for moulting, gonad maturation and egg production (Jónasdóttir 1999, Falk-Peterson et al. 2009). We observed a decrease in lipid content and dry mass in *C. glacialis* CV in December and January (Fig. 4.2). This may be explained by moulting of large CV into adult females, which consequently increased in abundance at this time (Fig. 4.1). The lipid catabolism is regulated by the enzyme 3-hydroxyacyl-CoA dehydrogenase (HOAD), which fuels the β-oxidation of fatty acids (Auerswald & Gäde 1999). In accordance with the drop in lipid content, we observed high HOAD activities in *C. glacialis* (**Manuscript III**). Combined measurements of the biochemical composition and activities of key metabolic enzymes allow to observe switches in energy utilization and metabolic pathways (Auerswald et al. 2009). Thus, we assessed the lipid catabolism by means of HOAD activity and the protein metabolism by means of aminoacyl-tRNA synthetase (AARS) activity (**Manuscript III**). AARS catalyses the first step of the protein synthesis in organisms. AARS activity was low when HOAD activity was high from October to January, which suggests that *C. glacialis* relied on internal lipids, while the protein metabolism was low. In accordance, the water-soluble protein content did not change profoundly during winter (**Manuscript III**), and the C:N ratio decreased only slightly from December to January (Fig. 4.2).

A decrease in lipid content in *C. glacialis* in the end of winter would hinder lipid-based buoyancy, since lipids are suggested to support upward migration in copepods (Yayanos et al. 1978, Visser & Jónasdóttir 1999). In our study, we suggest ion replacement as another mechanism to fine-tune buoyancy (**Manuscript I**). During ion replacement, ions that increase the density of copepods, e.g. Na^+ and Mg^{2+}, are exchanged by ions that reduce the density, e.g. NH_4^+ and Li^+, in winter and vice versa in summer (Sartoris et al. 2010, Schründer et al. 2013, 2014). *C. glacialis* showed high concentrations of Li^+ towards the end of the overwintering period, when the copepods ascend into surface waters (Fig. 4.2). Thus, Li^+ could support upward migration.

From March on, when the *C. glacialis* population resided in the upper 50 m, digestive and metabolic enzyme activities increased and individuals started feeding on ice algae. The chlorophyll *a* concentration in the water column increased from 2.9 mg m^{-2} in

January to 192.9 mg m^{-2} in May 2013, when also digestive enzyme activity peaked in *C. glacialis* CIV. The copepods feed in surface waters in spring- and summertime and accumulate energy reserves (Båmstedt 1984).

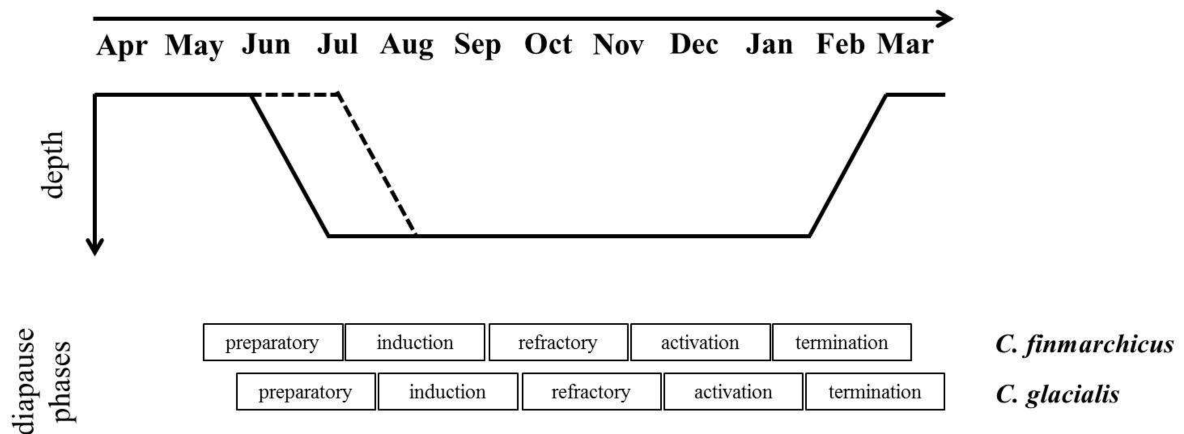

Fig. 4.3 Overview of the diapause phases and the seasonal vertical migration pattern of *Calanus finmarchicus* after Hirche (1996). The dashed line indicates the timing of descent of *C. glacialis* in Billefjorden, the ascent in both species matches. Diapause phases in *C. glacialis* are delayed by about one months in *C. glacialis* compared to *C. finmarchicus*.

Hirche (1996) assigned the seasonal changes in the physiology of *C. finmarchicus* to specific phases, i.e. the preparatory, induction, refractory, activation and termination phase (see chapter 1.2 for definition, Fig. 4.3). The present study on *C. glacialis* in Billefjorden showed clear seasonal patterns in the physiology and in the following, I compare the diapause phases of *C. finmarchicus* with those of its larger sibling species *C. glacialis* (Fig. 4.3). To illustrate and reveal if enzyme activities and the biochemical composition of *C. glacialis* CV were similar in certain months, a multi-dimensional analysis was performed (Fig. 4.4).

Hirche (1996) assigned the seasonal changes in the physiology of *C. finmarchicus* to specific phases, i.e. the preparatory, induction, refractory, activation and termination phase (see chapter 1.2 for definition). The present study on *C. glacialis* in Billefjorden showed clear seasonal patterns in the physiology, and in the following, I compare the diapause phases of *C. glacialis* with those of its smaller sibling species *C. finmarchicus* (Fig. 4.3). To illustrate and reveal if enzyme activities and the biochemical composition

of *C. glacialis* CV were similar in certain months, a multi-dimensional analysis was performed (Fig. 4.4).

According to Hirche (1996), the preparatory phase of diapause lasts from April to June in *C. finmarchicus*. In agreement, we observed that *C. glacialis* was actively feeding and growing in surface waters from April to July (Fig. 4.1 and Fig. 4.2). The duration and intensity of the productive season is critical for the development of *Calanus* spp. During the productive season, internal energy reserves are accumulated (Falk-Petersen et al. 2009). The lipid content was suggested as a possible cue for the timing of diapause (Irigoien 2004) and thus, food availability may indirectly play an important role in the timing of diapause (Søreide et al. 2008, 2010, Daase et al. 2013). Moreover, external cues like sea surface temperature (Kosobokova 1999, Niehoff & Hirche 2005) and photoperiod (Miller et al. 1991) may determine the timing of descent and explain discrepancies in the onset of diapause among *Calanus* species and populations from different habitats. The MDS analysis revealed a relatively scattered distribution of the productive months June and July 2012 and July 2013, which may indicate that the environmental conditions differed between the years and thus, the enzymatic and biochemical adjustments in the *C. glacialis* populations were dissimilar (Fig. 4.4).

Shortly after the descent, diapause is induced in *C. finmarchicus*, which is indicated by a cessation of feeding activity and low metabolic activity (Hirche 1996). Digestive enzyme activities of *C. glacialis* were low after the copepods reached the overwintering depth in Billefjorden. In comparison, also in *C. finmarchicus* from Gullmarfjorden, Sweden, and Korsfjorden, Norway, digestive enzyme activities were low in the beginning of overwintering (Hirche 1983). Metabolic enzyme activities in copepods at depths, i.e. CS and MDH activities, were not significantly lower compared to active individuals before November and December 2012 (Fig. 4.2). According to Hirche (1996), the copepods are in the refractory phase at that time, which is characterized by low metabolic activity and torpidity. Torpid behavior has been observed in overwintering *C. finmarchicus* (Hirche 1983) and *C. hyperboreus* (Conover 1962, Auel et al. 2003), however, *C. glacialis* showed no signs of torpidity after capture. The diapause intensity of the shelf species *C. glacialis*, which mainly overwinters in areas of less than 500 m water depth, may be less compared to *C. finmarchicus*, which is known to overwinter in water depth between 500 and 1500 m in the open ocean (Kaartvedt

1996, Dale et al. 1999). Correspondingly, metabolic enzyme activity decreased only by 50% (**Manuscript III**) and respiration rates in overwintering individuals were still one-third of those of active *C. glacialis* (Morata & Soreide 2013), while Hirche (1983) observed respiration rates in diapausing *C. finmarchicus* which were only one-fifth of those found in active individuals. Also digestive enzyme activities of *C. glacialis* in our study were only three to seven times lower in overwintering individuals compared to active ones, while activities of overwintering *C. finmarchicus* were 20 times lower compared to actively feeding copepods (Hirche 1983). The MDS plot revealed a clustering of August, October and November 2012 for enzyme activities (Fig. 4.4 a), however, it did not show a cluster for the biochemical composition (Fig. 4.4 b). This suggests that the metabolic activity is a better indicator for the state of diapause than the biochemical composition.

According to Hirche (1996), the activation and termination phase in *C. finmarchicus* lasts from December to March. During this time, the individuals moult, develop into males and females and ascent. By the end of the termination phase, the full metabolic potential is regained. We observed profound changes in dry mass and lipid content in *C. glacialis* during that time (Fig 4.2), which is probably due to an energy allocation into gonad maturation and moulting (Tande 1982). Digestive and metabolic enzyme activities were low from December to March, while HOAD activities were relatively high in *C. glacialis* compared to individuals during spring and summer.

Adaptations to the physical and biological environment of different habitats may explain the differences in the physiology of *C. finmarchicus* and *C. glacialis* during diapause (Hairston & Olds 1987, Dahms 1995). Both *Calanus* species, however, exhibit the five phases as described by Hirche (1996), which suggests an evolutionary establishment of diapause (Dahms 1995). Future studies should compare the physiology of the two *Calanus* species from the same habitat to assess to what extent environmental factors influence diapause behavior. In a first attempt, we investigated the physiology of *C. glacialis* from different habitats and compared enzyme activities and the biochemical composition of populations from different fjords. The results are described in the following chapter.

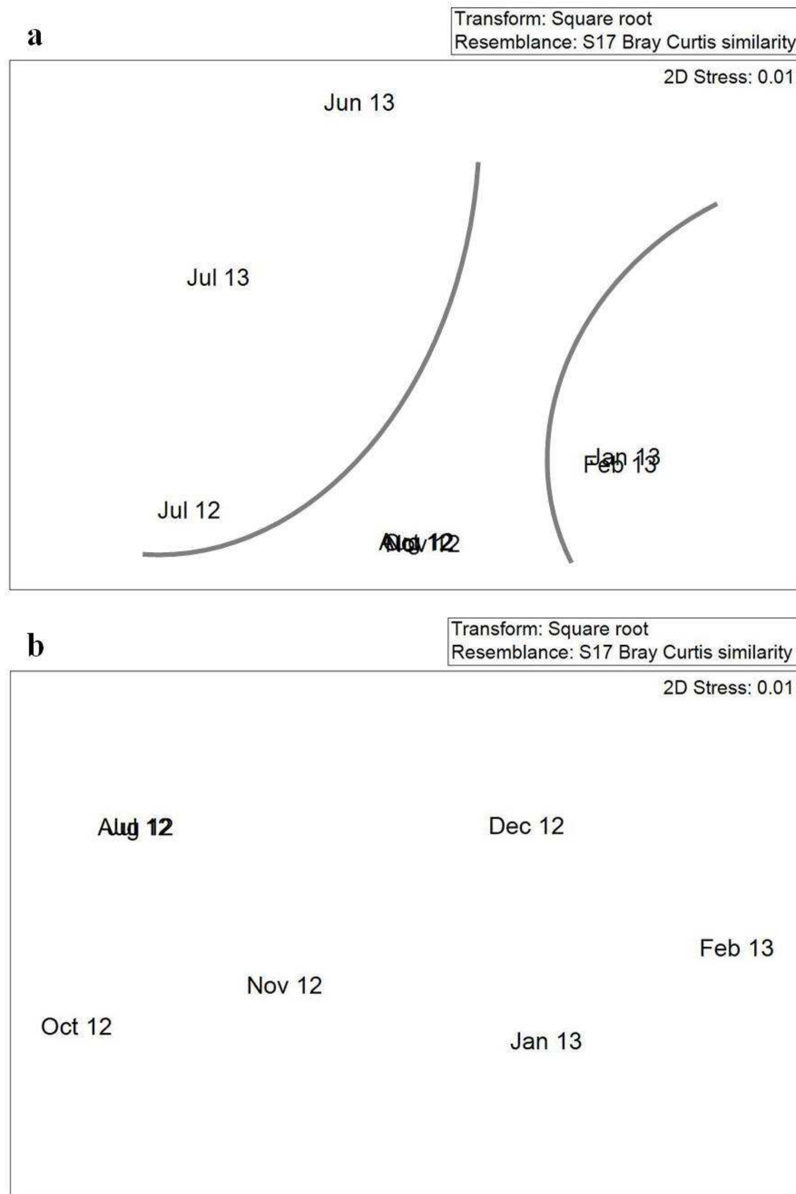

Fig. 4.4 Multi-dimensional scaling (MDS) plots based on Bray-Curtis similarity analysis on square root transformed digestive and metabolic enzyme activities (a) and biochemical composition data (b) of *Calanus glacialis* copepodite stage V from July 2012 to July 2013. CV were not sufficient to provide enough samples for enzyme activities and biochemical analysis in September 2012 and in March to May 2013. For (a), data on proteinase, lipase/esterase, citrate synthase, malate dehydrogenase, 3-hydroxyacyl CoA dehydrogenase and aminoacyl- tRNA-synthetase activities are included, and for (b), data on the lipid, water-soluble protein, carbon and nitrogen content and dry mass are included. The distance between months on the MDS plot shows the similarity, i.e. the closer two months are to each other, the more similar they are.

4.1.2 Spatial variations in the physiology of *Calanus glacialis*

The marine realm around Svalbard is a transition zone between Atlantic and Arctic water masses (Daase & Eiane 2007). The West Spitsbergen Current transports warm and saline Atlantic water northwards around the west of Svalbard, while the East Spitsbergen Current brings cold and less saline Arctic water from the Polar Basin (Svendsen et al. 2002, Schauer et al. 2004). This results in different hydrographical regimes in the fjords around Svalbard (Fig. 4.5). To study the effects of prevailing environmental conditions on the physiology of *C. glacialis*, we compared the populations of (i) Billefjorden, a sill fjord which is mainly influenced by locally formed Arctic water masses, (ii) Kongsfjorden, an open fjord which is strongly influenced by Atlantic water and (iii) Rijpfjorden, which opens towards the deep Polar Basin and is considered a true Arctic fjord (Kwasniewski et al. 2003, Nilsen et al. 2008, Wallace et al. 2010). Water temperatures differ among the fjords. Sea surface temperatures in Billefjorden ranged between -1.7°C and 5°C from July 2012 to July 2013 (Fig. 2.2). In Kongsfjorden in the same year, temperatures varied from 3°C to 6°C over the entire water column for more than seven months (Nahrgang et al. 2014). In contrast, sea surface temperatures in Rijpfjorden exceeded 3°C merely for one month, while during the rest of the year temperatures were around 0°C throughout the water column (Nahrgang et al. 2014). Sea ice conditions also vary among the fjords: Kongsfjorden is ice-free during the entire year (Cottier et al. 2007), while Billefjorden is ice-covered for around six months until May or early June and Rijpfjorden is covered by ice during most of the year from December to July (Søreide et al. 2010). As the start of the phytoplankton bloom is strongly dependent on the ice cover and light regime, the onset is delayed by several weeks in Rijpfjorden (Leu et al. 2006).

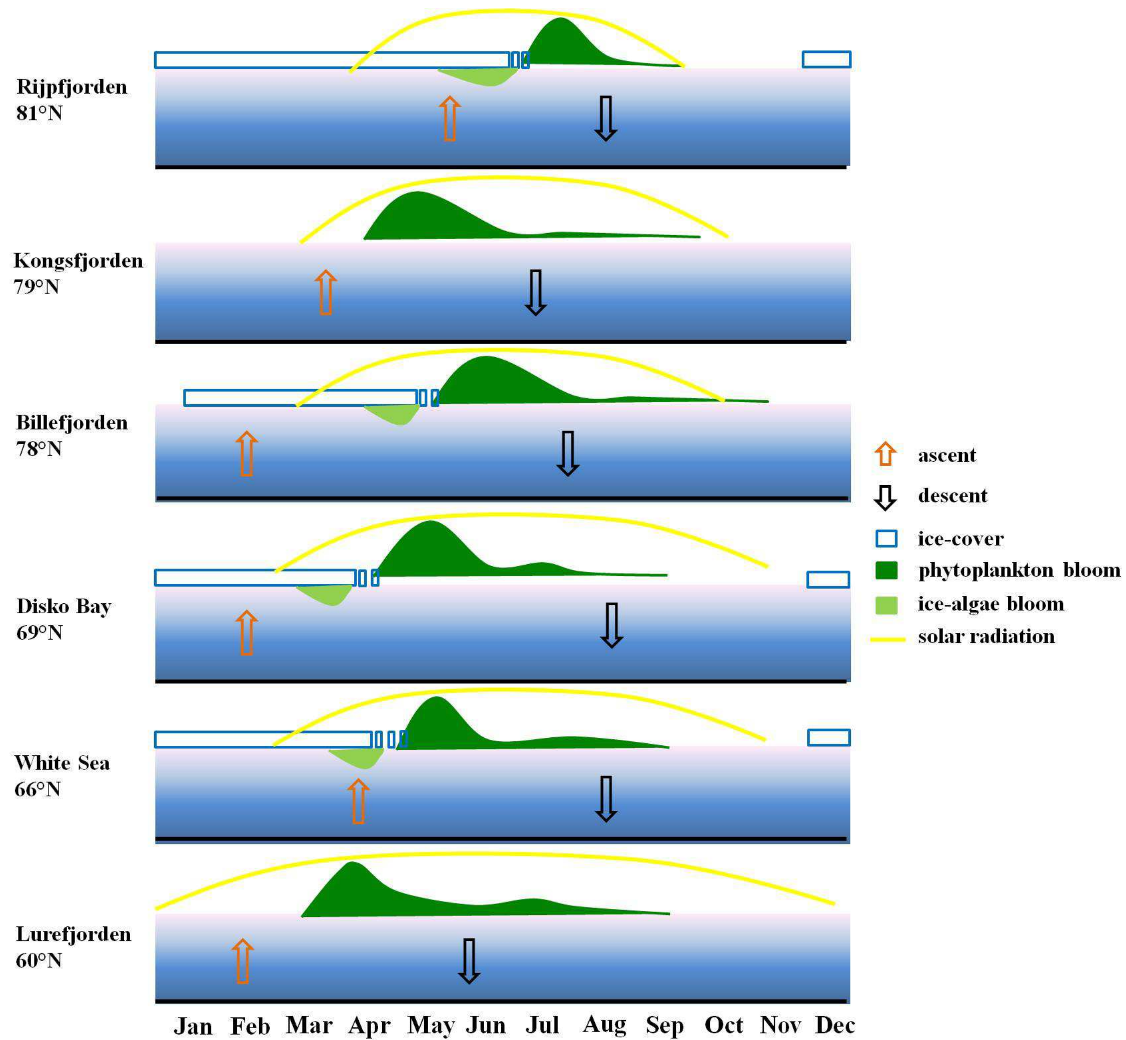

Fig. 4.5 Schematic comparison of the environmental conditions in six habitats of *Calanus glacialis* after Søreide et al. (2010) and Daase et al. (2013). Shown are the sea ice-cover, the solar radiation, the timing of ice algae and phytoplankton bloom and the timing of vertical migration of *Calanus glacialis* in three fjords in the Svalbard archipelago, i.e. Billefjorden, Kongsfjorden and Rijpfjorden, the Disko Bay (west Greenland), the White Sea and Lurefjorden (west Norway).

The prevailing water masses and environmental conditions determine the zooplankton population structures and species abundances in the three fjords. *C. finmarchicus* dominates the pelagic community in Kongsfjorden, since this species is mainly associated with Atlantic water (Daase & Eiana 2007, Daase et al. 2007). *C. glacialis* predominates in Billefjorden (Arnkværn et al. 2005) and also in Rijpfjorden (Søreide et al. 2008). *C. hyperboreus* prefers Arctic water masses and deeper waters and mainly occurs off the Svalbard shelf (Hassel 1986, Blachowiak-Samolyk et al. 2006). The adaptation to different habitats is reflected in the physiological characteristics of the three species, i.e. life cycle length, reproduction and size (Conover & Huntley 1991,

Falk-Petersen et al. 2009). By comparing *C. glacialis* populations from three fjords, we aimed to elucidate if environmental conditions cause intraspecific differences in physiological parameters. The focus of this study is on the CV as the main overwintering stage, but whenever these were not sufficiently abundant for biochemical analyses, adult females were analyzed. It has to be noted that due to logistical constraints sampling in Kongsfjorden and Rijpfjorden was not performed as frequently as in Billefjorden.

We observed a similar seasonal pattern in enzyme activities in all three fjords, i.e. low digestive and metabolic enzyme activities in autumn and winter and high activities in spring and summer and vice versa for catabolic enzyme activities (Fig. 4.6). There were, however, differences in the physiology of the three *C. glacialis* populations, which may be attributed to the prevailing environmental conditions.

In July 2012, *C. glacialis* CV were sampled in the upper 50 m of the water column and digestive and metabolic enzyme activities were relatively high in all fjords. However, proteinase, MDH and AARS activities of copepods from Rijpfjorden were significantly higher compared to CV in Kongsfjorden and in Billefjorden (one-way ANOVA $p<0.05$, Tukey post hoc test). The phytoplankton bloom starts later in Rijpfjorden compared to the other two fjords. The timing of the phytoplankton bloom strongly determines the time of descent in *C. glacialis*. In locations where the bloom starts early, e.g. in the ice-free Kongsfjorden and Lurefjorden (west Norway), the copepods descend as early as June, while in areas, which are ice-covered for several months, like e.g. the Disko Bay, the White Sea and Rijpfjorden, *C. glacialis* descends between July and August (Fig. 4.5, Søreide et al. 2008, Daase et al. 2013). Thus, maximal metabolic activities can be expected earlier in the more southerly located fjords as compared to Rijpfjorden.

In September individuals resided at their overwintering depth in Rijpfjorden (Fig. 4.5). Digestive enzyme activities were less than half of the activity in July, and also metabolic enzyme activities were only half of the activity of individuals from surface waters in July. HOAD activity, however, was ten times higher in CV at overwintering depth compared to individuals from the surface in Rijpfjorden (Fig. 4.6). In January and February, digestive and metabolic enzyme activities were still low, while HOAD activity was high in all three *C. glacialis* populations. In contrast to Rijpfjorden,

however, adult females appeared in higher abundances in Billefjorden and Kongsfjorden, possibly due to earlier moulting of the CV southwest off Svalbard, where warm Atlantic water prevails (Søreide et al. 2008). Indeed, CS and lipolytic activity of *C. glacialis* adult females in Kongsfjorden in February were already almost as high as for adult females in Rijpfjorden in May 2013 (Fig. 4.6). Generally, *C. glacialis* ascends and reproduces earlier in ice-free habitats with an early onset of the phytoplankton bloom (Fig. 4.5, Daase et al. 2013). Rijpfjorden is located very far north (81°N), which results in a shorter period of solar radiation and longer period of ice coverage compared to the more southerly, but also ice-covered Disko Bay (69°N) and White Sea (66°N) (Daase et al. 2013). The extreme environmental conditions in Rijpfjorden probably explain the long diapause period in the *C. glacialis* populations in Rijpfjorden compared to the populations of other locations (Fig. 4.5). In May, the copepods were feeding on ice algae in surface waters in Billefjorden and Rijpfjorden and the activities of all enzymes, except HOAD, were high.

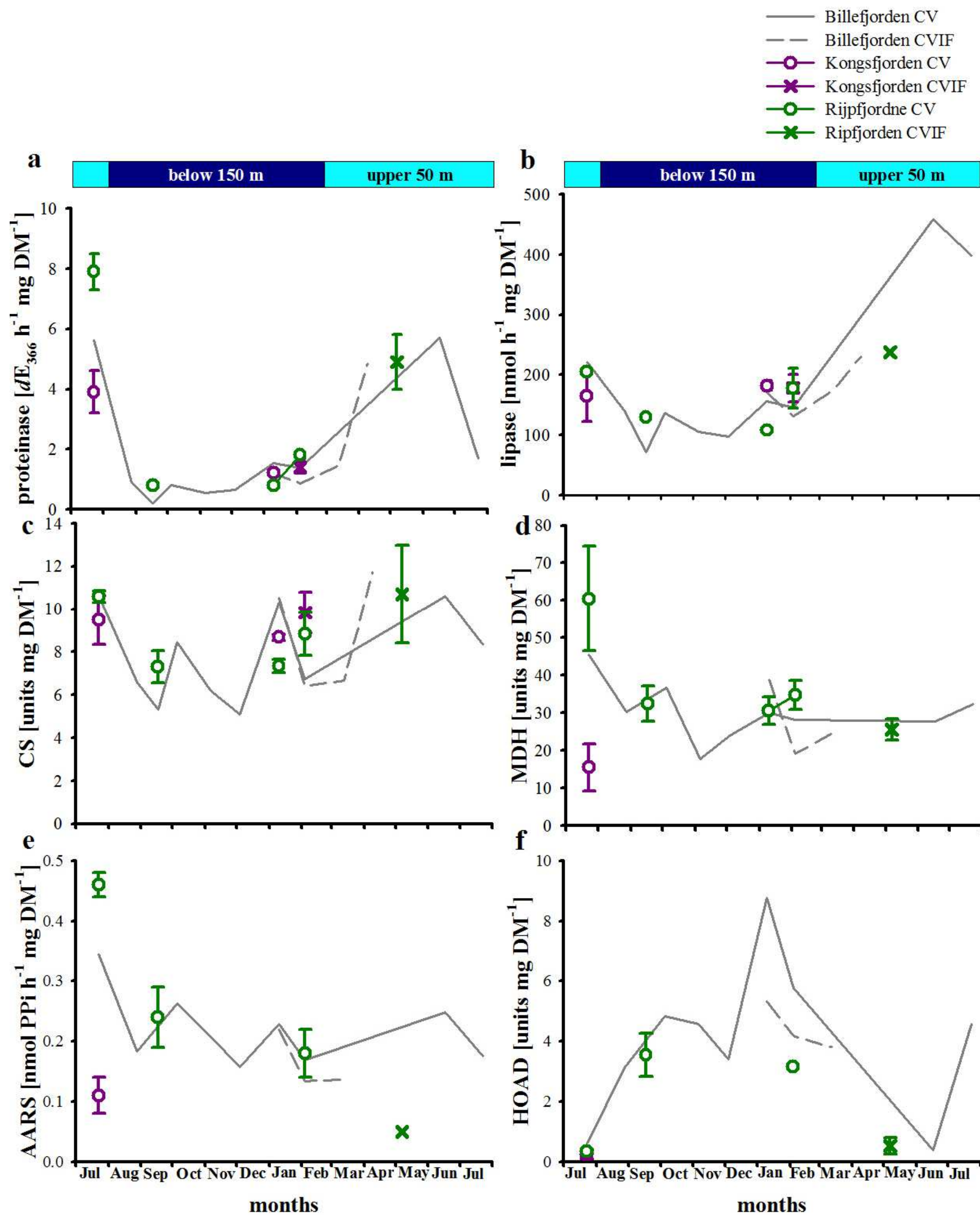

Fig. 4.6 Comparison of enzyme activities of *Calanus glacialis* copepodite stage V (CV) and adult females (CVIF) from Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013: proteinase (a), lipase/esterase (lipase) (b), citrate synthase (CS) (c), malate dehydrogenase (MDH) (d), aminoacyl-tRNA synthetase (AARS) (e) and 3-hydroxyacyl-CoA dehydrogenase (HOAD) (f) activity (mean ± SD, n varied between 2 and 3, for exact numbers see appendix Table A 4 - 9 in the appendix). Individuals were sampled in the upper 50 m of the water column in July 2012 and from March to July 2013 and below 150 m from August 2012 to February 2013 (for exact sampling depths see chapter 2.1).

Despite the difference in food availability between the fjords, dry mass and C and N content did not differ significantly among the *C. glacialis* populations (Fig. 4.7). Dry mass and C and N content were high in the beginning of the overwintering period in

July and September 2012 and significantly lower in January/February 2013, when individuals started to moult and to prepare for the ascent (Kruskal-Wallis p<0.05, Dunn's post hoc test). In the future, a higher sampling frequency would be desirable to tackle when exactly the biochemical composition of the copepods changes in Kongsfjorden and Rijpfjorden. Presumably, the drop in dry mass should be earlier in Kongsfjorden and Billefjorden than in Rijpfjorden, because copepods moult earlier in the southern fjords (Søreide et al. 2008).

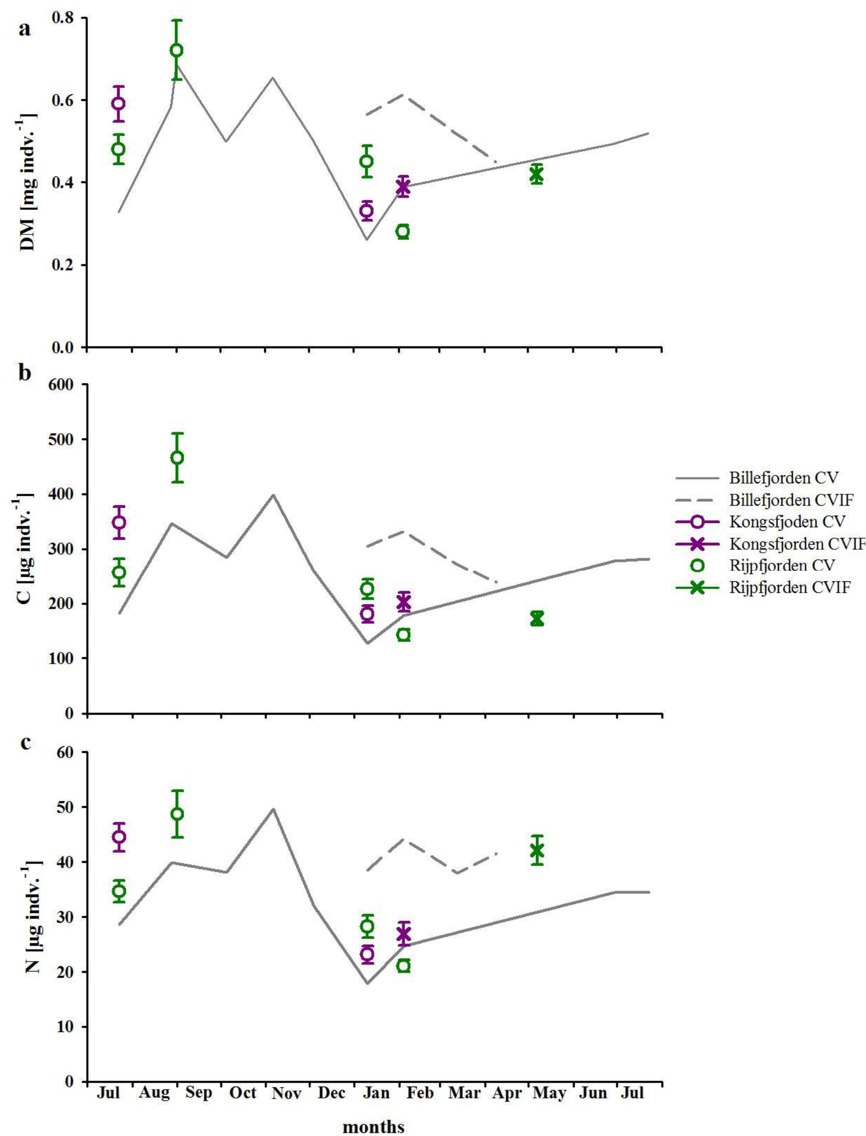

Fig. 4.7 Comparison of the biochemical composition of *Calanus glacialis* copepodite stage V (CV) and adult females (CVIF) from Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013: dry mass (DM) (a), carbon (C) (b) and nitrogen (N) content (c) (mean ± SE, n varied between 2 and 3, for exact numbers see Table A 10 - 12 in the appendix). Individuals were sampled in the upper 50 m of the water column in July 2012 and from March to July 2013 and below 150 m from August 2012 to February 2013 (for exact sampling depth see chapter 2.1).

Besides food availability, the temperature difference among the fjords may influence the physiology and timing of life cycle events of *C. glacialis*. For instance, the smaller species *C. finmarchicus* overwinters in diapause for nine months at 0°C (Saumweber & Durbin 2006), and this duration may shorten by one month at a temperature increase of 2°C (Pierson et al. 2013). In *C. glacialis*, in contrast, it was observed that the diapause duration is shorter in the cold Rijpfjorden compared to the warmer Kongsfjorden and Lurefjorden (Fig. 4.5, Daase et al. 2013). Other studies observed an effect of temperature on the reproduction period and showed that females stopped to reproduce and descended to their overwintering depth when sea surface temperatures increased to around 5°C in Lurefjorden and the White Sea (Kosobokova 1999, Niehoff & Hirche 2005). A study by Niehoff et al. (2002), however, found that *C. glacialis* remained in surface waters in Disko Bay even after the temperature had reached 6°C. In conclusion, the life cycle of *C. glacialis* is well adapted to ice-free areas with warmer water temperatures as well as to habitats with extensive ice coverage, less algal food and low temperatures. Future studies under controlled laboratory conditions should investigate if *C. glacialis* is also able to adjust its physiology on a short-term when temperature and food conditions change.

Key results of chapter 4.1:

- *C. glacialis* shows a clear seasonal pattern in digestive and metabolic enzyme activities, with high activities in spring and summer during the productive season and low activities in autumn and winter during diapause; and vice versa for catabolic enzyme activities.
- The biochemical composition, i.e. dry mass, C:N ratio and lipid content changed profoundly at the end of the overwintering period, possibly due to moulting, gonad maturation and the ascent.
- Diapause in *C. glacialis* can be categorized into phases, during which the copepods differ in their metabolic activity, and enzyme activities are a useful approach to characterize the physiological state of *C. glacialis*.
- Adjustments in metabolism are similar among *C. glacialis* populations of different environments, suggesting an evolutionary basis of diapause. Prevailing environmental conditions, however, result in differences in timing and intensity of metabolic activity.

4.2 Effect of different food and light conditions on the physiology of *Calanus glacialis*

In the framework of two incubation experiments at different food and light conditions, the physiological ability of *C. glacialis* CV to adjust to external cues during diapause was investigated. One experiment was performed in August/September 2012 (in the following referred to as August experiment) during this PhD thesis and another in November/December 2009 (in the following referred to as November experiment) during my Master thesis. According to the seasonal study in Billefjorden, *C. glacialis* was in the refractory phase with little changes in enzyme activities and biochemical composition in August, and copepods were in the activation phase, during which the biochemical composition changed profoundly, in November. It has to be noted that incubation experiments involve the risk of artifacts, i.e. stress and subsequent changes in the behavior of the copepods due to extensive handling (Ohman et al. 1998). Nevertheless, if the response of *C. glacialis* is dependent on the diapause phase, we

hypothesize that copepods will react faster during the November experiment than during the August experiment.

Digestive enzyme activities, i.e. proteinase and lipase/esterase activities, were measured to assess the potential feeding activity of the copepods. Citrate synthase activity was determined as a proxy for the metabolic activity (Meyer et al. 2002, Saborowski & Buchholz 2002, Kreibich et al. 2008). Moreover, to assess if *C. glacialis* assimilated the dietary components, the biochemical composition was determined by means of measuring dry mass and C and N content. In the following these parameters will be compared between the August and November experiment.

Proteinase activity increased in all treatments in both experiments regardless of food and light conditions (Fig. 4.8). The increase, however, was only significant for copepods from the food and light treatment of the November experiment (Kruskal-Wallis $p < 0.05$, Tukey post hoc test). In the August experiment, the increase in proteinase activity was stronger when copepods were exposed to food and darkness compared to the copepods kept with food and light. Thus, food availability intensified proteinase activity, regardless if light was present. During the November experiment, the copepods which were kept with food and in darkness were in bad shape and we assume that there was some contamination in the barrel. Unfortunately, we can thus not compare the response of this group in November with that in August. In the Arctic, the return of light in spring is followed by the occurrence of algal food (Arrigo et al. 2008). This suggests that a combination of both environmental factors causes a stronger increase of the metabolic activity of *C. glacialis* than light or food alone (Morata & Soreide 2013), which matches with the results of the November experiment.

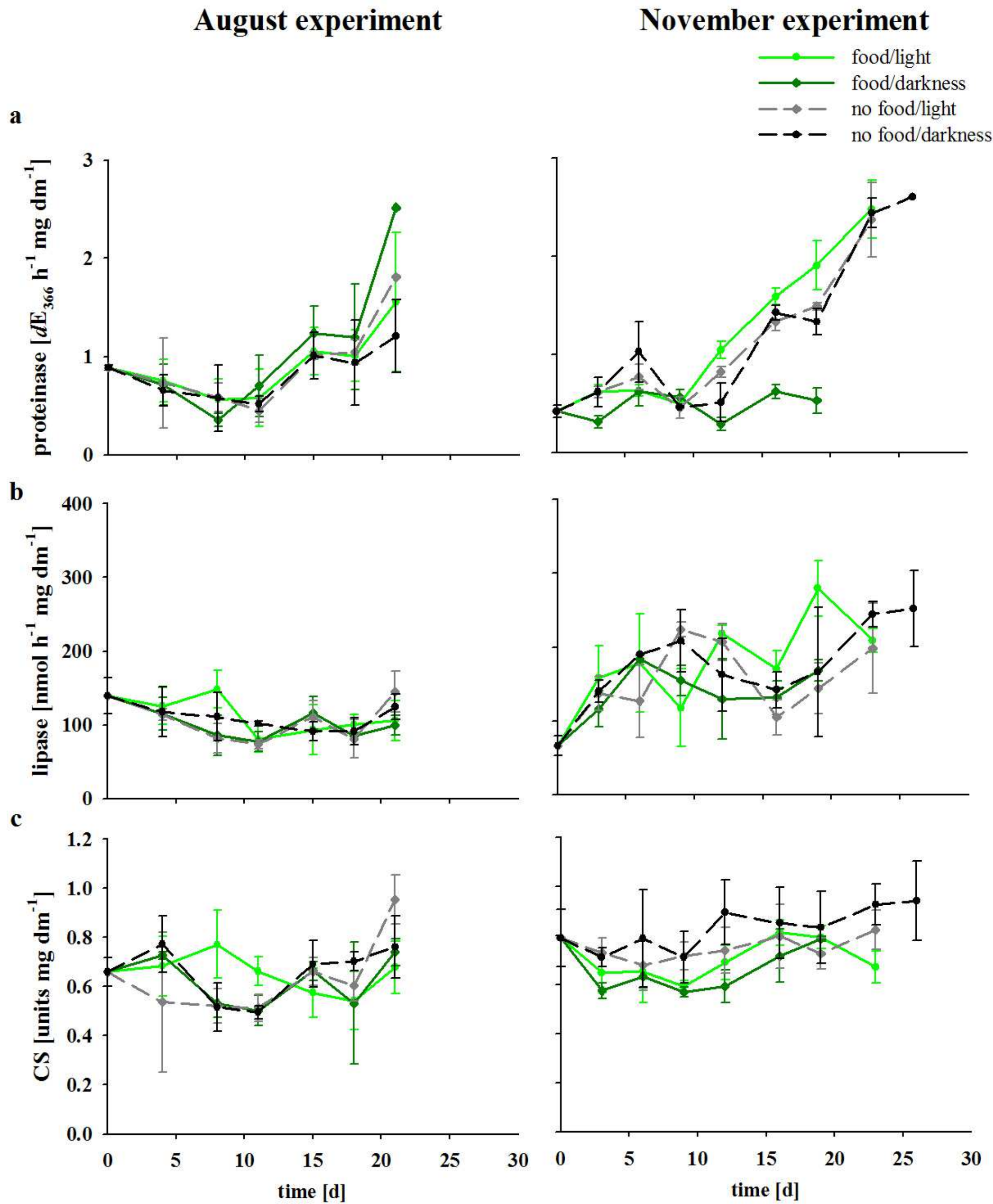

Fig. 4.8 Comparison of the proteinase (a), lipase/esterase (lipase; b) and citrate synthase (CS; c) activity of *Calanus glacialis* copepodite stage V kept with food and light (light green, solid line), with food and in darkness (dark green, solid line), without food and with light (light grey, dashed line) and without food and in darkness (dark grey, dashed line) in November/ December 2009 and August/ September 2012 (mean ± SD, n = 3, except for Nov., day 9 n = 2). In the August experiment individuals were kept in the laboratory for 21 days and the November experiment lasted for 26 days.

Proteinase activity increased in less than ten days in individuals of the November experiment, while it took more than ten days in individuals of the August experiment. One to two weeks has been suggested as acclimation period for enzyme activities in

copepods in other studies (Hassett & Laundry 1983, Head & Conover 1983). Our experiments suggest that diapausing copepods respond faster to external cues in November compared to August. At the end of both experiments, the maximum proteinase activities were more than twice as high as in *C. glacialis* CV from the field in September and December 2012. The highest proteinase activities of individuals from the experiments, however, were only half of those of actively feeding CV from Billefjorden in June 2013 (see chapter 4.1).

Lipase/esterase activities increased in *C. glacialis* during the November experiment (one-way ANOVA $p<0.05$, Holm-Sidak post hoc), while they did not change significantly throughout the August experiment (Fig. 4.8). At the end of the November experiment, lipolytic activity was the highest in individuals which were kept with food and light, and it was twice as high as the activity in copepods from Billefjorden in December 2012 (see chapter 4.1). This suggests an unspecific mobilization of digestive enzymes, which has already been found during incubation experiments with *C. hyperboreus* (Head & Conover 1983). The seasonal study in Billefjorden, however, did not reveal such an unspecific induction of digestive enzyme activity before food was available (**Manuscript II**).

CS activities were similar to the activities which were found in the field in August and September 2012 and November and December 2012 (see chapter 4.1). The CS activity revealed no clear differences among the treatments (Fig. 4.8). This contrasts other studies, which found significantly lower CS activities in starving compared to feeding crustaceans (Meyer et al. 2002, Saborowski & Buchholz 2002, Kreibich et al. 2008). Also the biochemical composition of *C. glacialis* did not change significantly in any of the experiments (Fig. 4.9), which suggests that the experimental period was too short for the copepods to assimilate the available food in detectable amounts into their body tissue. Thus, more extensive and comparative studies with copepods captured during the productive season should be performed in the future to further elucidate if the physiological response differs between active and diapausing copepods, and if *C. glacialis* is able to cope with changes in the food regime. An earlier onset of the phytoplankton bloom is a likely future scenario since duration and thickness of sea-ice cover change, with subsequent changes in light and food regime (Arrigo et al. 2008, Kahru et al. 2011).

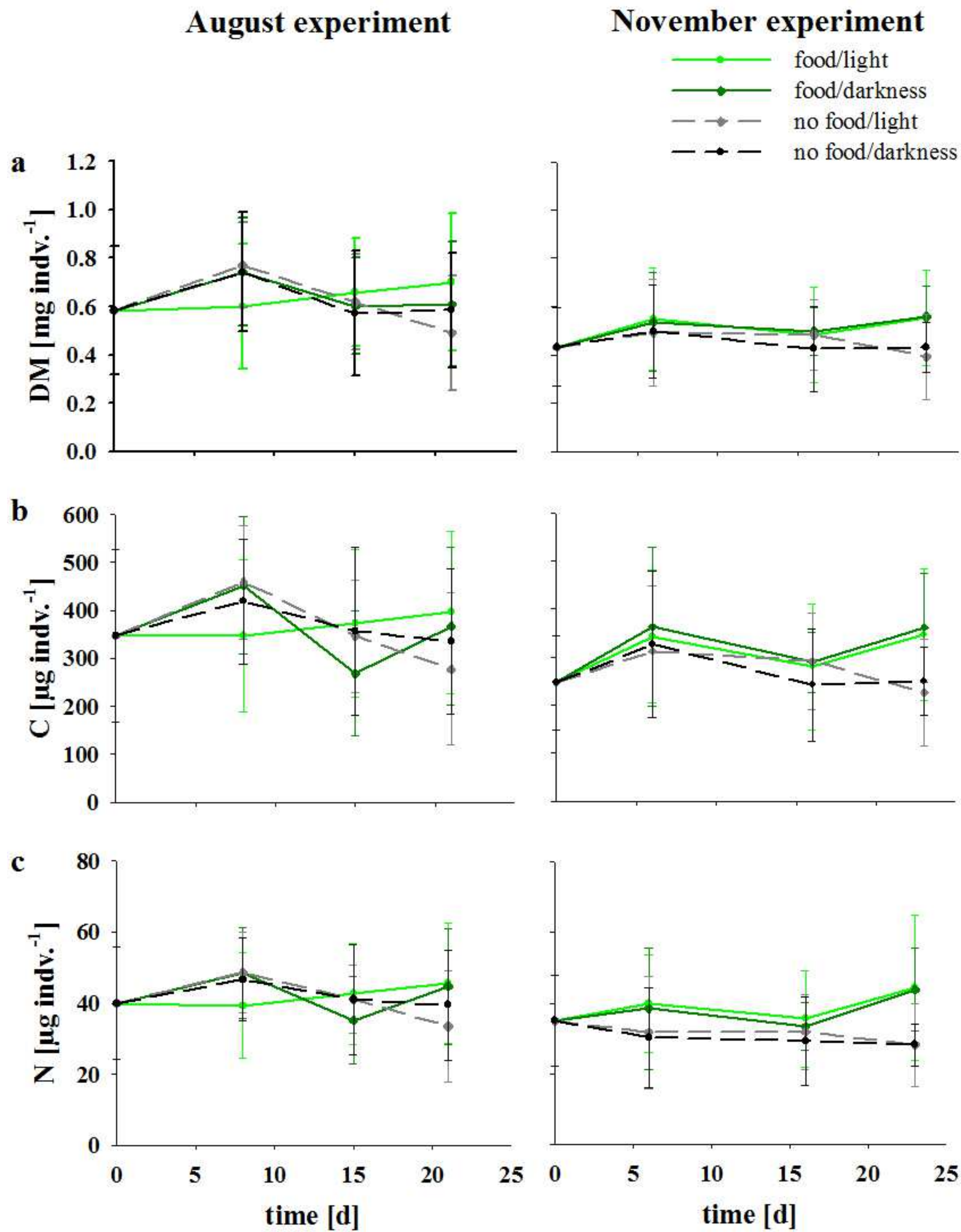

Fig. 4.9 Comparison of dry mass (DM; a), carbon content (C; b) and nitrogen content (N; c) of *Calanus glacialis* copepodite stage V kept with food and light (light green, solid line), with food and in darkness (dark green, solid line), without food and with light (light grey, dashed line) and without food and in darkness (dark grey, dashed line) in November/ December 2009 and August/ September 2012 (mean ± SD, n = 24, except for Nov., day 6 and 23, n = 19 and 12; Aug., day 15, n= 12). In the August experiment, the last sampling day for DM, C and N content was after 21 days and in the November experiment, the last sampling was performed after 23 days.

Key results of chapter 4.2:

- Digestive enzyme activities increased faster in *C. glacialis* which were in the activation phase of diapause (November experiment) compared to the ones of individuals, which were in the refractory phase (August experiment).
- Food availability intensified digestive enzyme activity independent of the light regime.
- Digestive enzyme activities, however, did not reach the same activities like in actively feeding copepods in surface waters in Billefjorden in spring and summer. Also, metabolic enzyme activity and the biochemical composition did not change significantly during the experiments, which may suggest that the experimental period was too short for the copepods to assimilate the food in detectable amounts into body tissue.

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5 Conclusions and future perspectives

This is the first study to characterize seasonal patterns in the physiology of the calanoid copepod *C. glacialis*. Diapause and activity were related to profound changes in metabolic and digestive enzyme activities as well as acid-base regulation and extracellular ion concentrations. In future studies, this set of physiological parameters could thus be used as a proxy for determining the activity level of copepods in relation to the timing and duration of diapause. It has to be noted, however, that our study investigated seasonal patterns in the physiology of a shelf species, which mainly inhabits fjords with less than 200 m depth. Other studies, for example, showed that respiration rates and enzyme activities are close to zero in diapausing individuals of the sibling species *C. finmarchicus* and *C. hyperboreus*, which overwinter at greater depths. It further has to be noted that the present study focused on CV, which prepare for moulting and gonad maturation in the end of overwintering. The younger overwintering stage CIV does not develop gonads and thus, the specific metabolic activities may increase later in the year compared to CV during winter. Future studies should investigate if the intensity of diapause is related to overwintering depth and stage. Comprehensive studies on species from different latitudes and locations should be performed to manifest a clear definition of the physiological adjustments in copepods during diapause.

The present study reflect that diapause is an evolutionary developed life history characteristic in copepods, however, the timing and intensity of diapause in *C. glacialis* is adjusted to prevailing environmental conditions. Depending on the habitat, *C. glacialis* is able to use either only the phytoplankton bloom (ice-free areas) or shows a life cycle that is synchronized with ice algae and phytoplankton bloom (ice-covered environments). *C. glacialis* should be able to respond flexible to shifts in the timing of algal blooms related to an earlier ice break-up, since digestive enzyme activities of the copepods correlated to food availability. The present study showed that the physiology of *C. glacialis* changes over the year triggered by an interplay of environmental factors and internal processes. To assess these factors and processes, higher sampling frequencies in future studies should be combined with molecular approaches to gain more precise information on the timing and intensity of physiological adjustments in copepods during diapause.

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Appendix

Table A 1 Chlorophyll *a* (Chl *a*) in Billefjorden, it was integrated over the water column from 75 m to surface, except for July 2013 (from 35 m to surface). Dry mass (DM; µg indv.$^{-1}$), carbon to nitrogen ratio (C:N) and lipid content (µg indv.$^{-1}$) of *Calanus glacialis* copepodite stages IV (CIV), V (CV) and adult females (CVIF) in Billefjorden from July 2012 to July 2013 (mean ± SE and number of replicates (n), if not shown then n = 1).

	Chl *a*	DM						C:N			Lipid content
		CV	n	CIV	n	CVIF	n	CV	CIV	CVIF	CV
23.07.2012		666.5 ± 46.2	24					8.7			208.0
28.08.2012		582.8 ± 54.3	24					8.7			212.0
17.09.2012		724.7 ± 69.8	10								
05.10.2012	39.0	497.9 ± 51.5	23					7.5			217.3
06.11.2012		653.5 ± 36.9	24					8.0			172.0
04.12.2012		502.2 ± 67.1	17					8.1			76.0
10.01.2013	2.9	242.4 ± 29.6	17			565	2	7.1		7.9	73.3
04.02.2013	3.8	389.2 ± 55.1	24			612.5 ± 28.2	24	7.1		6.9	52.0
13.03.2013				222.2 ± 26.7	23	517.5 ± 56.3	24		6.5	7.1	81.3
09.04.2013	39.5			108.3 ± 6.9	24	449.2 ± 61.2	12		6.1	5.8	42.7
26.04.2013	164.1			184.6 ± 11.7	13				5.7		
07.05.2013	192.9			163. 7 ± 9.1	24				5.9		
17.06.2013		493.0 ± 54.3	23					8.1			
23.07.2013	29.3	519.5 ± 49.2	20					8.2			

Table A 2 Proteinase activity (dE_{366} h^{-1} $indv.^{-1}$), lipase/esterase activity (nmol h^{-1} $indv.^{-1}$) and 3-hydroxyacyl-CoA dehydrogenase activity (HOAD, units $indv.^{-1}$) of *Calanus glacialis* copepodite stages IV (CIV), V (CV) and adult females (CVIF) in Billefjorden from July 2012 to July 2013 (mean ± SE, n = 3, except for HOAD activity of CIV in May n = 2).

	Proteinase			**Lipase/esterase**			**HOAD**		
	CV	**CIV**	**CVIF**	**CV**	**CIV**	**CVIF**	**CV**	**CIV**	**CVIF**
23.07.2012	0.87 ± 0.13			106.27 ± 11.26			0.39 ± 0.13		
28.08.2012	0.52 ± 0.01			74.99 ± 7.02			0.61 ± 021		
17.09.2012	0.14 ± 0.02			50.67 ± 4.48					
05.10.2012	0.40 ± 0.03			68.53 ± 0.32			0.80 ± 0.02		
06.11.2012	0.36 ± 0.01			75.3 ± 1.03			0.99 ± 0.19		
04.12.2012	0.35 ± 0.12			53.86 ± 2.49			0.57 ± 0.09		
10.01.2013	0.46 ± 0.22		0.49 ± 0.10	48.91 ± 5.08		73.01 ± 4.60	0.98 ± 0.16		0.73 ± 0.73
04.02.2013	0.54 ± 0.02		0.53 ± 0.11	62.52 ± 0.87		85.01 ± 6.59	0.75 ± 0.08		0.85 ± 0.85
13.03.2013		0.12 ± 0.04	0.76 ± 0.18		18.14 ± 3.48	78.33 ± 18.31			0.66 ± 0.66
09.04.2013		0.61 ± 0.30	2.03 ± 0.33		18.74 ± 3.55	104.75 ± 3.10		0.41 ± 0.09	
26.04.2013		0.95 ± 0.02			33.79 ± 4.53			0.17 ± 0.02	
07.05.2013		1.36 ± 0.19			59.94 ± 0.46			0.05	
17.06.2013	2.93 ± 0.16			246.59 ± 3.87			0.07 ± 0.03		
23.07.2013	0.88 ± 0.23			168.35 ± 11.02			0.79 ± 0.18		

Table A 3 Citrate synthase activity (CS, units indv.$^{-1}$) and malate dehydrogenase activity (MDH, units indv.$^{-1}$) of *Calanus glacialis* copepodite stages IV (CIV), V (CV) and adult females (CVIF) in Billefjorden from July 2012 to July 2013 (mean ± SE, n = 3, except for MDH activity of CVIF in February n = 2). The extracellular pH (pH_e) and Li^+ concentration (mmol L^{-1}) are shown for CV 2013 (mean ± SE, n = 6, except for Li+ in June n = 2).

	CS			**MDH**			**pH_e**	**Li^+**
	CV	**CIV**	**CVIF**	**CV**	**CIV**	**CVIF**	**CV**	**CV**
23.07.2012	0.48 ± 0.04			12.38 ± 1.52			7.9 ± 0.3	69.9 ± 18.4
28.08.2012	0.38 ± 0.02			8.83 ± 1.64			6.7 ± 03	75.3 ± 31.8
17.09.2012	0.35 ± 0.03							
05.10.2012	0.42 ± 0.02			9.17 ± 1.29				
06.11.2012	0.41 ± 0.02			5.83 ± 0.57			6.3 ± 0.6	60.9 ± 69.1
04.12.2012	0.27 ± 0.01			6.05 ± 1.08				
10.01.2013	0.31 ± 0.03		0.43 ± 0.01	5.14 ± 0.65		8.00 ± 0.35	5.7 ± 0.5	132.2 ± 58.9
04.02.2013	0.26 ± 0.01		0.39 ± 0.03	5.49 ± 0.19		5.88	5.9 ± 0.5	197.2 ± 101.8
13.03.2013		0.14 ± 0.01	0.43 ± 0.00			6.42 ± 1.05	5.5 ± 0.7	128.5 ± 106.9
09.04.2013		0.12 ± 0.01	0.49 ± 0.02		2.18 ± 0.35		6.3 ± 1.2	104.5 ± 103.9
26.04.2013		0.18 ± 0.01			2.71 ± 056		5.9 ± 0.6	160.7 ± 85.7
07.05.2013		0.30 ± 0.01			4.34 ± 068		7.0 ± 0.8	106.8 ± 72.9
17.06.2013	0.54 ± 0.02			7.13 ± 0.39			7.9 ± 0.4	9.9
23.07.2013	0.43 ± 0.02			8.38 ± 0.46			7.8 ± 0.5	8.8 ± 8.9

Table A 4 Proteinase activity (dE_{366} h^{-1} mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	5.6 ± 0.3		3.9 ± 0.7		7.9 ± 0.6	
28.08.2012	0.9 ± 0.0					
17.09.2012	0.2 ± 0.0				0.8 ± 0.1	
05.10.2012	0.8 ± 0.1					
06.11.2012	0.5 ± 0.0					
04.12.2012	0.7 ± 0.4					
10.01.2013	1.5 ± 1.3	1.2 ± 0.4	1.2 ± 0.1		0.8 ± 0.0	
04.02.2013	1.4 ± 0.1	0.9 ± 0.3		1.4 ± 0.2	1.8 ± 0.1	
13.03.2013		1.5 ± 0.6				
09.04.2013		4.9 ± 1.3				
07.05.2013						4.9 ± 0.9
17.06.2013	5.7 ± 0.6					
23.07.2013	1.7 ± 0.8					

Table A 5 Lipase/esterase activity (nmol h^{-1} mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3, except for CV in Rijpfjorden July 2012 and CVIF in Rijpjforden in May n = 2).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	221.3 ± 43.2		163.9 ± 41.3		204.8	
28.08.2012	139.5 ± 24.0					
17.09.2012	71.7 ± 13.4				129.8 ± 3.7	
05.10.2012	136.1 ± 2.8					
06.11.2012	105.9 ± 16.2					
04.12.2012	97.6 ± 17.8					
10.01.2013	155.9 ± 29.1	169.9 ± 18.6	182.1 ± 8.1		108.0 ± 4.5	
04.02.2013	146.0 ± 25.5	130.7 ± 19.2		178.3 ± 22.8	177.8 ± 33.03	
13.03.2013		172.3 ± 56.5				
09.04.2013		230.5 ± 36.1				
07.05.2013						237.6
17.06.2013	458.4 ± 29.7					
23.07.2013	398.8 ± 47.9					

Table A 6 Citrate synthase activity (units mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	10.7 ± 0.3		9.5 ± 1.2		10.6 ± 0.3	
28.08.2012	6.6 ± 0.6					
17.09.2012	5.3 ± 0.7				7.3 ± 0.7	
05.10.2012	8.5 ± 0.6					
06.11.2012	6.2 ± 0.5					
04.12.2012	5.1 ± 0.2					
10.01.2013	10.3 ± 1.8	10.5 ± 0.5	8.7 ± 0.1		7.3 ± 0.3	
04.02.2013	6.7 ± 0.6	6.4 ± 0.9		9.8 ± 0.9	8.8 ± 1.0	
13.03.2013		6.7 ± 2.8				
09.04.2013		11.7 ± 0.9				
07.05.2013						10.7 ± 2.3
17.06.2013	10.6 ± 0.8					
23.07.2013	8.4 ± 0.8					

Table A 7 Malate dehydrogenase activity (units mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	45.6 ± 10.8		15.5 ± 6.2		60.5 ± 13.9	
28.08.2012	30.3 ± 9.7					
17.09.2012					32.5 ± 4.7	
05.10.2012	36.9 ± 8.9					
06.11.2012	17.9 ± 3.0					
04.12.2012	24.1 ± 7.5					
10.01.2013	30.0 ± 6.6	38.8 ± 2.9			30.6 ± 3.6	
04.02.2013	28.2 ± 1.7	19.2 ± 8.8			34.7 ± 3.9	
13.03.2013		24.8 ± 10.1				
09.04.2013						
07.05.2013						25.6 ± 2.8
17.06.2013	27.8 ± 2.6					
23.07.2013	32.3 ± 3.0					

Table A 8 Aminoacyl-tRNA synthetase activity (nmol PPi h^{-1} mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3, except for CVIF in Rijpfjorden in May n = 2).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	0.3 ± 0.0		0.1 ± 0.0		0.5 ± 0.0	
28.08.2012	0.2 ± 0.1					
17.09.2012					0.2 ± 0.1	
05.10.2012	0.3 ± 0.0					
06.11.2012	0.2 ± 0.1					
04.12.2012	0.2 ± 0.0					
10.01.2013	0.2 ± 0.1	0.2 ± 0.0				
04.02.2013	0.2 ± 0.0	0.1 ± 0.0			0.2 ± 0.0	
13.03.2013		0.1 ± 0.0				
09.04.2013						
07.05.2013						0.5
17.06.2013	0.2 ± 0.0					
23.07.2013	0.2 ± 0.0					

Table A 9 Three-hydroxyacyl-CoA dehydrogenase activity (units mg DM^{-1}) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SD, n = 3, except for CVIF in Kongsfjorden in July 2012 and CVIF in Rijpfjorden in February n = 2).

	Billefjorden		**Kongsfjorden**		**Rijpfjorden**	
	CV	**CVIF**	**CV**	**CVIF**	**CV**	**CVIF**
23.07.2012	0.6 ± 0.5		0.2 ± 0.2		0.3 ± 0.1	
28.08.2012	3.1 ± 1.9					
17.09.2012					3.6 ± 0.7	
05.10.2012	4.8 ± 0.2					
06.11.2012	4.6 ± 1.5					
04.12.2012	3.4 ± 0.9					
10.01.2013	8.8 ± 2.4	38.8 ± 2.9				
04.02.2013	5.8 ± 1.1	19.2 ± 8.8			3.2	
13.03.2013		24.8 ± 10.1				
09.04.2013						
07.05.2013						0.5 ± 0.3
17.06.2013	0.4 ± 0.3					
23.07.2013	4.6 ± 1.8					

Table A 10 Dry mass (µg indv.$^{-1}$) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SE and number of replicates (n)).

	Billefjorden				Kongsfjorden			Rijpfjorden			
	CV	**n**	**CVIF**	**n**	**CV**	**n**	**CVIF**	**CV**	**n**	**CVIF**	**n**
23.07.2012	328.3 ± 46.3	24			594.7 ± 47.7	24		476.5 ± 35.4	24		
28.08.2012	582.8 ± 54.3	24									
17.09.2012	686.9 ± 69.8	10						720.3 ± 71.7	24		
05.10.2012	497.9 ± 51.5	23									
06.11.2012	653.5 ± 36.9	24									
04.12.2012	502.2 ± 67.1	17	565.0	2							
10.01.2013	260.7 ± 29.6	17	612.5 ± 28.2	24	330.9 ± 22.7	24		447.5 ± 37.7	16		
04.02.2013	389.2 ± 55.1	24	517.5 ± 56.3	24			393.3 ± 24.6	279.2 ± 15.7	24		
13.03.2013			449.2 ± 61.2	12							
09.04.2013											
07.05.2013										418.8 ± 22.9	24
17.06.2013	49.3 ± 54.3	23									
23.07.2013	519.5 ± 49.2	20									

Table A 11 Carbon content (µg indv.$^{-1}$) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SE and number of replicates (n)).

	Billefjorden				**Kongsfjorden**				**Rijpfjorden**			
	CV	**n**	**CVIF**	**n**	**CV**	**n**	**CVIF**	**n**	**CV**	**n**	**CVIF**	**n**
23.07.2012	181.6 ± 16.1	24			347.9 ± 28.9	24			256.3 ± 25.2	24		
28.08.2012	347.0 ± 36.9	24										
17.09.2012		24							465.5 ± 44.4	23		
05.10.2012	284.8 ± 35.2	24										
06.11.2012	398.9 ± 26.8	21										
04.12.2012	259.1 ± 30.2	17	305.2	2								
10.01.2013	127.1 ± 15.4	15	331.9 ± 15.2	23	180.5 ± 15.3	24			226.1 ± 18.2	16		
04.02.2013	177.3 ± 17.6	24	271.3 ± 22.1	24			202.9 ± 17.5		142.5 ± 9.7	24		
13.03.2013			238.7 ± 34.9	13								
09.04.2013												
07.05.2013											172.0 ± 12.2	24
17.06.2013	277.7 ± 36.7	23										
23.07.2013	282.3 ± 34.7	21										

Table A 12 Nitrogen content (µg indv.$^{-1}$) of *Calanus glacialis* copepodite stages V (CV) and adult females (CVIF) in Billefjorden, Kongsfjorden and Rijpfjorden from July 2012 to July 2013 (mean ± SE and number of replicates (n)).

	Billefjorden				**Kongsfjorden**			**Rijpfjorden**			
	CV	**n**	**CVIF**	**n**	**CV**	**n**	**CVIF**	**CV**	**n**	**CVIF**	**n**
23.07.2012	28.6 ± 1.4	24			44.5 ± 2.5	24		34.6 ± 1.9	24		
28.08.2012	39.9 ± 3.2	24									
17.09.2012		24						48.7 ± 4.3	23		
05.10.2012	38.1 ± 3.4	24									
06.11.2012	49.7 ± 4.7	21									
04.12.2012	31.9 ± 3.3	17	38.5	2							
10.01.2013	17.9 ± 2.0	15	44.2 ± 1.7	23	23.1 ± 1.6	24		28.2 ± 2.0	16		
04.02.2013	24.7 ± 1.9	24	37.9 ± 2.6	24			26.9 ± 2.1	21.0 ± 1.1	24		
13.03.2013			41.5 ± 5.5	13							
09.04.2013											
07.05.2013										42.1 ± 2.6	24
17.06.2013	34.5 ± 3.6	23									
23.07.2013	34.4 ± 3.1	21									

Eidesstattliche Erklärung

(Gem. § 6 (5) Nr. 1-3 PromO)

Hiermit versicher ich, dass ich

1. die vorliegende Arbeit ohne erlaubte fremde Hilfe angefertigt habe,
2. keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und
3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremerhaven, den 18.02.2015

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Daniela Freese