Turnover of trophic markers and lipid carbon in Arctic marine food webs

The contribution of key zooplankton species

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Turnover of trophic markers and lipid carbon in Arctic marine food webs

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CONTENTS

Preface

This cumulative dissertation is composed of three publications as listed below (for more details on the author contributions, see the Contribution section, at the end of this thesis). It further includes a general introduction, an overview of the methods, a synoptic discussion and concluding remarks. This study was partially funded by the Research Council of Norway (Arctic Field Grant, Svalbard Science Forum, project ID 235913/E10 and CLEOPATRA II, project ID 216537/E10).

Manuscript I

Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods

Lauris Boissonnot, Barbara Niehoff, Wilhelm Hagen, Janne E. Søreide and Martin Graeve

The concept was developed by Martin Grave, Barbara Niehoff, Janne E. Søreide and myself. I carried out the fieldwork. I designed and performed the experiments and analyzed the data with the help of Martin Graeve and Barbara Niehoff. I wrote the manuscript in close cooperation with all co-authors. This manuscript was published in Journal of Plankton Research in 2016.

Manuscript II

Year-round population dynamics of *Limacina helicina* and *L. retroversa* in a high-Arctic fjord

Lauris Boissonnot, Janne E. Søreide, Barbara Niehoff, Peter Kohnert, Eike Stübner, Michael Schrödl and Martin Graeve

The idea of this study was conceived by Janne E. Søreide, Barbara Niehoff and myself, from previous field observations performed by Janne E. Søreide and Eike Stübner on the entire mesozooplankton community. I analyzed the data on thecosome pteropod distribution as well as the meteorological data from the Norwegian Meteorological Institute, together with Barbara Niehoff and Janne E. Søreide. Peter Kohnert performed molecular analyses with the help of Michael Schrödl and wrote the corresponding section of the material and methods. I wrote the manuscript with scientific advice from Barbara Niehoff and Janne E. Søreide. This manuscript is in preparation for Polar Biology.

Manuscript III

Lipid and fatty acid turnover of the pteropods *Limacina helicina*, *L. retroversa* and *Clione limacina* from Svalbard waters

Lauris Boissonnot, Benedikt Ehrenfels, Barbara Niehoff, Janne E. Søreide, Wilhelm Hagen and Martin Graeve

This study was conceived by Martin Graeve and myself. I designed the experiments with by Martin Graeve, Barbara Niehoff and Janne E. Søreide. Fieldwork was carried out by Benedikt Ehrenfels and myself. Benedikt Ehrenfels and I performed the experiments with Janne E. Søreide, and analyzed the data with the help of Martin Graeve. I wrote the manuscript in close collaboration with all co-authors. The manuscript is submitted to Marine Ecology Progress Series.

Glossary and abbreviations

ArW	Arctic water
AT	atom percent
ATE	atom percent excess
AW	Atlantic water
C	carbon
CI - CV	copepodite stage I-V
Chl *a*	chlorophyll *a*
CSIA	compound specific isotope analysis
DAGE	diacylglycerol ether
DMSP	dimethylsulphoniopropionate
EFA	essential fatty acid
FA	fatty acid
Falc	fatty alcohol
FAME	fatty acid methyl ester
FAS	fatty acid signature
FATM	fatty acid trophic marker
GC	gas chromatography
IRMS	isotope ratio mass spectrometry
LC MUFA	long chain monounsaturated fatty acid
NAC	Norwegian Atlantic Current
PUFA	polyunsaturated fatty acid
QFASA	quantitative fatty acid signature analysis
SST	sea surface temperature
TAG	triacylglycerol
TAW	transformed Atlantic water
TL	total lipids (sum of fatty acids and fatty alcohols)
WE	wax ester
WSC	west Spitsbergen current

Summary

The Arctic pelagic food web is characterized by a high seasonality in terms of light and therefore primary production. To cope with the long winter periods of low food availability, many species have developed the ability to store large amounts of lipid reserves. These high-energy compounds are of major importance in different processes such as somatic growth, survival, development, reproduction and metabolism independently on ambient food levels. In the Arctic pelagic food web, zooplankton plays a crucial role linking primary production and higher trophic levels. The efficiency of zooplankton species to transfer lipids and fatty acids in the food web depends on a combination of ecological and physiological aspects such as distribution, life cycle strategies, lipid content and lipid assimilation rapidity. In the context of climate warming, severe shifts in the phyto- and zooplankton communities, and thus changes in trophic interactions, are expected. It is therefore essential to better understand the lipid and fatty acid turnover in the in the lipid-driven Arctic food web. This study aims at evaluating the role of zooplankton in the transfer of lipids from primary producers to higher trophic levels. It combines field observations and experimental work to fill the gaps of knowledge in the ecology and lipid biochemistry of Arctic zooplankton key species, i.e. the copepods *Calanus glacialis*, *Pseudocalanus minutus* and *Oithona similis*, the thecosome pteropods *Limacina helicina* and *L. retroversa* and the gymnosome pteropod *Clione limacina*.

The life cycle and the distribution of thecosome pteropods were investigated by field observations that were conducted year-round in 2012 and 2013 in Svalbard waters. These studies aimed at relating the distribution of *L. helicina* and *L. retroversa* to environmental parameters and examining the growth of veligers and juveniles. To study the metabolic capacities of key zooplankton species in terms of lipid and fatty acid turnover, feeding experiments were conducted with animals that were collected in Svalbard waters during the late productive season (summer/early autumn) in 2014 and 2015. Consumers of the first trophic level were fed a ^{13}C labeled diatom-flagellate mixed diet for one week (thecosome pteropods) and for three weeks (copepods). The consumer representing the second trophic level, i.e. *C. limacina*, was fed ^{13}C labeled *Limacina* spp. for 3 weeks. The ^{13}C incorporation into fatty acids and alcohols was monitored by compound specific isotope analyses (CSIA). The use of CSIA in combination with labeling experiments allowed for a precise evaluation of the lipid and fatty acid turnover in zooplankton organisms.

The results showed that lipid and fatty acid assimilation rates of zooplankton species clearly reflect their life cycle strategies. The Arctic herbivorous species *C. glacialis* and *P. minutus* exhibited a rapid total lipid turnover (1.3 and 2.6% d^{-1}), combined with a high *de novo* synthesis of wax esters (1.2 and 3.3% d^{-1}). This reflects that these species need to quickly store large energy reserves to successfully complete their life cycle. These species assimilated diatom fatty acids trophic markers (FATM) (2.4 and 4.6% d^{-1}) at a much higher rate than flagellate FATM (1.2 and 1.3% d^{-1}), suggesting a high importance of this food source. The omnivorous species *O. similis* and *L. helicina* showed lower total lipid turnover rates (0.1 and 0.5% d^{-1}), reflecting a life strategy that is less dependent on lipid reserves than that of the herbivorous species. *L. retroversa* exhibited a surprisingly rapid lipid turnover considering that its feeding strategy is similar to that of *L. helicina*. Omnivorous zooplankton *de novo* synthesized significant amounts of long-term energy storage, such as fatty alcohols (*O. similis*: 0.2% d^{-1}) and long chain monounsaturated fatty acids (MUFA) (thecosome pteropods: 0.3-0.5% d^{-1}). *O. similis* and *L. helicina* had similar ingestion/assimilation rates of flagellate and diatom FATM (*O. similis*: 0.7 vs. 0.5% d^{-1}, *L. helicina*: 0.1 vs. 0.1% d^{-1}), indicating an opportunistic feeding strategy. In contrast, *L. retroversa* seemed to prefer flagellates over diatoms (5.3 vs. 0.2% d^{-1}). The carnivorous species *C. limacina* exhibited a very slow lipid turnover (0.07% d^{-1}), which in this study may have been related to the investment of energy into metabolism but not into storing reserves after a long period of starvation. *C. limacina de novo* synthesized substantial amounts of odd chain fatty acids (0.03% d^{-1}) that may be used as long-term energy reserves to overcome the variability of the presence of its unique food source *Limacina* spp..

The lipid assimilation rates of zooplankton organisms assessed in this study are representative only for the investigated stages and only for the late productive season in the Arctic. Lipid turnover may, however, be characterized by pronounced variability with highest rates in spring and lowest rates during overwintering. Also, energetic needs vary quantitatively and qualitatively among developmental stages since processes such as growth, reproduction, and metabolism during food scarcity require different amounts of lipids as well as specific compounds. Essential fatty acids (EFA) that are synthesized by primary producers are largely transferred by zooplankton species to higher trophic levels. During the late productive season the transfer are mainly facilitated by calanoid copepods as a result of their high lipid content, efficient lipid turnover and high abundances. Pteropods, however, provide different essential fatty acids to higher trophic levels, suggesting their complementary function in the food web.

Global changes that will occur in the Arctic pelagic ecosystem will affect the primary production of lipids and fatty acids since the phytoplankton community will likely shift to a dominance of small cells. Therefore, particularly herbivorous zooplankton species will be affected by a change of their diet. Species such as *C. glacialis* and *P. minutus*, which preferentially ingest/assimilate diatoms may decrease in abundance while species feeding on flagellates may thrive. In addition, thecosome pteropod species may disappear since there are highly vulnerable to acidification. These future changes in zooplankton population dynamics and community composition may negatively impact the transfer of lipids and specific EFA to higher trophic levels. It is hence of major importance to conduct detailed studies on lipid turnover capacities of Arctic zooplankton and higher trophic levels to better assess the vulnerability of the lipid-driven Arctic pelagic food web to its changing environment.

Zusammenfassung

Das arktische Nahrungsnetz zeichnet sich durch eine starke Saisonalität des Lichteinfalls und der damit verbundenen Primärproduktion aus. Viele Arten haben daher die Fähigkeit entwickelt, große Mengen an Lipiden zu speichern und so lange Phasen ohne Nahrung zu überdauern. Im Pelagial nimmt das Zooplankton eine zentrale Rolle als Bindeglied zwischen den Primärproduzenten und den höheren trophischen Ebenen ein. Dabei hängt die Effektivität, mit der Lipide und Fettsäuren zwischen den Komponenten des Nahrungsnetzes transferiert werden, von einer Reihe ökologischer und physiologischer Aspekte ab, zum Beispiel der geographischen Verteilung, dem Lebenszyklus, dem Lipidgehalt und davon, wie schnell Lipide assimiliert werden können. Aufgrund der Klimaerwärmung werden sich die Zusammensetzung sowohl der Phyto- als auch der Zooplanktongmeinschaften und damit die trophischen Interaktionen ändern. Es ist daher essentiell, den Lipid- und Fettsäureumsatz des arktischen pelagischen Nahrungsnetzes besser zu verstehen. Die vorliegende Studie untersucht die Rolle des Zooplanktons für den Lipidtransfer zwischen Primärproduzenten und höheren trophischen Ebenen. Um Wissenslücken in der Ökologie und Lipidbiochemie von einiger Schlüsselarten des arktischen Zooplanktons und zwar *Calanus glacialis*, *Pseudocalanus minutus* und *Oithona similis* (Copepoda), *Limacina helicina* und *L. retroversa* (thecosome Pteropoden) und *Clione limacina* (gymnosomen Peteropoden) zu schließen, wurden Felduntersuchungen mit Experimenten und biochemischen Methoden kombiniert.

Der Lebenszyklus und die Verteilung der thecosomen Pteropoden wurden im Rahmen von Feldstudien, die 2014 und 2015 in den Küstengewässern Spitzbergens durchgeführt wurden, untersucht. Diese Untersuchungen hatten zum Ziel, die Verteilung von *L. helicina* und *L. retroversa* mit Umweltparametern zu korrelieren und das Wachstum von Veligern und Juvenilen zu erfassen. Um die Fähigkeiten verschiedener Zooplanktonarten, Lipide und Fettsäuren umzusetzen, zu untersuchen, wurden mehrere Nahrungsexperimente durchgeführt. Die Tiere wurden dazu am Ende der produktiven Jahreszeit im Sommer und im frühen Herbst gefangen. Konsumenten erster Ordnung wurden eine Woche (thecosome Pteropoden), beziehungsweise drei Wochen (Copepoden), mit einer Mischung aus ^{13}C-markierten Diatomeen und Dinoflagellaten gefüttert. Der Konsument zweiter Ordnung - *C. limacina* - wurde drei Wochen lang mit ^{13}C-markierten *Limacina*-Arten gefüttert. Die Aufnahme von ^{13}C in Fettsäuren und Alkohole wurde mithilfe der isotopenanalytischen Methode CSIA (Compound Specific Isotope Analyses) erfasst. Diese Kombination von CSIA und Markierungsexperimenten erlaubte eine genaue

Analyse der Lipid- und Fettsäureumwandlung in zooplanktischen Organismen.

Die Ergebnisse der vorliegenden Studie zeigen, dass die Lipid- und Fettsäureassimilation der Zooplanktonarten deren Lebenszyklus wiederspiegeln. Die arktischen herbivoren Arten *C. glacialis* und *P. minutus* setzten Lipide sehr schnell um (1,3 bzw. 2,6% d^{-1}) und synthetisierten *de-novo* Wachsesther mit relativ hohen Raten (1,2 bzw. 3,3% d^{-1}). Die spiegelt wieder, dass diese Arten in kurzer Zeit große Energiereserven anlegen müssen, um ihren Lebenszyklus erfolgreich abschließen zu können. Beide Arten nahmen bevorzugt die Fettsäurebiomarker der Diatomeen (2,4 bzw. 4,6% d^{-1} versus 1,2 bzw. 1,3% d^{-1} Fettsäurebiomarker der Flagellaten) auf bzw. assimilieren diese, was die hohe Wichtigkeit der Diatomeen als Nahrungsquelle unterstreicht. Im Vergleich dazu zeigten die omnivoren Arten *O. similis* und *L. helicina* geringere Lipidumsatzraten (0,1 bzw. 0,5% d^{-1}), was eine von Lipidreserven unabhängigere Lebensstrategie wiederspiegelt. *L. retroversa* hingegen setzte Lipide überraschend schnell um, obwohl die Strategie der Nahrungsaufnahme dieser Art der von *L. helicina* entspricht. Durch *De-novo*-Synthese bildeten alle omnivoren Zooplankter bedeutende Mengen an Langzeitenergiespeichern, zum Beispiel Fettalkohole (*O. similis*: 0,2% d^{-1}) und langkettige einfach ungesättigte Fettsäuren (thecosome Pteropoden: 0,3-0,5% d^{-1}). *O. similis* und *L. helicina* nahmen Fettsäurebiomarker in gleichem Maße aus Flagellaten und Diatomeen auf bzw. assimilierten diese (*O.similis*: 0,7 vs. 0,5% d^{-1}, *L. helicina*: 0,1 vs. 0,1% d^{-1}), was ihre opportunistische Nahrungsstrategie reflektiert. Im Gegensatz dazu bevorzugte die subarktische Art *L. retroversa* Flagellaten im Vergleich zu Diatomeen (5,3 vs. 0,2% d^{-1}). Bei der carnivoren Art *C. limacina* wurde ein sehr langsamer Umsatz der Lipide gemessen (0,07% d^{-1}), der damit erklärt werden kann, dass nach der langen Hungerperiode in dieser Studie die Energie nur für den Stoffwechsel und nicht zur Speicherung von Lipiden genutzt wurde. Durch *De-novo*-Synthese bildete *C. limacina* substantielle Mengen an ungeradzahligen Fettsäuren (0,03% d^{-1}), die als Langzeitenergiereserven verwendet werden könnten, um die Schwankungen im Vorkommen der einzigen Nahrungsquelle *Limacina* spp. zu überstehen.

Die Daten dieser Studie repräsentieren nur die Lipidassimilation der untersuchten Entwicklungsstadien und nur am Ende der produktiven Jahreszeit. Wahrscheinlich unterliegt jedoch der Lipidstoffwechsel ausgeprägten jahreszeitlichen Schwankungen. Es ist davon auszugehen, dass die höchsten Lipidumsatzraten im Frühjahr und die niedrigsten Raten während der Überwinterung zu messen sind. Ebenso sind Abweichungen zwischen verschiedenen Entwicklungsstadien von Zooplanktonorganismen wahrscheinlich, da sich deren Bedürfnisse an den

Energiegehalt und an spezifischen Komponenten, die über die Nahrung aufgenommen werden, unterscheiden.

Die essentiellen Fettsäuren, die die Primärproduzenten synthetisieren, werden durch das Zooplankton an Konsumenten höherer Ordnungen weitergegeben. Diesen Transfer gewährleisteten in meiner Studie am Ende der produktiven Zeit in der Arktis hauptsachlich die calanoiden Copepoden, da sie sich durch einen hohen Lipidgehalt, einen effizienten Lipidumsatz und hohe Abundanzen auszeichneten. Pteropoden lieferten andere essentielle Fettsäuren als die Copepoden und könnten damit eine komplementäre Funktion im Nahrungsnetz bekleiden.

Es ist möglich, dass aufgrund von Klimaveränderungen in Zukunft die kleinen Algenarten die Phytoplanktongemeinschaft dominieren. Besonders das herbivore Zooplankton wäre von Veränderungen an der Basis des Nahrungsnetzes betroffen. Die Abundanzen von Arten wie *C. glacialis* und *P. minutus*, die bevorzugt Diatomeen aufnehmen/assimilieren, könnten sinken, während die Abundanzen von Arten, die Flagellaten als Nahrungsquelle nutzen, zunehmen könnten. Zusätzlich könnten die thecosomen Pteropoden aufgrund der Ozeanversauerung völlig aus dem Ökosystem verschwinden. Diese Veränderungen hätten große Auswirkungen auf den Transfer von Lipiden und essentiellen Fettsäuren im pelagischen Nahrungsnetz. Es ist essentiell, dass die Kapazitäten des arktischen Zooplanktons, Lipide aufzubauen und trophischen Ebenen verfügbar zu machen, detailliert untersucht werden, um die Anfälligkeit des auf Lipiden basierenden pelagischen Nahrungsnetzes gegenüber veränderten Umweltbedingungen besser beurteilen zu können.

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1

INTRODUCTION

1.1 Importance of lipids in the Arctic pelagic food web

1.1.1 The Arctic pelagic food web

Primary production in the Arctic marine food web is supported by algae growing under the sea ice and phytoplankton in the open sea. Arctic primary producers are mainly composed of diatoms and flagellates (Bursa 1963, Gradinger 1999, Leu et al. 2011). The secondary production of the food web is performed by zooplankton species, of which copepods, amphipods and, at times, pteropods are the most abundant (Iken et al. 2005, Węsławski et al. 2007, Blachowiak-Samolyk et al. 2008) (Fig.1). While some zooplankton species are herbivorous and feed strictly on phytoplankton, others are omnivorous to carnivorous and feed on organic matter and smaller zooplankton species (Graeve et al. 1994a). Zooplankton is a major food source for various higher levels of consumers. Dominant fish species such as Arctic char feed on zooplankton as well as on smaller fish species (Runge 1988, Heath and Lough 2007). Birds (i.e. little auk, guillemot and kittiwake) also rely on zooplankton for their survival (Karnovsky et al. 2008). Whales depend particularly on large amounts of copepods and pteropods (Meisenheimer 1905, Gilmer and Harbison 1991, Moore and Laidre 2006) while seals mainly feed on fish (Finley and Evans 1983) and are preyed upon by the apex predator of the Arctic food web, the polar bear (e.g. Stirling and Øritsland 1995).

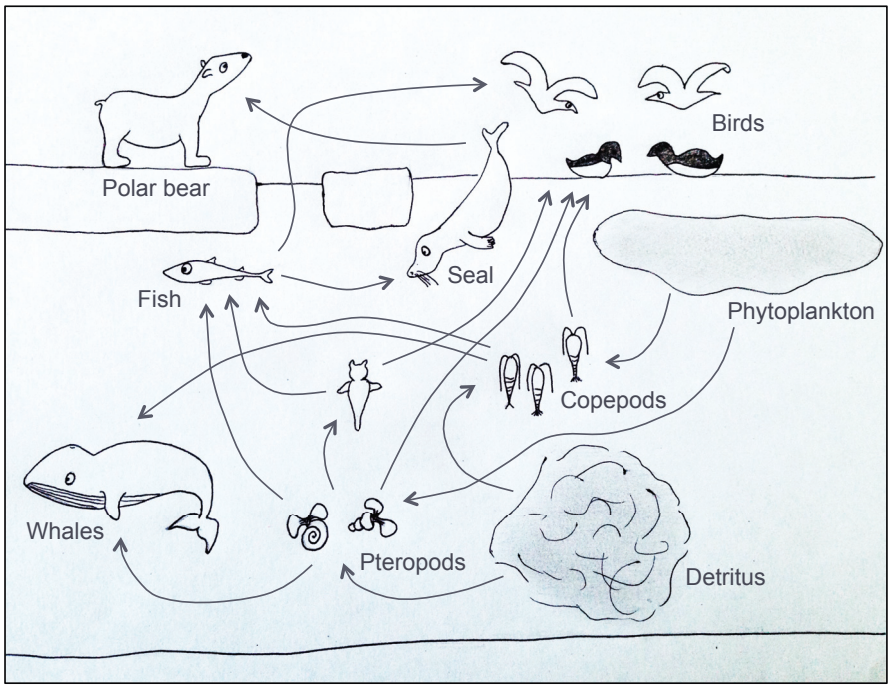


Figure 1: Schematic representation of the Arctic pelagic food web. Arrows represent the transfer of energy.

The Arctic food web is characterized by low species richness as compared to that of lower latitudes (Rosenzweig 1995, Gaston and Blackburn 2000, Willig et al. 2003). The poor diversity in Arctic ecosystems may be the result of complex interactions between various biotic and abiotic factors. The reduced availability of energy is considered to be among the main drivers of these interactions (Rohde 1992). In addition, the slow evolutionary speed caused by low temperatures combined with the relatively young age of Arctic ecosystems result in a short time for diversification (Rohde 1992, Mittelbach et al. 2007). The fact that only few species channel the bulk of food to top predators implies that each species occupies an essential place in the food web (Smetacek and Nicol 2005). Specifically, zooplankton plays a crucial role as the link between primary producers and higher trophic levels (Tande and Båmsted 1985, Falk-Petersen et al. 1990, Gradinger et al. 2010, Bluhm et al. 2011).

1.1.2 Seasonality in high latitude ecosystems and adaptations of organisms

High latitude environments are characterized by an extreme seasonality of the light regime, with midnight sun in summer and darkness in winter. In the high Arctic (>70 °N), the sun first rises mid-February and sets in late October. The summer period, from mid-April to mid-August, is characterized by continuous light (Fig.2). The increase of light intensity in spring

triggers an intense bloom of primary producers. Ice algae begin to grow at low light intensity in March and continue to grow until their sea ice substrate melts, in April/May (Hegseth 1998).

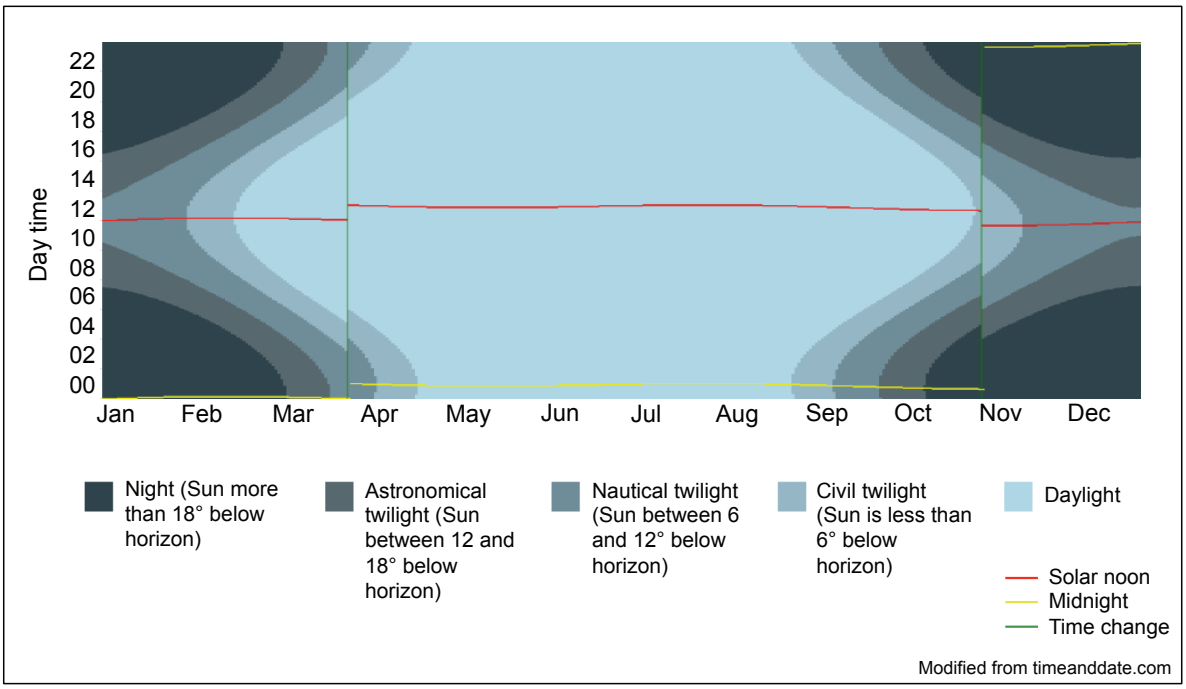


Figure 2: Sun graph for the region of Longyearbyen, Svalbard (78 °N).

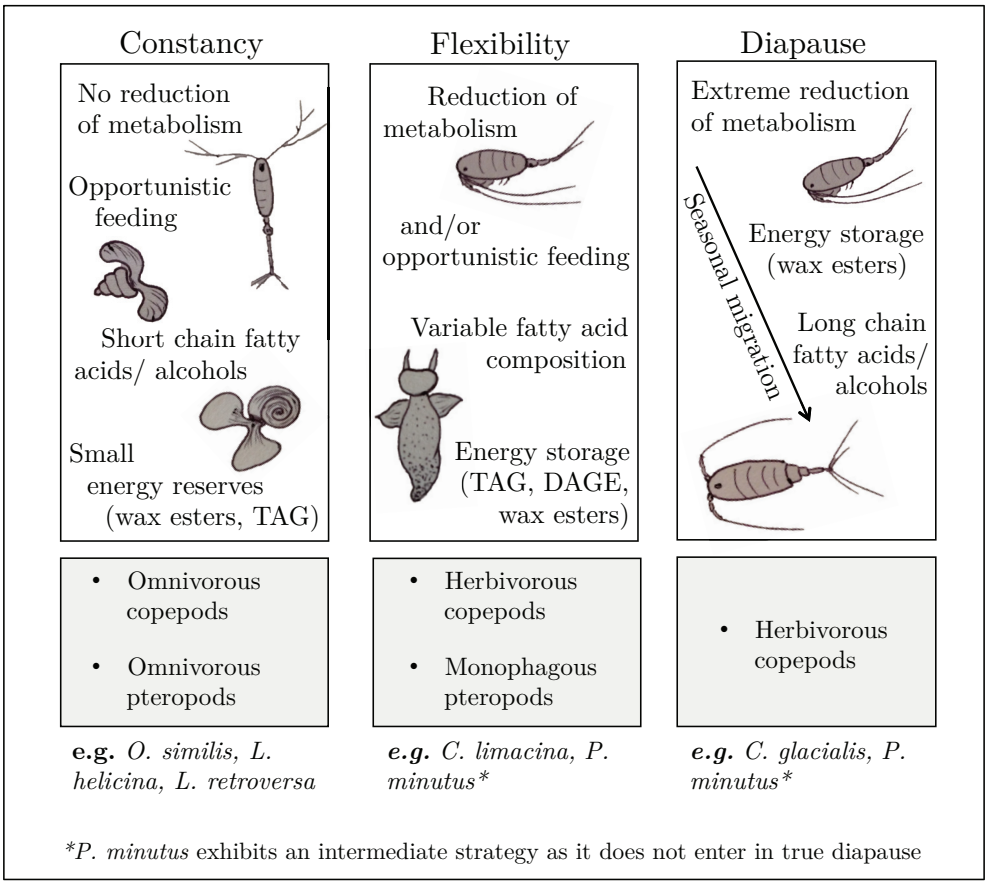
Pelagic phytoplankton production starts after the ice break-up and reaches maximal concentrations in May/June (Falk-Petersen et al. 2000, Madsen et al. 2001, Ringuette et al. 2002, Hansen et al. 2003). A temporal succession of different dominant taxa is observable, since diatoms dominate in spring (April/May) and flagellates are most abundant in summer (July/August) (Leu et al. 2006, Søreide et al. 2010, Hegseth and Tverberg 2013). Due to nutrient depletion and decreasing light intensity in autumn (September/October), phytoplankton concentrations decrease to negligible levels that persist throughout winter (Vader et al. 2015).

The dominant factor to which Arctic species have to adapt is the extreme seasonality in food supply (Clarke 1983). Most species from polar regions can withstand long periods of starvation with the help of lipid reserves (Lee et al. 1971, Lee and Hirota 1973). Particularly zooplankton species exhibit a large diversity of energy storage, which reflects their contrasting life strategies (Sargent and Falk-Petersen 1988, Scott et al. 1999, Hagen and Auel 2001) (Box.1). Whether their efficiency to assimilate lipids also varies depending on life strategies remains however unknown.

Excess of available food during the short production period is converted into depot lipids by zooplankton species, and largely transferred up the food web to the highest trophic levels (Scott

et al. 1999, Dahl et al. 2003, Thiemann et al. 2007, 2008). This energy storage is utilized by organisms to fuel metabolism and major life events, independently of ambient food levels (Kattner and Hagen 1995). The survival of all trophic levels depends on their ability to store sufficient amounts of energy from their diet in which zooplankton occupies a large fraction (Dalsgaard et al. 2003, section 1.1.1).

Box 1: Lipid diversity and strategy of adaptations of Arctic zooplankton during food scarcity periods. *Modified from Wilhelm Hagen and Holger Auel.*



1.1.3 Biosynthesis of lipids reserves

Types of storage lipids

Storage lipids provide energy for various processes such as ontogenic development, gonad maturation, reproduction and metabolism during periods of low food supply (Kattner and Hagen 1995, Lee et al. 2006). The high-energy content of lipids (ca. 39 kJ g^{-1}) offers an advantage over proteins and carbohydrates (both ca. 17 to 18 kJ g^{-1}) (Hagen and Auel 2001). Four typical classes of storage lipids are found in marine zooplankton: phospholipids, triacylglycerols (TAG), wax esters and diacylglycerol ethers (DAGE) (Fig.3) (Lee et al. 2006). Phospholipids

are key components of biomembranes. In zooplankton these polar lipids consist of 3 major fatty acids: 16:0, 20:5(n-3) and 22:6(n-3) (Box.2), which are always present in high amounts in organisms, independently of dietary changes (Lee et al. 1971, Falk- Petersen et al. 2000). A portion of these phospholipids is also used as reserves, fueling for example gonad development in copepods (Jónasdóttir et al. 2009).

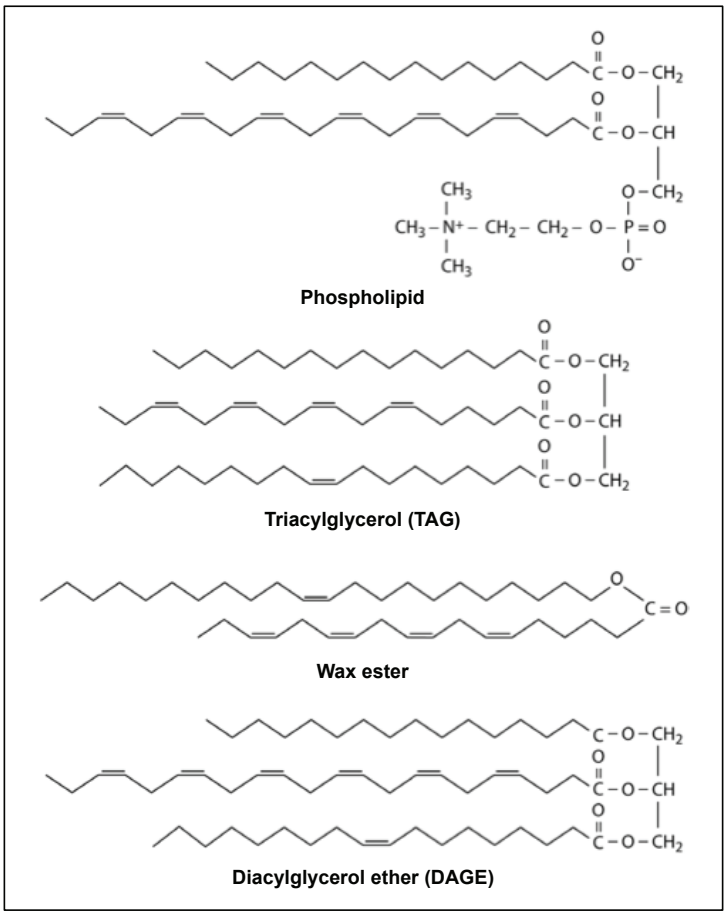


Figure 3: Chemical structures of the four major lipid classes in zooplankton: phospholipid (16:0 and 22:6 fatty acids); triacylglycerol (TAG) (16:0, 18:4 and 18:1 fatty acids); wax ester (22:1 alcohol and 18:4 fatty acid); diacylglycerol ether (DAGE) (16:0 alcohol, 22:6 and 17:1 fatty acids). Modified from Lee et al. (2006).

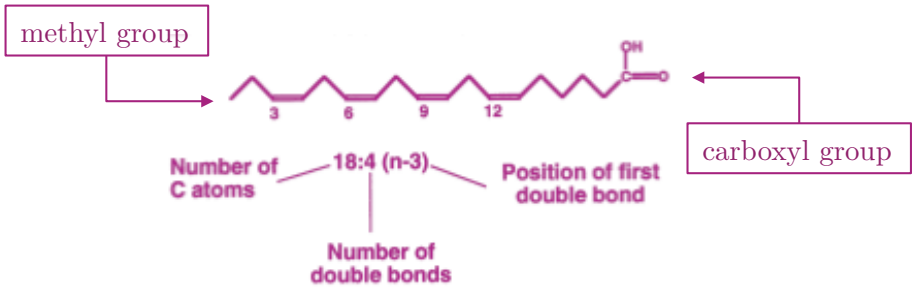
TAG are the most common storage lipids in animals and are usually used as short term energy reserves. They are characterized by a glycerol backbone esterified with 3 fatty acids (Lee et al. 2006). Wax esters are major long term storage lipids in high latitude species. They consist of simple esters of one long chain alcohol and one long chain fatty acid. DAGE are composed of one ether-linked alkyl chain and 2 fatty acid esters at the glycerol backbone. Among Arctic zooplankton, DAGE have been mainly found in the gymnosome pteropod *C. limacina*, in which they reach considerable amounts (Lee 1974, 1975, Phleger et al. 1997, Falk- Petersen et al. 2001, Böer et al. 2005). They are used as long term energy reserves and are considered to be *de novo* synthesized (Kattner et al. 1998, Böer et al. 2007). The composition of DAGE of *C.*

limacina is unique, comprising a very large portion of odd chain fatty acids, such as 15:0, 17:0 and 17:1(n-8).

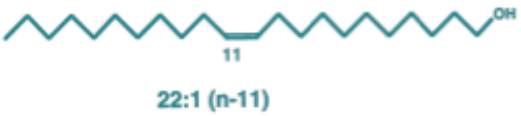
Box 2: Nomenclature of fatty acids and fatty alcohols.

Fatty acids and their radicals are named according to the IUPAC (International Union of Pure and Applied Chemistry) rules for the Nomenclature of Organic Chemistry.
Fatty acids are always numbered with the carboxyl group as C_1.

For unsaturated fatty acids, the number of double bounds is indicated just after the numbering of carbon atoms. The position of double bonds is indicated in the form (n-x) where n represents the number of carbon atoms in the chain and x is the x^{th} carbon bond counting from the terminal methyl end. For example, the position of the double bonds of stearidonic acid (trivial nomenclature), may be given as (n-3).



Long-chain **alcohols** and the radicals derived from them are designated according to systematic nomenclature, but not by trivial names that are derived from those of fatty acids.



Transfer of lipids in the food web

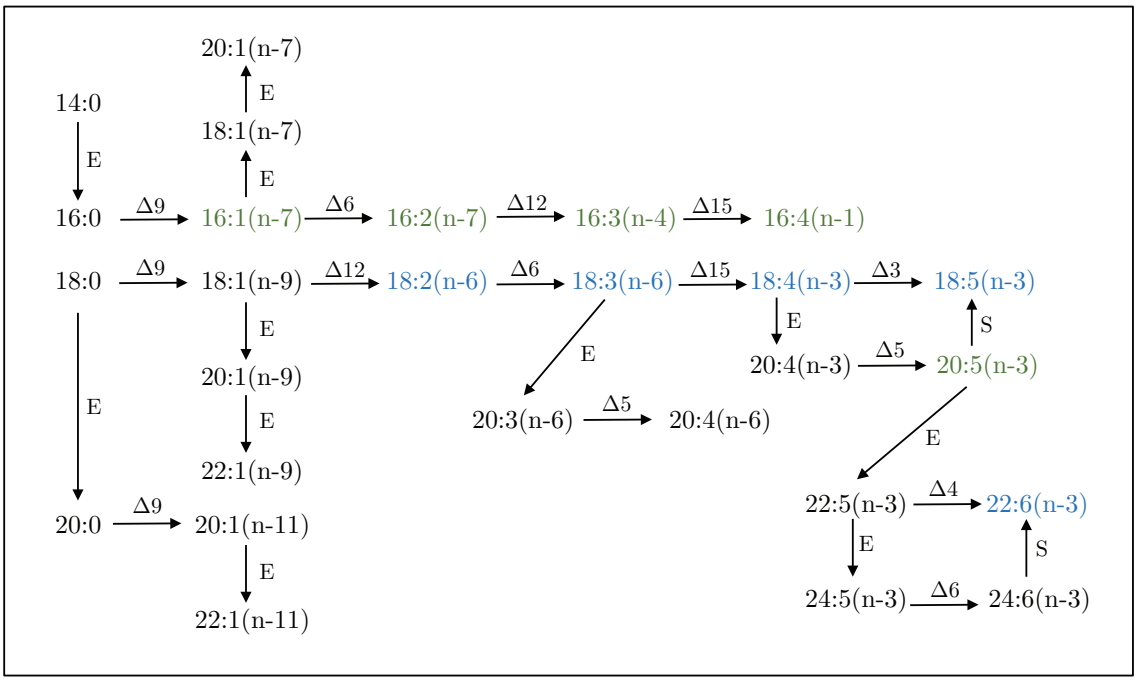
Ice algae and phytoplankton constitute the primary energy source in the Arctic pelagic food web (Parsons 1963). They produce high-quality food that is best at the onset of their blooms, when the irradience is low to moderate and the water characterized by high nutrient concentrations (Skerratt et al. 1995, Reuss and Poulsen 2002, Leu et al. 2011, Hessen et al. 2008). These primary producers biosynthesize all of their constituents *de novo*, including a large portion of fatty acids (Sargent and Henderson 1995, Cook 1996). Fatty acids in ice algae and phytoplankton mainly consist of even numbered, saturated or unsaturated compounds with 12 to 24 carbon atoms (Pohl and Zurheide 1979, 1982, Wood 1988, Cobelas and Lechado 1989,

Harwood and Jones 1989, Kayama et al. 1989). Among them, the polyunsaturated fatty acids (PUFA) (n-6) and (n-3), which are only biosynthesized by primary producers, are essential fatty acids (EFA) for heterotrophic organisms (Pohl and Zurheide 1979, Gurr and Harwood 1991, Cook 1996, Smith and Fitzpatrick 1996). The concentration of (n-3) and (n-6) PUFA in primary producers and subsequent levels of the food web is therefore an important food quality indicator (Jónasdóttir et al. 1995, Müller-Navarra 1995, Müller-Navarra et al. 2000, Wacker and von Elert 2001). Previous studies reported a strong seasonality of fatty acid composition in Arctic phytoplankton (Mayzaud et al. 1989, Skerratt et al. 1995, Reuss and Poulsen 2002). This temporal dynamic is mainly driven by the succession of algal taxa, which are characterized by different fatty acid profiles (Leu et al. 2006). Diatoms are enriched in 16:1(n-7), C_{16} PUFA and 20:5(n-3) while flagellates contain high amounts of C_{18} PUFA and 22:6(n-3) (Kates and Volcani 1966, Harrington et al. 1970, Fernandez-Reiriz et al. 1989, Volkman et al. 1989).

Most fatty acids are transferred without modification from primary producers to zooplankton storage lipids and are referred to as trophic markers (FATM), as first described by Lee et al. (1971). Herbivorous species exhibit a FATM signature that clearly reflects their diet (Sargent and Henderson 1986). Therefore, the proportion of, for example, 16:1(n-7) and 18:4(n-3) in their storage lipids can be used to evaluate the relative importance of diatoms and dinoflagellates in their diet (Graeve et al. 1994b, Scott et al. 1999). Omnivorous and carnivorous species have much more complex diets, resulting in more diverse lipid signatures than herbivorous species (Daalsgard et al. 2003). A typical marker of these feeding strategies is however, the presence of the fatty acid 18:1(n-9) in high amounts (Falk-Petersen et al. 2000). Some zooplankton species are capable of *de novo* synthesizing specific lipid compounds (Daalsgard et al. 2003), (Box.3). For example, the large Arctic calanoid copepods synthesize high amounts of long chain monounsaturated fatty acids and fatty alcohols with 20 and 22 carbon atoms (Sargent and Henderson 1986). These compounds are recognized as markers for a calanoid-dominated diet in higher trophic levels, specifically fish and birds (Graeve et al. 1994a, 1994b, Kattner et al. 2007). The gymnosome pteropod *C. limacina* also has the capacity to *de novo* synthesize fatty acids. It is the only known zooplankton taxa to synthesize odd chain fatty acids (Kattner et al. 1998, Falk-Petersen et al. 2001, Böer et al. 2005).

The lipid compounds that are assimilated and *de novo* synthesized by zooplankton (Lee 1975, Sargent and Henderson 1986, Falk-Petersen et al. 1987, 2000, Lee et al. 2006) are transferred through the food web without major modification (Falk-Petersen et al. 1990, 2004, Dahl et al. 2003). While the crucial role of zooplankton in the lipid-based Arctic pelagic food web is well

Box 3: Fatty acid biosynthesis of Arctic phytplankton and zooplankton. Diatom FATM are indicated in green and flagellate FATM in blue. Elongation (E), $\beta-$oxidation (S) and desaturation (Δ) are the main processes involved in fatty acid modifications in organisms. Fatty acids are elongated by an elongase enzyme. Desaturation is done by a fatty acid desaturase enzyme. The number associated to Δ indicates the position where a double bond is created, from the carboxyl end. Fatty acids are broken down by means of $\beta-$oxidation, primarily facilitated by the mitochondrial trifunctional enzyme complex. *Modified from Martin Graeve.*



recognized, there is nearly no information about how quickly the community is able to transfer lipids from primary producers to higher trophic levels (but see Graeve et al. 2005).

1.2 A changing Arctic

The Arctic region is experiencing intense warming (ACIA 2004, Kaplan and New 2006, Chepurin and Carton 2012), which has accelerated during the last decades (Comiso et al. 2008). Since 1982, a warming of 0.5 °C per decade has been reported for Arctic sea surface temperatures (SST) (Timmermans and Proshutinsky 2015, 2016). Particularly the European part is warming at a rate 2-3 times faster than the global average trend (Manabe et al. 1992, ACIA 2004, IPCC 2007). Rising air temperatures, increasing precipitation and higher river inflows are leading to a large and rapid change in the upper Arctic ocean layers (Li et al. 2009). This change is accelerated by the decrease of surface albedo due to ice and snow melt (Overpeck et al. 1997) and the elevated inflow of abnormally warm Atlantic waters by the intensified North Atlantic Current over the past decades (Dickson et al. 2000, Hansen et al. 2004, Holliday et al. 2008, 2009).

The dramatic losses of ice include a decrease of the perennial sea ice cover by 9-14% per decade (Johannessen et al. 1999, Comiso 2002, Nghiem et al. 2007, Serreze et al. 2007, AMAP 2011, Stroeve et al. 2012). The full ice cover, i.e. the combination of perennial and seasonal sea ice, has also been decreasing, at the lower rate of 3% per decade (Bjorgo et al. 1997, Parkinson et al. 1999, Parkinson and Cavalieri 2002). Reduction in sea ice thickness and area will alter the current primary production regime due to earlier ice break-up and earlier onset of the phytoplankton bloom (Tremblay et al. 2006, Arrigo et al. 2008, Pabi et al. 2008, Søreide et al. 2010). Also, as the period with available light becomes longer in the water, an earlier and enhanced primary production can be expected throughout the year (Kahru et al 2011). However, the combination of warmer SST and increased inflow of freshwater in the surface layers will cause a stronger stratification. Phytoplankton may hence be affected by a depletion of nutrients in surface waters, resulting in a lower primary production (Li et al. 2009). Also the community composition may change: under low nutrient conditions, small cells may thrive as they are more efficient to assimilate nutrients than larger cells due to their larger surface area/volume ratio (Li et al. 2009, Tremblay et al. 2009). In future decades, the Arctic ecosystem may hence be characterized by a low production and biomass of large phytoplankton cells such as diatoms and a high abundance of smaller cells such as flagellates (Li et al. 2009, Ardyna et al. 2011). These changes will likely have large consequences on the quantity and quality of lipid compounds available for zooplankton, with possible repercussions on the energy transfer to higher trophic levels.

The increased temperatures combined with the elevated Atlantic inflow result in the introduction of non-native species to the Arctic ecosystem (Bollens et al. 2002 and references therein). The thecosome pteropod community for example, might shift from a dominance of the Arctic *L. helicina* towards a dominance of the smaller Atlantic species *L. retroversa* (Bauerfeind et al. 2014). Another aspect of the changing Arctic is the acidification of seawater. Anthropogenic CO_2 is absorbed in the water and induces a decrease in pH and carbonate ion concentration (Orr et al. 2005, McNeil and Matear 2008), which will reduce the calcite and aragonite saturation state. Local observations as well as modeled projections suggest that Arctic surface waters will become regionally and/or seasonally under-saturated within less than a decade (Orr et al. 2005, Steinacher et al. 2009, Yamamoto-Kawai et al. 2009). Ocean acidification has been proved to affect calcification, growth, development and metabolism of several marine species, such as calcifying phytoplankton, echinoderms, bivalves, coral and crustaceans (e.g. Riebesell et al. 2000, Kurihara 2008, Pörtner 2008). In the Arctic pelagic food web, thecosomes pteropods are expected to be the most vulnerable organisms to acidification due to their aragonite shell (Lalli

and Gilmer 1989, Orr et al. 2005, Seibel et al. 2007). A possible decline in the population may have large impacts on their predators and therefore on the entire lipid-based food web as well as on the biogeochemical cycling of carbon.

1.3 The studied species

1.3.1 Copepods

Copepods constitute one of the major zooplankton taxa in Arctic seas (Iken et al. 2005, Węsławski et al. 2007). While large copepod species of the genus *Calanus* are dominant with regard to biomass (Conover and Huntley 1991, Mauchline 1998, Ringuette et al. 2002, Nielsen et al. 2007, Søreide et al. 2008), small copepods species such as *Pseudocalanus minutus* and *Oithona similis* prevail in terms of abundance (Auel and Hagen 2002, Hopcroft et al. 2005, Svensen et al. 2011). The copepod community plays a key role in the Arctic pelagic food web and hence largely influences the energy flow and functioning of productive marine ecosystems (Tande 1991, Longhurst 1998). This section briefly describes the species and their life cycle characteristics. Information about their lipid strategies is gathered in Table 1.

Calanus glacialis

C. glacialis is one of the three major large calanoid copepods (maximum size of 3.5-5.2 mm) (Fig.4) in the Arctic Ocean, together with *C. hyperboreus* and *C. finmarchicus* (Conover and Huntley 1991, Ringuette et al. 2002, Søreide et al. 2008). Among zooplankton species in the Arctic shelf seas, *C. glacialis* prevails, accounting for up to 80% of the zooplankton biomass (Tremblay et al. 2006, Blachowiak-Samolyk et al. 2008, Søreide et al. 2008).

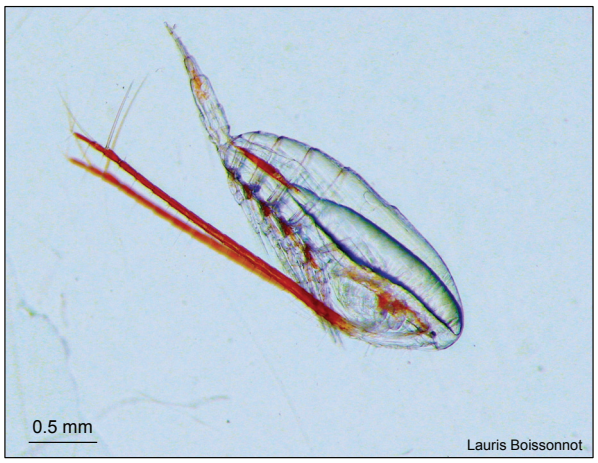


Figure 4: The Arctic calanoid copepod *Calanus glacialis*.

C. glacialis is a herbivorous filter-feeder that mainly feeds on diatoms (Falk-Petersen et al. 2009, Søreide et al. 2010). The species has a 1 to 3 year life cycle and reproduces in April-June. It relies on the phytoplankton bloom to support its growth from nauplii to copepodites, mainly during spring and summer. In late summer, the species descends to deep waters to survive the long and dark food-depleted winter in diapause (Hirche 1998, Freese 2015). The main overwintering stages are copepodite stages CIV and CV (Falk-Petersen et al. 2009). Overwintering individuals develop into females in mid-winter and ascend to surface waters in spring to feed and reproduce (Kosobokova 1999).

C. glacialis is one of the only 3 species (together with *C. hyperboreus* and *C. finmarchicus*) for which lipid turnover rates have been investigated, from a diet exclusively composed of diatoms, and estimated at about 3% d^{-1} (Graeve et al. 2005).

Pseudocalanus minutus

P. minutus is a calanoid copepod that reaches a maximum size of 1.4-2.2 mm (Fig.5). It is mainly distributed in the Arctic Ocean with southward extensions in the western North Atlantic and western North Pacific (Grainger 1965, Frost 1989). In Arctic shelf seas the species is very numerous, contributing to 75% of the total zooplankton abundance at times (Hopcroft et al. 2010).

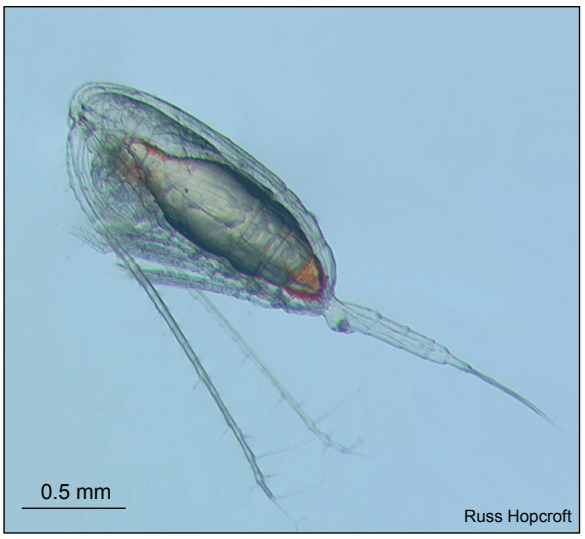


Figure 5: The Arctic calanoid copepod *Pseudocalanus minutus*.

P. minutus is described as a mainly herbivorous filter feeder (Corkett and McLaren 1979, Norrbin et al. 1990) that feeds on diatoms in spring and summer and on flagellates and small metazoans in autumn and winter (Lischka and Hagen 2007). The species is characterized by

short developing season in spring-summer and a long resting period in winter. The life cycle is considered to last 1 to 1.5 years (Norrbin 1991, Lischka and Hagen 2005). Reproduction occurs during the phytoplankton bloom in May-June (Marshall 1949, Pertsova and Kosobokova 1996, Lischka and Hagen 2005). Food supplies appear to be essential for egg production and spawning (Corkett and McLaren 1979, Niehoff and Runge 2003). *P. minutus* overwinters mainly as copepodite stages CIII-CV (Kwasniewski 1990, Norrbin 1991, Lischka and Hagen 2005). The species does not enter true diapause, but feeds omnivorously throughout the winter although with a considerably reduced metabolism (Davis 1976, 1984, Norrbin et al. 1990, Norrbin 1994). Migration toward deep layers has been observed between November and January (Lischka and Hagen 2005).

Oithona similis

O. similis is a cyclopoid copepod that reaches a maximum size of 0.7-1.0 mm (Fig.6). It is one of the most frequently reported species in the Arctic (Auel and Hagen 2002, Walkusz et al. 2003, Møller et al. 2006, Daase and Eiane 2007, Madsen et al. 2008), contributing up to 40% of the total zooplankton abundance (Kosobokova and Hirche 2000). It can hence constitute a significant part of copepod biomass and secondary production (Nielsen and Andersen 2002). This small copepod is an ambush feeder and has an omnivorous to carnivorous feeding mode (Drits and Semenova 1984, Nielsen and Sabatini 1996). It feeds preferentially on motile preys such as flagellates and protists (Drits and Semenova 1984, Svensen and Kiørboe 2000). In addition, some studies suggest that it could feed on suitably shaped diatoms (Kattner et al. 2003, Lischka and Hagen 2007).

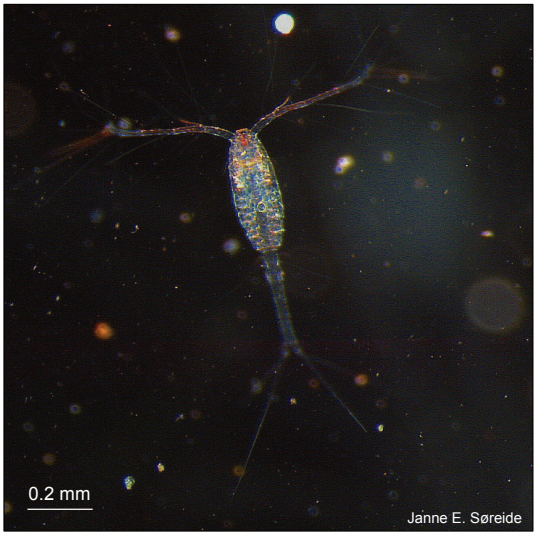


Figure 6: The Arctic cyclopoid copepod *Oithona similis*.

The species is considered to have a 1 year life cycle (Lischka and Hagen 2005). Its life strategy differs from that of the Arctic calanoid copepods, as does not overwinter in diapause, but stays active in the upper layer of the water column (Conover and Huntley 1991, Lischka et al. 2007). In the high Arctic, the species shows a continuous reproduction with two major reproductive events, one in June and one in August/September (Lischka and Hagen 2005, Narcy et al. 2009). Hence, two generations co-occur: the first generation appears in July as stages CI-CIII and complete its development until August/September (Corkett and McLaren 1986). The second generation occurs as nauplii and early copepodites in November and develops to adults until May (Lischka and Hagen 2005). Such fairly rapid completion of a single generation cycle of *O. similis* has also been described by Grainger (1959).

1.3.2 Pteropods

In Arctic waters, holoplanktonic mollusks are only represented by few species of the class Gastropoda (Lalli and Gilmer 1989). They include the shelled pteropods (thecosomes), which are commonly known as "sea butterflies" and the naked pteropods (gymnosomes), usually referred to as "sea angels". Even though these organisms can reach high biomass and have significant impacts on the ecology of pelagic Arctic communities (Dadon 1990, Hunt et al. 2008), they are little studied. The distribution and life cycle strategies of the species are described in this section Their lipid strategies are summarized in Table 2 for easier comparison with those of copepods (Table 1).

Thecosome pteropods - *Limacina helicina* and *L. retroversa*

Thecosome pteropods are significant contributors to marine biogeochemical cycles. They fix inorganic carbon in surface waters by producing an aragonite shell and export it to the sea floor by sinking after death (Berner 1977, Fabry 1990). They can reach high abundances at times, and trend to gather in large swarms due to processes that remain poorly understood (Sakthivel 1972, Gilmer and Harbison 1991, Falk-Petersen et al. 2001, Karnovsky et al. 2008). The two species *L. helicina* and *L. retroversa* are the only thecosome pteropods observed in Arctic waters (Borchgrevink 1905, Lalli 1970, Conover and Lalli 1972, Smidt 1979, Boysen-Ennen and Piatkowski 1988, Lalli and Gilmer 1989, Kattner et al. 1998) (Fig.7). While *L. helicina* reaches a maximum size of 8 mm (Gannefors et al. 2005), *L. retroversa* is much smaller and does not grow larger than 3 mm (Hsiao 1939, Katter et al. 1998, Lischka et al. 2012). *L. helicina* is

Table 1: Dry mass and lipid storage strategies of the copepods *Calanus glacialis*, *Pseudocalanus minutus* and *Oithona similis*.

Species	Dry mass	Lipid reserves	Main lipid class	Major fatty acids and alcohols	Utilization
C. glacialis	0.1 mg ind^{-1} (Falk Petersen et al. 2009)	Large amounts ($\leq$60% of the dry mass) (Scott et al. 2000) Stored in a lipid sac in the body cavity (Sargent and Falk-Petersen 1988, Miller et al. 1998, 2000, Lee et al. 2006)	Wax esters (Falk-Petersen et al. 2009)	Fatty acids 16:0, 16:1(n-7), 20:5(n-3) and 22:6(n-3), fatty acids and alcohols 20:1(n-9) and 22:1(n-11) (Graeve et al. 2005)	Molting, gonad maturation, reproduction (Lee et al. 2006, Søreide et al. 2008, Freese et al. 2017, Boissonnot et al. in prep.)
P. minutus	ca. 10 μg ind^{-1} (estimated for *P. acutus*, Norrbin et al. 1990)	Large amounts Stored in a lipid sac in the body cavity (Lischka and Hagen 2007)	Wax esters (Fraser et al. 1989, Kattner and Krause 1989, Norrbin et al. 1990)	Fatty acids 16:1(n-7), 18:0, 18:1(n-9), 20:5(n-3) and 22:6(n-3), fatty alcohols 14:0 and 16:0 (Lischka and Hagen 2007)	Metabolism during winter, reproduction and development (Lischka and Hagen 2007)
O. similis	0.01 μg ind^{-1} (Sabatini and Kiørboe 1994)	Small amounts (Lischka and Hagen 2007) Stored in a lipid droplet in the body cavity (Narcy et al. 2009)	Wax esters (Kattner et al. 2003, Lischka and Hagen 2007)	Fatty acids 18:1(n-9) and 22:6 (n-3), fatty alcohols 14:0, 16:0 and 20:1(n-9) (Kattner et al. 2003, Lischka and Hagen 2007)	Reproduction and winter metabolism, in complement to feeding (Kattner et al. 2003, Lischka and Hagen 2007, Zamora-Terol et al. 2013)

a polar species and can occur in high densities in Arctic marine ecosystems (Kerswill 1940, Kobayashi 1974, Lalli and Gilmer 1989, Cooney et al. 2001). In contrast, *L. retroversa* is a boreal species and is usually associated, in the Arctic, to Atlantic water inflow (Chen and Bé 1964, van der Spoel 1967, Bé and Gilmer 1977, Hopkins 1985, 1987).

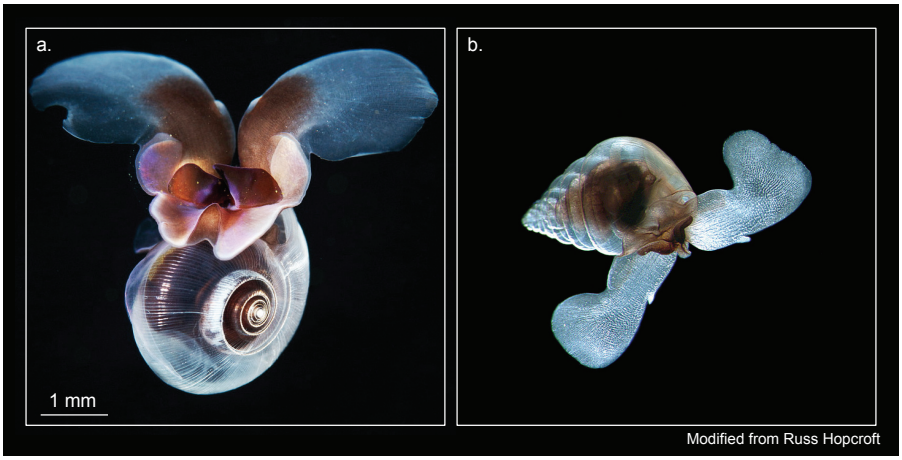


Figure 7: The thecosome pteropods (a.) *Limacina helicina* and (b.) *L. retroversa*.

Both *L. helicina* and *L. retroversa* are omnivorous and feed by excreting a mucous web, in which food particles become entangled (Gilmer 1972, Harbison and Gilmer 1992). Their diet mainly consists of diatoms and flagellates, but also of detritus and small zooplankton (Gilmer and Harbison 1991, Gannefors et al. 2005). They graze at high rates and can thus impact phytoplankton biomass (Perissinotto 1992, Bernard and Froneman, 2009).

The life cycle duration of *L. helicina* is still under debate. Some studies suggest that individuals reach maturity in one year and die after reproduction (Fabry 1989, Gannefors et al. 2005, Hunt et al. 2008) while others suggest a longer life span, with at least two overwintering periods (Kobayashi 1974, Bednaršek et al. 2012). *L. helicina* develops into adults in summer and reproduces in late summer/autumn (Gannefors et al. 2005). Veligers and juveniles constitute the main overwintering stages. Whether or not they grow and develop during this period or during the following spring/summer remains unclear (Gannefors et al. 2005, Lischka et al. 2012, Bednaršek et al. 2012). The life cycle of *L. retroversa* is poorly understood. Some studies conducted in sub-polar environments suggested a 1 year life cycle, with a reproductive event in spring (Hsiao 1939) or in autumn (Meinecke and Wefer 1990). More likely however, they reproduce constantly through the light season, with peaks in spring and autumn (Lebour 1932, Dadon and De Cidre 1992). Whether or not *L. retroversa* is only an expatriate or able to complete its life cycle successfully in Arctic waters is still unknown (Lischka et al. 2012).

Gymnosome pteropods - *Clione limacina*

Clione limacina is a bipolar species (Fig.8). It is the only representative of gymnosome pteropods in Arctic waters and its distribution extends southwards into sub-Arctic waters in both the North Atlantic and the North Pacific (Lebour 1931). It can reach a maximum size of 70-80 mm (Böer et al. 2005). *C. limacina* is considered as a monophagous species, feeding exclusively on *L. helicina* in Arctic waters and on *L. retroversa* in Atlantic waters (Meisenheimer 1905, Lalli 1970, Conover and Lalli 1972, Hopkins 1985). In turn, *C. limacina* constitutes an important food source for baleen whales, seabirds and planktivorous fish (Lebour 1931, Lalli 1970).

When hunting, *C. limacina* swims in circles around its prey, with its 6 buccal cones everted, which are to capture the shell of *Limacina* spp. (Lalli and Gilmer 1989). Once the prey captured, *C. limacina* holds the shell with these buccal cones and inserts chitinous hooks, evaginated from paired sacs, in combination with its radula to extract *Limacina* spp. from its shell (Conover and Lalli 1972). *C. limacina* has never been observed to feed on dead *Limacina* spp. or empty shell, suggesting that the trigger of predation behavior involves chemical processes and/or a response to the wing movements of the prey (Lalli and Gilmer 1989).

Figure 8: The gymnosome pteropod *Clione limacina*. Illustration of the buccal cones evagination, typical of a hunting behavior.

The life cycle of *C. limacina* is poorly understood, but may last at least 2 years in the high Arctic (Böer et al. 2005). The species seems to reproduce at low levels throughout the entire year, with an intense peak of spawning in spring/summer (Mileikovsky 1970). However, while most

of the stages are present year-round, the overall population structure shows a clear change from larvae in spring to adults in summer, with the largest specimens occurring in autumn (Böer et al. 2005).

Table 2: Dry mass and lipid storage strategies of the pteropods *Limacina* spp. and *Clione limacina*.

Species	**Dry mass**	**Lipid reserves**	**Main lipid class**	**Major fatty acids and alcohols**	**Utilization**
Limacina spp.	15 mg ind^{-1} (*L. helicina* adult, Gannefors et al. 2005)	Moderate amounts (<20% of the dry mass) (Falk-Petersen et al. 2001, Gannefors et al. 2005)	TAG (Kattner et al. 1998, Gannefors et al. 2005)	Fatty acids 16:0, 18:0, 20:5(n-3) and 22:6(n-3) (Gannefors et al. 2005)	Reproduction (Kattner et al. 1998, Gannefors et al. 2005)
C. limacina	20 mg ind^{-1} (Falk-Petersen et al. 2001, Gannefors et al. 2005)	Large amounts (≤50% of dry mass) (Ikeda 1972, Lee 1974, Phleger et al. 1997) Stored in lipid droplets in the entire body (Böer et al 2005)	TAG and DAGE (Lalli and Gilmer 1989, Kattner et al. 1998, Falk-Petersen et al. 2001, Böer et al. 2007)	Fatty acids 16:0, 18:0, 20:5(n-3), 22:6(n-3), 17:0 and 17:1(n-8) (Falk-Petersen et al. 2001, Böer et al. 2006)	Growth, maturation, gonad production and spawning during summer (Phleger et al. 1997, Böer et al. 2005)

1.4 Aims and outlines of this thesis

In a context of climate warming, Arctic seas are affected by several changes at physical, chemical and biological levels, possibly leading to dramatic shifts in the lipid-driven pelagic food web. It is therefore essential to better understand how lipids are transferred from the base of the food web up to high trophic levels, via zooplankton. The capacity of zooplankton to efficiently transfer lipids within the food web depends on several aspects, including the importance of species in terms of abundance and biomass, their life cycle strategies, ingestion rates, and rapidity to convert food into lipid reserves. Several gaps exist in the comprehension of the ecology and biochemistry of main zooplankton species. Regarding their distribution and life cycle strategies, several studies focused on the large copepods of the genus *Calanus* and, at a lower level, on the small copepods *P. minutus* and *O. similis*. However there is a major lack of knowledge regarding the life history of pteropods. Their population structure, life span and growth rates are poorly understood and still under debate. Regarding zooplankton lipid biochemistry, the time that organisms need to turnover their fatty acids and fatty alcohols has only been determined for *Calanus* spp. (Graeve et al. 2005). No previous effort has been made to evaluate the lipid turnover rates of other main components of the zooplankton community, namely small copepods and pteropods.

This study combines field observations and experimental work with innovative methods to fill the gaps of knowledge in the ecology and lipid biochemistry of zooplankton. It ultimately aims at better comprehending the overall zooplankton capacity to transfer lipids in the Arctic pelagic food web. The main objectives of this thesis are:

I. To relate the lipid turnover of the small copepods species *P. minutus* and *O. similis* to their life-cycle strategies (Manuscript **I**)
Feeding experiments with copepods were conducted during 3 weeks, using a ^{13}C labeled diatom-flagellate mix diet. The lipid and fatty acid assimilation was investigated by compound specific isotope analyses (CSIA). The underlying hypothesis was that herbivorous copepods such as *P. minutus* exhibit a more efficient lipid turnover than omnivorous copepods such as *O. similis*.

II. To investigate the distribution and life-cycle patterns of the Arctic thecosome pteropods *L. helicina* and *L. retroversa* (Manuscript **II**)
Field observations were conducted during 2 complete years in a Svalbard fjord. The aim was

to relate the distribution of *L. helicina* and *L. retroversa* to environmental parameters and to investigate annual growth of veligers and juveniles. This study was based on the hypothesis that *L. helicina* is present year-round and fulfills its life cycle in Svalbard whereas *L. retroversa* is only advected by Atlantic water masses and cannot reproduce in high latitudes.

III. To understand the lipid turnover of the thecosome pteropods *L. helicina* and *L. retroversa* and estimate the transfer of lipids to their predator *C. limacina* (Manuscript **III**)
Feeding experiments with thecosome pteropods were conducted during one week, using the same protocol as in the experiment with copepods. In addition, *C. limacina* was fed ^{13}C labeled *L. retroversa* during 3 weeks. The lipid and fatty acid assimilation was analyzed by CSIA. The hypothesis was that both *L. helicina* and *L. retroversa* have a low lipid turnover corresponding to their omnivorous feeding strategy whereas the monophagous *C. limacina* exhibits a very efficient lipid turnover.

2

MATERIAL AND METHODS

This chapter gives a brief overview of the sampling procedure, experimental work and analytic methods, which were used in this thesis. Detailed descriptions are given in the respective manuscripts (I - III).

2.1 Study area

This study is based on field observations and sampling performed in fjords on the west coast of Svalbard and in the Arctic Ocean, north of Svalbard. Svalbard archipelago is located at 76-80 °N and surrounded by the Fram Strait, the Barents Sea, the Arctic Ocean, and the Norwegian Sea (Fig.9). The major import of heat is the West Spitsbergen Current (WSC), the northernmost branch of the Norwegian Atlantic Current (NAW), which forms a continuation of the Gulf Stream (Aagaard et al. 1987, Schauer et al. 2004). The WSC flows northward along the continental shelf of West Spitsbergen (Schauer et al. 2004). It transports Atlantic water (AW) that is characterized by a temperature >3 °C and a salinity >34.9 psu (Swift and Aagaard 1981, Swift 1986, Hopkins 1991, Daase and Eiane, 2007). The other major water mass around Svalbard is the cold and less saline Arctic water (ArW), which flows northward in a west coastal current (Schauer et al. 2004). ArW is characterized by a temperature <1 °C and a salinity <34.7 psu (Svendsen et al. 2002, Daase and Eiane, 2007).

The shelves are normally dominated by ArW, whereas off-shelf and deep waters are often

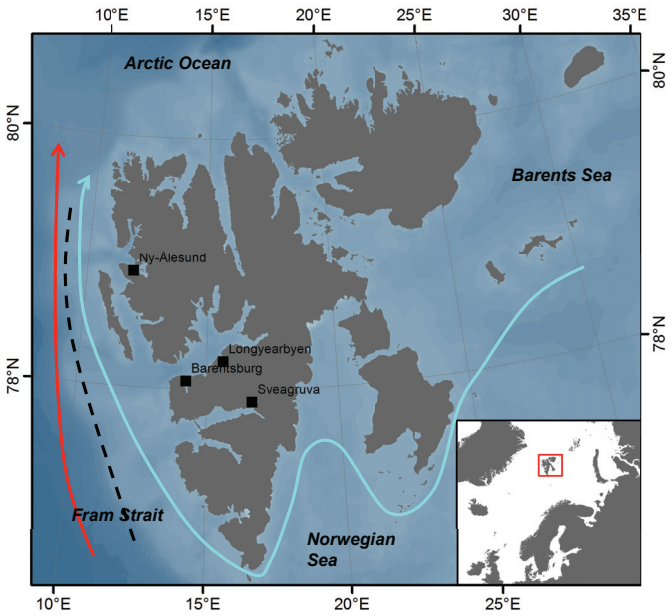


Figure 9: Map of Svalbard showing the major currents. Off the coast of western Spitsbergen is the West Spitsbergen Current (WSC, red) and the Arctic coastal water (ArW, blue). The dashed black line indicates the frontal area between the two currents.

strongly influenced by AW (Løyning 2000, Sundfjord et al. 2008). However these water masses are substantially modified by different processes including mixing, atmospheric heating and cooling, ice freezing and melting, precipitation and evaporation (Pfirman et al. 1994). As a result of mixing, AW circulating near shores and entering in the fjords is colder and less saline ($1<T<3$ °C, $34.7<S<34.9$ psu) than the water in the core of the WSC and is considered as Transformed Atlantic Water (TAW) (Svendsen et al. 2002). During summer, ice melting, river runoff and precipitations create an additional water mass that is referred to as surface water (SW). This layer is characterized by low salinity ($S<34.3$ psu) (Loeng 1991, Harris et al. 1998). AW and ArW are a major driving force in controlling the distribution of species and the trophic structure of the marine ecosystem of Svalbard and the Barents Sea (Wassmann et al. 2006).

2.2 Fieldwork

Fieldwork was conducted year round in 2012/2013 for the thecosome distribution study and in summer/autumn in 2014 and 2015 for feeding experiments and lipid investigations. Table 3 gives an overview of all samples collected in the frame of this thesis, both for field observations and feeding experiments.

Table 3: Compilation of the main sampling events.

Date	Location	Operating platform	Sampling gear	Species	Fixation	Purpose
27 Dec 2011 - 13 Dec 2012	IsA	PolarCirkel	WP2 - 60 μm	*Limacina* spp.	Formalin	Distribution
27 Dec 2011 - 13 Dec 2012	IsA	PolarCirkel	WP2 - 60 μm	*Limacina* spp.	70% EtOH	Genetics
23 Jan 2013 - 06 Oct 2013	IsA	PolarCirkel	WP2 - 60 μm	*Limacina* spp.	Formalin	Distribution
23 Jan 2013 - 06 Oct 2013	IsA	PolarCirkel	WP2 - 60 μm	*Limacina* spp.	70% EtOH	Genetics
July 2014	Arctic Ocean	RV Lance	MIK net - 1500 μm	*C. limacina* adult	Frozen -80 °C	Lipids and stable isotopes
July 2014	Arctic Ocean	RV Lance	MIK net - 1500 μm	*C. limacina* adult	–	Feeding experiment
24 July 2014	BAB	PolarCirkel	WP2 - 200 μm	*C. glacialis* CIV	Frozen -80 °C	Lipids and stable isotopes
24 July 2014	BAB	PolarCirkel	WP2 - 200 μm	*C. glacialis* CIV	–	Feeding experiment
29 July 2014	BAB	PolarCirkel	WP2 - 60 μm	*P. minutus* CV	Frozen -80 °C	Lipids and stable isotopes
29 July 2014	BAB	PolarCirkel	WP2 - 60 μm	*P. minutus* CV	–	Feeding experiment
29 July 2014	BAB	PolarCirkel	WP2 - 60 μm	*O. similis* female	Frozen -80 °C	Lipids and stable isotopes
29 July 2014	BAB	PolarCirkel	WP2 - 60 μm	*O. similis* female	–	Feeding experiment
20 Sept 2014 - 10 Oct 2014	Grønnfjorden	PolarCirkel	WP2 - 1000 μm	*L. retroversa* adult	–	Feeding of *C. limacina*
23 Sept 2014	KB3	RV Helmer Hanssen	WP3 - 1000 μm	*L. retroversa* adult	Frozen -80 °C	Lipids and stable isotopes
23 Sept 2014	KB3	RV Helmer Hanssen	WP3 - 1000 μm	*L. retroversa* adult	–	Feeding experiment
28 July 2015	IsA	PolarCirkel	WP3 - 1000 μm	*C. limacina* adult	Frozen -80 °C	Lipids and stable isotopes
28 July 2015	IsA	PolarCirkel	WP3 - 1000 μm	*C. limacina* adult	–	Feeding experiment
July 2015	Adventfjorden	Snorkeling	Scoop net	*L. helicina* adult	–	Feeding of *C. limacina*
28 July 2015	Adventfjorden	Snorkeling	Scoop net	*L. helicina* adult	Frozen -80 °C	Lipids and stable isotopes
28 July 2015	Adventfjorden	Snorkeling	Scoop net	*L. helicina* adult	–	Feeding experiment
29 Sept 2015	IsA	PolarCirkel	WP2 - 60 μm	*L. helicina* juvenile	Frozen -80 °C	Lipids and stable isotopes
29 Sept 2015	IsA	PolarCirkel	WP2 - 60 μm	*L. helicina* juvenile	–	Feeding experiment

2.2.1 Distribution study of thecosome pteropods

The zooplankton community was sampled every month (when the meteorological conditions allowed) in 2012 and 2013, in Isfjorden/Adventfjorden, at IsA station (78.26 °N, 15.54 °E) (Fig.10). Sampling was done on board of a PolarCirkel from UNIS, using a WP2 net (60 μm mesh size, 0.25 m^2 aperture) for vertical hauls (65-0 m).

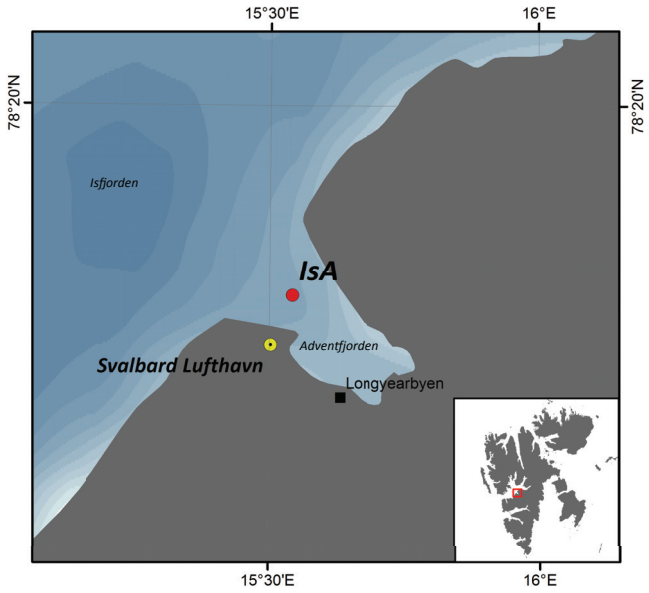


Figure 10: Map of Isfjorden and Adventfjorden showing the sampling station IsA (78.26 °N, 15.54 °E) and the meteorological station Svalbard Lufthavn (78.24 °N, 15.50 °E).

The water mass properties (salinity, temperature, and density) and fluorescence were measured vertically at each sampling event at IsA station, from bottom to surface using a hand-held CTD with a fluorometer attached (CTD, SAIV A/S). The data of air temperature, precipitation, wind direction and wind speed were provided by the Norwegian Meteorological Institute. This data set includes measurements conducted every 6 hours from January 2012 to December 2013 at the station Svalbard Lufthavn, (78.24 °N, 15.50 °E) (Fig.10).

2.2.2 Sampling for feeding experiments and lipid investigations

C. glacialis was sampled in Billefjorden, at BAB station (78.66 °N, 16.74 °E) (Fig.11), on board of a PolarCirkel from UNIS, by vertical hauls from 100 m to surface using a WP2 net (200 μm mesh size, 0.25 m^2 aperture). *P. minutus* and *O. similis* were sampled on 29 July 2014 at BAB station, following the same protocol as for *C. glacialis* but using a 60μm mesh size WP2.

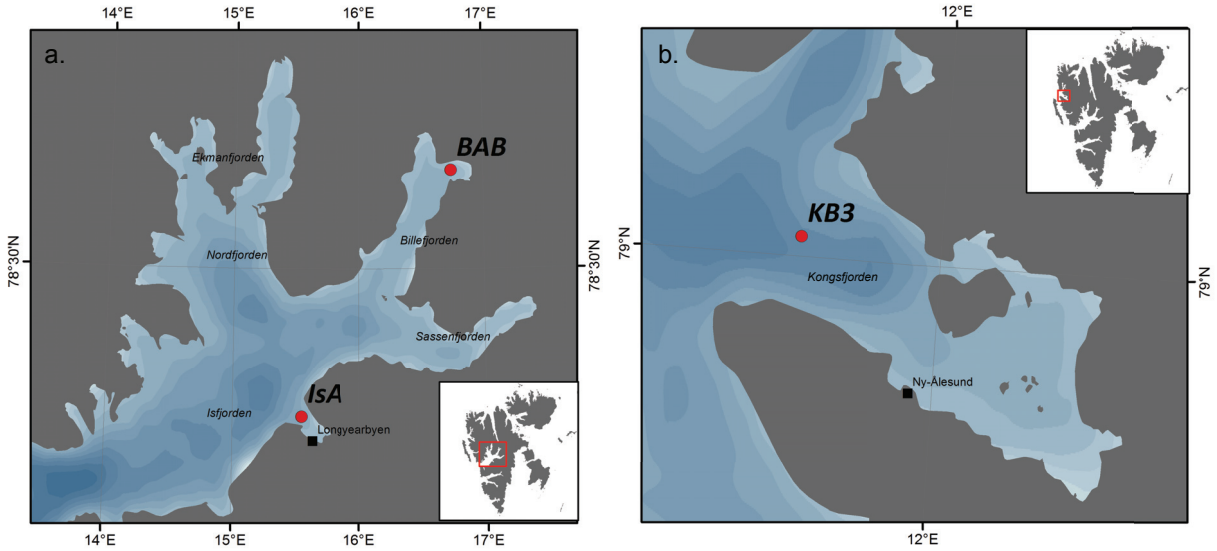


Figure 11: Sampling stations (a.) BAB in Billefjorden (78.66 °N, 16.74 °E), IsA in Isfjorden/Adventfjorden (78.26 °N, 15.54 °E) and (b.) KB3 in Kongsfjorden (79.00 °N, 11.23 °E).

The thecosome pteropods *L. retroversa* (adults) and *L. helicina* (juveniles and adults) were sampled in 2014 and 2015, in Kongsfjorden, at KB3 station (79.00 °N, 11.23 °E) and in Isfjorden/Adventfjorden, at IsA station (Fig.11).

The field campaign for thecosome pteropods started in early July 2014. No thecosome was found until 14 August of the same year despite an intensive sampling in Isfjorden, using WP2 and WP3 nets on board of a PolarCirkel. From this date on, *L. retroversa* was sampled mainly in Grønnfjorden where its presence was relatively constant. Regular sampling of *L. retroversa* continued until October 2014, and the specimens were used for various preliminary feeding experiments (see section 2.3.2). Specimens sampled for the feeding experiment based on ^{13}C labeled diet were captured on 23 September in Kongsfjorden, at KB3 station (Fig.11) on board of RV Helmer Hanssen, by vertical hauls (100-0 m) using a WP3 net. In turn, specimens that were used as food for *C. limacina* were sampled following the same procedure, in Isfjorden, every 3-4 days from 20 September to 10 October. During the entire field campaign of 2014, only one single specimen of *L. helicina* adult was observed (27 July, Billefjorden).

In contrast to 2014, 2015 was characterized by high abundances of *L. helicina* and almost no *L. retroversa* (3 specimens in total). *L. helicina* adults appeared in Isfjorden as early as May 2015, but for logistic reasons, sampling started in early July. Adults were at first sampled with WP3 nets, by oblique trawling (80-0 m during 15 min.), but this method was abandoned

due to extremely high mortality of animals apparently caused by damaged shells. Horizontal trawling with WP3 was also tried and abandoned due to similarly high mortalities. Finally, *L. helicina* adults were sampled by snorkeling, using a scoop net, in Adventfjorden on 28 July 2015 (Fig.11). Further specimens used as food for *C. limacina* were captured with the same procedure in Adventfjorden, in July/August. *L. helicina* juveniles were found from mid-August on and were sampled at IsA station (Fig.11) on board of a PolarCirkel on 22-29 September, using WP2 nets since they were less fragile than the adults.

The gymnosome pteropod *C. limacina* was very rarely found in Isfjorden in 2014, despite intensive sampling on board of a PolarCirkel, from early July to mid-October. Only three animals were captured in total, by oblique trawling with WP3 on 15 July 2014. In 2014, most *C. limacina* individuals were therefore sampled in the Arctic Ocean, north of Svalbard, in July 2014 on board of RV Lance, by vertical hauls from bottom to surface with a MIK net (method Isaac Kid; opening 3.14m^2, mesh size 1.5 mm and net bag 7 m long).
In 2015, *C. limacina* individuals were regularly observed in Isfjorden in summer-autumn. They were sampled for a feeding experiment based on *L. helicina* adults on 28 July 2015 on board of a PolarCirkel by oblique trawling with WP3 net (80-0 m during 15 minutes).

2.3 Experimental work

Feeding experiments with the copepods *C. glacialis*, *P. minutus* and *O. similis* and the pteropods *L. helicina*, *L. retroversa* and *C. limacina* were conducted in UNIS laboratories in 2014 and 2015. All experiments but one consisted in a 2-levels food web with a primary producer represented by a flagellate-diatom mix and a primary consumer, i.e. copepod or thecosome pteropod. The only exception was a feeding experiment with a 3-levels food web including the algal mixture, a thecosome pteropod and *C. limacina*.

2.3.1 Algal cultures

Single cultures of diatoms (*Chaetoceros debilis* and *Contricribra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*) were grown under similar conditions for all feeding experiments in 2014 and 2015. Light conditions were set at a 12h light:12h dark day cycle (50 μmol m^{-2} s^{-1} for the light period incident radiation was measured with surface reference sensor in air LI-190, LI-COR). *R. salina* and *C. debilis* were kept at 4 °C, *C. weissflogii* and

D. salina were kept at 15 °C since they were growing faster than at 4 °C. All algae were grown in a medium constituted of 0.7 μm filtered seawater enriched with f/2 Guillard medium (15 mL L^{-1}). Extra silicate was added to the medium for diatoms (2 mL L^{-1}) to ensure optimal growth. The culture medium was labeled with ^{13}C sodium bicarbonate prior addition to the algal cultures, with a concentration that was decreased between the experiments conducted in 2014 and the ones of 2015. This is discussed into more details in section 4.1. Prior to feeding of the animals, algal cells were counted with a haemacytometer (Schoen 1988) and Chl *a* concentrations were measured by fluorometry using methanol as the extracting solvent (Holm-Hansen and Riemann 1978). Thereafter algal cultures were added to the bottles containing the animals in excess concentrations and with a ratio 1:1 of flagellate cells:diatom cells. In addition, samples for lipid analyses were taken by filtrating duplicates of 5 mL of each algal culture on 0.7 μm GF/F filters.

2.3.2 Set up of feeding experiments

Immediately after arrival at UNIS, living and clearly active (swimming behavior) animals were sorted using a methodology summarized in Table 4.
Similar experimental conditions were set up for all feeding experiments with copepods and pteropods, in terms of diet (see section 2.3.1), light regime (24:24 h, 50 μmol m^{-2} s^{-1}) and temperature (3-4 °C).

One experiment was set up with 50 *O. similis* females and 25 *P. minutus* CV, which were transferred together into 15 glass bottles containing 1 L of 0.7 μm filtered seawater (three replicates for each of the five sampling dates). Another experiment was set up with 15 *C. glacialis* that were also transferred into 15 glass bottles. For these two experiments, three *in situ* replicates (Day 0) with the same number of copepods were immediately frozen at -80°C (without any preservative). (Table 5). Two thirds of the water in all bottles was changed every 3-4 days in order to feed the copepods and maintain ambient oxygen concentrations. Simultaneously (from Day 6-7 on), 3 of the bottles were completely emptied and the animals were counted and deep-frozen in triplicates at -80 °C for subsequent lipid analyses (Table 5). The experiments lasted 21 days in total.

Thecosome pteropods were less easy to handle in experimental conditions than copepods. At first, in August 2014, *L. retroversa* adults were transferred into aquaria containing 110 L of 1 μm

Table 4: Identification of the zooplankton species and stages.

Species	**Dominant stage used for experiment**	**Sorting tools**	**Identification criteria**	**References**
C. glacialis	CIV	Dissecting microscope	3.2-4.9 mm total lenght; Lipid sac prominent; Urosome length ∼25% prosome length; Antenna equal or longer than total length	Jashnov 1955, Boxshall 2001
P. minutus	CV	Dissecting microscope	1.15-1.95 mm total length; Slender prosome and protruding, nearly angular cephalosome; Lipid sac often well visible; Long urosome (∼40% of prosome); Antenna longer then prosome but less than total length	Frost 1989, Boxshall 2001
O. similis	Female	Dissecting microscope	0.7-1.0 mm total length; Elongated prosome; Lipid sac often visible; Long urosome (40-50% of prosome); Antenna longer then prosome but less than total length	Claus 1866, Boxshall 2001
L. helicina	Adult	Sight	50-80 mm shell diameter; Left flat-coiled shell	Phipps 1774, van der Spoel 1967, Kobayashi 1974, Lalli and Wells 1978
L. retroversa	Adult	Sight	1-70 mm shell diameter; pointy spiral shell	Fleming 1823, Hsiao 1939, van der Spoel 1967, Brisco 1977, Lalli and Wells 1978
L. helicina/L. retroversa	Juvenile	Dissecting microscope and molecular analyses	<1 mm shell diameter; for individuals >0.5 mm: identification based on the same morphological aspects as for adults; for individuals <0.5 mm: no morphological differences between the 2 species, molecular identification based on nuclear histone marker 3	Hsiao 1939, Kobayashi 1974, Lischka pers. comm.
C. limacina	Adult	Sight	10-80 mm total length; Oval-shaped body with lateral wings; Brown stomach	Phipps 1774, Morton 1957, Böer et al. 2005

Table 5: List of samples for lipid analyses.

Feeding time	***C. glacialis* CIV**	***P. minutus* CV**	***O. similis* female**	***L. helicina* juv.**	***L. helicina* adult**	***L. retroversa* adult**	***C. limacina* adults (nb *L. retroversa* eaten) - 2014**	***C. limacina* adults (nb *L. helicina* eaten) - 2015**
Day 0	3x15	3x25	3x50	3x40	3x1	3x5	3x1 (0)	3x1(0)
Day 1	-	-	-	-	-	-	-	6x1(1)
Day 2	-	-	-	45;50;52	3x1	8;8;9	-	3x1(1)
Day 3	-	-	-	-	3x1	-	-	2x1(1)
Day 4	-	-	-	49;50;56	3x1	10;10	-	-
Day 6	-	20;21;22	28;29;46	46;50;56	3x1	5;5;5	-	-
Day 7	13;15;16	-	-	-	-	-	-	-
Day 9	-	14;20;21	19;31;32	-	-	-	-	-
Day 11	14;14;15	-	-	-	-	-	3x1(9);2x1(10)	-
Day 13	-	21;23;24	11;14;27	-	-	-	-	-
Day 14	16;15;16	-	-	-	-	-	-	-
Day 16	-	13;14;22	11;11;13	-	-	-	-	-
Day 17	-	-	-	-	-	-	1(5);1(9);1(10)	-
Day 18	11;12;13	-	-	-	-	-	-	-
Day 20	-	-	-	-	-	-	2x1(8);1(9)	-
Day 21	10;12;13	15;17;23	11;16;28	-	-	-	-	-
Day 23	-	-	-	-	-	-	1(7);1(9);3x1(10);1(17); 2x1(18)	-

filtered seawater (Fig.12). These aquaria were specifically designed to culture pteropods, with a pump system creating a continuous circular flow intended to keep the animals in suspension (see Howes et al. 2014). However, massive mortalities occurred in this system (no survival after 24h, $n_{thecosome} = 200$), independently of the flow speed that was progressively decreased from 120 to 80 L h^{-1}. A further trial was done, with the addition of antibiotics in the water ((JBL Ektol bac, JBL GmbH & Co. KG, 1 mL 100 L^{-1})), as recommended by Howes et al. (2014). This treatment induced even higher mortalities of the thecosomes with the totality of individuals dying in less than 10 hours ($n_{thecosome} = 200$). This culturing method was therefore abandoned. Instead, thecosome pteropods were kept in 1 L glass bottles, with as little disturbances as possible. Since survival rates were much improved, at least for 5-6 days, this method was selected.

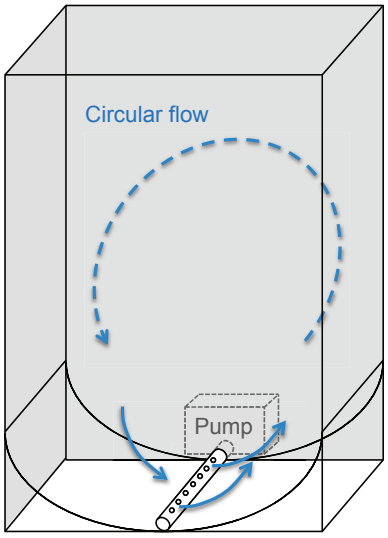


Figure 12: Schematic representation of the aquaria designed for culturing pteropods. The arrows represent the flux of water generated by the pump.

A successful first experiment was conducted with *L. retroversa* adults, starting on 23 September 2014 and lasting 6 days. At Day 0, three *in situ* replicates of 5 animals were frozen at -80 °C (Table 5). Two thirds of the water in all bottles were changed every other day to feed the thecosomes with the labeled algal mix-diet and to maintain high oxygen concentrations. In parallel, three bottles were emptied and the animals deep-frozen in triplicates for lipid analyses. The same experimental protocol was followed in 2015 in the experiments with *L. helicina* adults and juveniles (Table 5).

C. limacina were transferred in the 110 L aquaria (40-50 animals x 3 aquaria) and were kept in filtered sea water while waiting for feeding with *Limacina* spp. In 2014, due to the adjustments of *L. retroversa* culturing as explained in the previous paragraph, *C. limacina* were kept without

food for 10 weeks. During this period, half of the water was renewed twice a week to maintain high oxygen concentrations. During the 10 weeks of starvation, mortality was quasi-negligible in aquaria. A side trial using antibiotics (JBL Ektol bac, JBL GmbH & Co. KG, 1 mL 100 L^{-1}) was done with 5 *C. limacina* that were each kept in a glass bottle containing 1 L of filtered seawater, bu as with thecosomes, survival was higher without antibiotics.

Feeding started on 23 September 2014 with *L. retroversa* that had been previously fed with labeled algae for 3 days. To keep track of the feeding success, *C. limacina* were individually kept into glass bottles containing 1 L of 0.7 μm filtrated seawater. Five (on 23 and 25 September) and thereafter three (on 8,10 and 13 October) active *L. retroversa* were transferred into each bottle. *C. limacina* showed an aggressive predator behavior, feeding on most of the offered preys. The ones that were not eaten were removed when not swimming anymore since *C. limacina* apparently only feeds on active pteropods (Lalli 1970). Feeding rates were determined by counting the number of emptied shells after 24 h and 48 h. The feeding experiment lasted for 23 days. Each ndividual was frozen 3 days after the last feeding episode (Table 5). In 2015, an additional experiment was conducted, feeding *C. limacina* with *L. helicina* adults. The same set up as in 2014 was used, but the behavior of *C. limacina* was completely different. Few animals showed a predator behavior (ca. 20%), and among the ones that did, ca. half successfully caught their prey. No *C. limacina* fed on more than 1 *L. helicina* and none survived more than 2 days after ingestion of their unique prey. The feeding experiment therefore lasted for 3 days, since individuals were frozen only 1 day after their last feeding episode (Table 5).

2.4 Analytical work

2.4.1 Distribution and growth rates of thecosome pteropods

This section gives a brief overview of the methods used to investigate the thecosome population structure and life cycle in the Arctic environment. A comprehensive description can be found in manuscript II.

Since *L. retroversa* and *L. helicina* look alike during the first development stages (see Tab. 4), species determination of the small individuals (<0.5 mm shell diameter) was done with molecular tools, based on the Nuclear Histone 3 marker (H3), which contains a diagnostic nucleotide to distinguish *L. helicina* from *L. retroversa* (Kohnert, unpublished data).

Investigation of the environmental forcing on the thecosomes distribution patterns was done with multivariate analyses, using the R package FactoMineR (Lê et al. 2008).

Frequency distributions of the shell diameter of *L. helicina* were modeled as mixture distributions for which parameters (mean values, and standard deviations of sub-distributions) were estimated by the Kernel density estimation. At this step, generations were identified and used as a basis for estimation of growth rates. The shell size distribution of *L. retroversa* was too heterogeneous to reflect different generations.

2.4.2 Analyses of fatty acids and fatty alcohols

Total lipids of copepods and pteropods were extracted and analyzed following the method detailed in manuscript I. No pre-treatment was done on the algae/animals prior to lipid extraction except for *C. limacina*, whose guts were removed to exclude non-assimilated contents since the prey was clearly visible and occupying 1/3 of the body volume. Total lipids were extracted according to Folch et al. (1957) with slight modifications, and separated using a gas chromatograph (HP 6890N, Agilent Technologies Deutschland GmbH & Co. KG). The chromatograms were evaluated using ChemStation software (Agilent Technologies Deutschland GmbH & Co. KG). Total lipid mass per individual was calculated by summing up single fatty acid and fatty alcohol masses.

2.4.3 Compound specific isotope analyses

The same protocol of CSIA was used for all samples. A detailed explanation of the method is presented in manuscript I. The ^{13}C isotopic enrichment in fatty acids and fatty alcohols of algae, copepods and pteropods was measured using a Thermo GC-c-IRMS (gas chromatography-combustion-isotope-ratio mass spectrometry) system (Thermo Scientific Corporation, Bremen, Germany). The chromatographic peak areas and carbon isotope ratios were obtained with the instrument-specific software Isodat 3.0.

Isotopic ratios of each fatty acid and fatty alcohol are normally expressed in δ notation according to the formula (1).

$$\delta^{13}C\ (‰) = (\frac{R_{sample}}{R_{standart}} - 1) \times 1000 \quad (1)$$

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

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

$$AT\ (\%) = \frac{R_{sample}}{R_{sample} + 1} \times 100 \tag{2}$$

AT includes the enrichment of samples as well as their natural background (Brenna et al. 1997). To exclude the natural enrichment and consider only the experimental one, the atom percent excess (ATE) was calculated according to (3).

$$ATE\ (\%) = AT_{t=i} - AT_{t=0} \tag{3}$$

where t=i is the number of days since the beginning of the ^{13}C feeding experiment and t=0, the starting day of the experiment. $AT_{t=0}$ is therefore an average of the background AT in all fatty acids.

Since the dietary fatty acids were composed of both ^{13}C and ^{12}C, to calculate the percentage of carbon (^{13}C + ^{12}C) assimilated by the consumers in their fatty acids and fatty alcohols (PA), their ATE was divided by the diet AT (4).

$$PA\ (\%\ fatty\ acid\ mass\ ) = \frac{ATE}{AT(diet)} \tag{4}$$

For copepods and thecosome pteropods, average fatty acid AT of the algae mixture was used in the calculation:

– AT(algae, 2014)$_{copepods\ expmt}$ = 15.28%

– AT(algae, 2014)$_{thecosomes\ expmt}$ = 13.7%

– AT(algae, 2015)$_{thecosomes\ expmt}$ = 3.7%

Average AT of *L. retroversa* fatty acids at Day 3 was used to estimate the portion of carbon assimilated by *C. limacina*:

– AT(*L. retroversa*, Day 3) = 2.2%

To estimate the assimilation of carbon (C_{assi}) as mass (μg C_{assi} ind^{-1}) (5) by consumers, PA was multiplied by the mass of each fatty acid and fatty alcohol (B) expressed as carbon mass (in μg C ind^{-1}). B was derived from the number of moles of fatty acid and fatty alcohol in the consumers. The molecular mass of each labeled fatty acid and fatty alcohol was calibrated by its AT to incorporate the carbon mass according to the $^{13}C/^{12}C$ ratio (De Troch et al. 2012).

$$C_{assi}\ (\mu g\ C\ ind^{-1}) = B \times PA = B \times \frac{ATE}{AT(diet)} \tag{5}$$

The relative assimilation of fatty acids and fatty alcohols by consumers was calculated as (6).

$$Relative_{Cassi}\ (\%\ total\ fatty\ acid\ assimilated\ ) = \frac{C_{assi}}{TL} \times 100 \tag{6}$$

where TL is the total lipid mass (sum fatty acids and fatty alcohols).

Finally, the capacity of zooplankton communities to provide fatty acids and fatty alcohols for higher trophic levels was calculated as (7).

$$C_{provided}\ (\mu g\ C\ d^{-1}\ m^{-3}) = C_{assi} \times abundance_{species/stage} \tag{7}$$

where the abundances of species/stages (ind m^{-3}) were derived from literature and from this study's results.

2.4.4 Statistics

All statistical analyses were performed using the free software R 3.2.1 (team RDC, 2010). To test data for normal distribution, a Shapiro-Wilk test was applied. Significance of the results was tested with one-way ANOVA followed by a Fisher test and Tukey HSD tests that were respectively performed on linear regressions (growth rates of *L. helicina*, manuscript II) or polynomial regressions of degree 2 (lipid assimilation, manuscript I, III). Portions were $\arcsin(\sqrt{x})$ transformed prior to tests. The significance level was set at 5% (alpha = 0.05). Results were referred to as statistically significant and the null hypothesis was rejected if the P-value was lower than the alpha-level.

Principal component analyses (PCA) were performed to explore multivariate datasets (manuscripts II, III). To investigate the influence of environmental data on biological data (manuscript II), PCA were done using the R package FactoMineR (Lê et al. 2008). The environmental data

were computed as active variables while biological data were added as supplementary variables. Missing values were estimated with the package missMDA (Husson and Josse 2010), using the relations between all variables, from 2 dimensions of the PCA. The R package mixdist (Macdonald and Du 2012) was used to fit mixture distribution models to the shell diameter distributions of *L. helicina* and *L. retroversa*, using the Kernel density estimation.

3

MANUSCRIPTS

3.1 Manuscript I

Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods

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Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods

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This study aimed at understanding how life-cycle strategies of the primarily herbivorous *Pseudocalanus minutus* and the omnivorous *Oithona similis* are reflected by their lipid carbon turnover capacities. The copepods were collected in Billefjorden, Svalbard, and fed with ^{13}C labeled flagellates and diatoms during 3 weeks. Fatty acid (FA) and fatty alcohol compositions were determined by gas chromatography, ^{13}C incorporation was monitored using isotope ratio mass spectrometry. Maximum lipid turnover occurred in *P. minutus*, which exchanged 54.4% of total lipid, whereas 9.4% were exchanged in *O. similis*. In *P. minutus*, the diatom markers 16:1(n-7), 16:2(n-4) and 16:3(n-4) were almost completely renewed from the diet within 21 days, while 15% of the flagellate markers 18:2(n-6), 18:3(n-3) and 18:4 (n-3) were exchanged. In *O. similis*, 15% of both flagellate and diatom markers were renewed. *P. minutus* exhibited typical physiological adaptations of herbivorous copepod species, with a very high lipid turnover rate and the ability to integrate FAs more rapidly from diatoms than from flagellates. *O. similis* depended much less on lipid reserves and had a lower lipid turnover rate, but was able to ingest and/or assimilate lipids with the same intensity from various food sources, to sustain shorter periods of food shortage.

KEYWORDS: *Oithona similis*; *Pseudocalanus minutus*; fatty acids and alcohols; ^{13}C labeling; lipid assimilation

INTRODUCTION

The Arctic Ocean is characterized by an extreme seasonal variability in solar radiation, inducing a short and intense period of primary production during summer and an extended period of food scarcity in winter, especially for marine herbivorous species (Lee *et al.*, 2006; Falk-Petersen *et al.*, 2009). High-latitude zooplankton have developed specific adaptive biochemical pathways to cope with this strong seasonality (Sargent and Henderson, 1986; Hagen, 1999; Lee *et al.*, 2006), allowing them to deposit neutral lipids, either wax esters or triacylglycerols, as energy reserves that may be used for maintenance during winter and for reproductive processes (Lee *et al.*, 1971a; 1971b;

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Clarke, 1983; Kattner and Hagen, 1995). Fatty acids (FA) and alcohols biosynthesized by zooplankton are rapidly transferred through the food web and supply higher trophic levels with the required calories (Falk-Petersen *et al.*, 1990). This lipid-based flux of energy is central in the lipid-driven Arctic food web. The various biochemical processes, that produce lipid reserves of different compositions, enable species to utilize different ecological niches, and are major determinants of biodiversity in polar zooplankton (Falk-Petersen *et al.*, 2000). FAs can also be used as trophic markers (FATM) and thus capture changes in feeding behavior (Dalsgaard *et al.*, 2003 and references therein). In herbivorous species, mainly calanoids, it is generally accepted that phytoplankton FATM are incorporated largely unchanged, making it possible to determine their recent diet (Sargent and Henderson, 1986; Graeve *et al.*, 1994b). In contrast, omnivorous species have a much more complex diet, resulting in more diverse lipid signatures than herbivorous species.

The calanoid copepod *Pseudocalanus minutus* mainly inhabits arctic-boreal seas (Peters *et al.*, 2004; Lischka and Hagen, 2007). The cyclopoid *Oithona similis* has been described as the most abundant and ubiquitous copepod species in the world's oceans (Gallienne and Robins, 2001). Both species prevail in Arctic shelf seas in terms of abundance (Nielsen and Andersen, 2002; Daase and Eiane, 2007). Also considering their high population turnover rates, these species play an important role in Arctic marine food webs, despite their small size (McLaren and Corkett, 1978; Auel and Hagen, 2002; Hopcroft *et al.*, 2005). Particularly in autumn, when the larger herbivorous copepods of the genus *Calanus* leave the upper layers of the water column to overwinter at depth, a niche is created for smaller copepods (Svensen *et al.*, 2011), "niche" being defined as "the actual place and role in an ecosystem an organism or species occupies" (Lawrence, 1989). Thus particularly in autumn and winter, the smaller species are ecologically important, restructuring the grazer chain and ensuring a continuous food supply for higher trophic levels (Conover and Huntley, 1991; Hansen *et al.*, 1999; Møller *et al.*, 2006; Zamora-Terol *et al.*, 2013). Due to their suitable size they are also important food items of various predators such as fish larvae (Hubold, 1985; Kellermann, 1987). *P. minutus* is a herbivorous species (Corkett and McLaren, 1979; Norrbin *et al.*, 1990), mainly feeding on diatoms in spring and on flagellates in summer, autumn and winter (Lischka and Hagen, 2007). Correspondingly, its FA composition is dominated by 16:1(n-7), 18:0, 18:1(n-9), 20:5(n-3) and 22:6(n-3). Fatty alcohols such as 14:0 and 16:0 are synthesized *de novo* and esterified with dietary FAs to wax esters (Lischka and Hagen, 2007). During the winter season, *P. minutus* continues feeding, although this is combined with a reduced metabolism and the utilization of wax esters (Davis, 1976; Norrbin *et al.*, 1990, 1991). *O. similis* is an ambush feeder and has an omnivorous to carnivorous feeding mode (Drits and Semenova, 1984; Nielsen and Sabatini 1996). Its FA composition is highly dominated by 18:1(n-9) and 22:6 (n-3) and reflects a diet based on flagellates and metazoans (Kattner *et al.*, 2003; Lischka and Hagen 2007). Lischka and Hagen (2007) suggested that wax esters, mainly composed of 14:0 and 16:0 but also 20:1 (n-9) alcohols, are accumulated during summer in Kongsfjorden and are largely used to fuel the reproduction peak in May/June, while the reproduction peak in August/September is mainly fueled by direct dietary input. During winter, consumption of protozooplankton allows the individuals to maintain a rather high metabolic activity (Zamora-Terol *et al.*, 2013).

Most investigations on lipid metabolism have been carried out on primary producers and biomass-dominating zooplankton organisms such as the large Arctic *Calanus* species (Turner, 2004; Graeve *et al.*, 2005). In contrast, few studies exist on the lipid biochemistry of small copepods such as *P. minutus* and *O. similis* (Fraser *et al.*, 1989; Narcy *et al.*, 2009). Lischka and Hagen (2007) monitored *in situ* FA and fatty alcohol compositions of both species over the course of a year, but no study focused on the turnover of lipid carbon. As reported for the Southern Ocean, the annual production of small copepods seems to be higher than that of the biomass-dominant copepod species (Fransz and Gonzalez, 1995; Metz, 1995). Since the role of small copepods in lipid carbon turnover in the Arctic remains largely unknown, food web models may underestimate their contribution to carbon flux. It is therefore of great interest to understand the significance of these smaller copepod species and their ecophysiological capacities with regard to their lipid synthesis and energy transfer to higher trophic levels. This will allow a better perception of ecosystem dynamics and improve modeling approaches in the light of climate change, especially in the rapidly changing Arctic. Since the different life-cycle strategies of both *P. glacialis* and *O. similis* are likely to influence their carbon assimilation capacities, this study aims at elucidating the abilities of these small-sized copepods to channel lipid carbon from their food. Until recently, ^{14}C was used to label food when monitoring the lipid biosynthesis of various zooplankton species (Sargent and Lee, 1975; Dall *et al.*, 1993; Cowie and Hedges, 1996). In this study, a ^{13}C labeled diatom-flagellate mix was fed to the copepods during 3 weeks to follow carbon FA assimilation and possible *de novo* synthesis of FAs and alcohols.

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METHOD

Sampling

Copepods were collected in Billefjorden (78°66 N, 16°74 E), an Arctic sill fjord, part of the larger Isfjorden, on the west coast of Spitsbergen (Norway). The fjord consists of two basins. An 80 m deep sill separates the outer basin of Billefjorden from Isfjorden. The maximum depth of this basin is around 230 m. The inner basin, separated by a 45 m deep sill from the outer basin, has a maximum depth of 190 m (Arnkvaern *et al.*, 2005; Nilsen *et al.*, 2008).

The two copepod species *P. minutus* and *O. similis* were sampled in the inner basin on board of a small boat from UNIS (University Centre in Svalbard) on 29 July 2014. Animals were collected by vertical hauls from 100 to 0 m using WP2 nets (200 μm mesh size, 0.25 m^2 net opening). Live specimens were transferred to containers filled with filtered seawater (at 4°C) and brought back to UNIS laboratory within 5 hours for subsequent experiments and analyses. They were kept in thermos boxes at close to *in situ* temperatures during transportation to the laboratory.

Experimental set-up

Immediately after arrival at UNIS, living and healthy-looking *P. minutus* and *O. similis* were sorted under a dissecting microscope. Identification of species and stages was based on morphology and size criteria (Lischka and Hagen, 2005). The most dominant stage of each species was chosen for the experiment, i.e. females of *O. similis* and copepodite stage V (CV) of *P. minutus*. The copepods were immediately transferred in groups of 50 *O. similis* females together with 25 *P. minutus* CV in 15 glass bottles containing 1 L of 0.7 μm filtered seawater (three replicates for each of the five sampling dates) and kept at 4°C. In addition, three *in situ* replicates (t_0) with the same number of copepods were immediately frozen at −80°C (without any preservative).

During the 21 days experiment, the copepods were fed with a mixture of diatoms (*Chaetoceros debilis* and *Contricribra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*) at concentrations ≫ 1000 cells mL^{-1} (≫ 20 μg Chl *a* L^{-1}). Algal cells were counted with a haemacytometer (Schoen, 1988) and Chl *a* concentrations were measured by fluorometry, using methanol as the extracting solvent (Holm-Hansen and Riemann, 1978). The copepods were fed with the same cell concentrations of flagellates and diatoms, which exceeded those of a typical spring bloom (Sakshaug *et al.*, 2009), ensuring surplus feeding conditions despite high numbers of copepods per bottle. Algae were cultured in 0.7 μm filtered seawater with f/2 Guillard medium (15 mL L^{-1}) and labeled with ^{13}C sodium bicarbonate (15 mg L^{-1}). Extra silicate was added to the medium for diatoms (2 mL L^{-1}) to ensure optimal growth. *C. debilis and R. salina* were kept at 4°C, *C. weissflogii* and *D. salina* at 15°C for faster growth than at 4°C. All cultures were grown at a 12 h light:12 h dark cycle. Samples for lipid analyses were taken by filtrating 5 mL duplicates of each algal monoculture on filters (0.7 μm pore diameter), at each feeding date.

The copepods were kept under continuous light to imitate ambient conditions, i.e. polar day. Light intensity was around 50 μmol m^{-2} s^{-1} (incident radiation measured with surface reference sensor in air (LI-190, LI-COR). Two-thirds of the water in all bottles was changed every 3–4 days in order to feed the copepods and maintain high oxygen concentrations. Simultaneously (from Day 6 on), three of the bottles were emptied completely and the animals counted and deep-frozen in triplicates at −80°C for subsequent lipid analyses. Mortality rates of *P. minutus* were constant over time, at 1.3 ± 0.8% day^{-1}. Mortality rates of *O. similis* were highest during the first 9 days (3.1 ± 0.7% day^{-1}) and low thereafter until termination of the experiment (0.9 ± 0.5% day^{-1}). Overall, the average mortality of *O. similis* was 3.0 ± 0.8% day^{-1}. Samples for lipid analyses contained between 14 and 25 specimens of *P. minutus* and between 11 and 50 specimens of *O. similis*. Despite the small size of the individuals, their number in each sample was sufficient for lipid analyses, providing a lipid content that was detectable with the methods we used.

Analyses of FAs and fatty alcohols

Total lipid was extracted by homogenizing animal tissues and filters in a solution of dichloromethane : methanol (2:1,v:v), modified after Folch *et al.* (1957). As internal standard, a known amount of the tricosanoic acid methyl ester (23:0) was added to each sample. A 0.88% solution of KCl (potassium chloride) was added to easily differentiate the biphasic system. Trans esterification of the lipid extracts was performed by heating the samples with 3% sulfuric acid H_2SO_4 in methanol for 4 h at 80°C under nitrogen atmosphere. The fatty acid methyl esters (FAME) were extracted with cyclohexane. FAME and fatty alcohols were determined using a gas chromatograph (HP 6890 N, Agilent Technologies Deutschland GmbH & Co. KG) equipped with a 30 m × 0.25 mm i.d. wall-coated open tubular capillary column (film thickness: 0.25 μm; liquid phase: DB-FFAP), a split/splitless injector (250°C) and a flame ionization detector (280°C),

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according to the method of Kattner and Fricke (1986). The oven program was set from 60 to 160°C with a rate of 30°C min^{-1}, reaching a final temperature of 240°C at 1.5°C min^{-1}. Helium 5.0 was used as carrier gas at a flow rate of 1.0 mL min^{-1}. To identify unknown peaks, additional GC-mass spectrometry runs were carried out. The chromatograms were evaluated using the ChemStation software from Agilent. Total lipid mass per individual was calculated by summing up FA and fatty alcohol masses. The percentage of wax esters in total lipid was calculated from the proportion of alcohols on a mole basis, assuming that copepods contain no free fatty alcohols (Kattner and Krause, 1989).

Carbon isotopic ratios

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

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

Isotopic ratios of each FA and fatty alcohol are normally expressed in δ notation according to the formula (1):

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

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

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

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

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

To only consider the enrichment that resulted from the assimilation of labeled food, the atom percent excess (ATE) was calculated according to (3):

$$\mathrm{ATE}(\%) = \mathrm{AT}_{t=i} - \mathrm{AT}_{t=0} \qquad (3)$$

where $t = i$ is the number of days since the beginning of the ^{13}C feeding experiment and $t = 0$, the starting day of the experiment. $AT_{t=0}$ is therefore an average of the AT in all FAs and alcohols *in situ*.

Since the dietary FAs did not only contain ^{13}C but also ^{12}C, to calculate the proportion of carbon assimilated in the copepods FAs and alcohols (PA), the ATE was divided by the total average labeling in algal FAs, L (atom%), which was constant during the experiment ($L = 15.28\%$) (4):

$$\mathrm{PA} = \frac{\mathrm{ATE}}{L} \qquad (4)$$

To estimate the assimilation of carbon (C_{assi}) as mass (µg C_{assi} ind^{-1}) (5), the proportion of carbon assimilated (PA) was multiplied by the mass of each FA and alcohol (B) expressed as carbon mass (in µg C ind^{-1}). The carbon mass was derived from the number of moles of FAs and alcohols in the copepods. The molecular mass of each labeled FA and alcohol was calibrated by its carbon atom percentage to incorporate the carbon mass variation according to the $^{13}C/^{12}C$ ratio (De Troch *et al.*, 2012):

$$C_{assi}\,(\mu g\ C\ ind^{-1}) = B \times PA = B \times \frac{\mathrm{ATE}}{L} \qquad (5)$$

Statistics

Statistical analyses were performed using the free software R 3.2.1 (team RDC, 2010). Normal distribution

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was tested with Shapiro–Wilk test. One-way ANOVA followed by Tukey HSD tests were performed on polynomial regressions (degree 2). FA and alcohol proportions were arcsin transformed prior to tests. The significance level was set at 5% ($\alpha = 0.05$). Results were referred to as statistically significant and the null hypothesis was rejected if the *P*-value was lower than the α-level.

RESULTS

FA compositions and labeling of algal cultures

Major FAs in the algal food were 16:0 (13.7%), 20:5(n-3) (21.1%) and 22:6(n-3) (10.3%), reflecting a contribution of diatoms and flagellates to these FA masses at a ratio of ~70 vs. 30%, respectively (Table I). In addition, the monounsaturated 16:1(n-7) (16.2%) and the polyunsaturated (PUFA) 16:2(n-4) (3.6%) and 16:3(n-4) (7.2%) were almost exclusively provided by diatoms (>99% of FA mass). The PUFA 18:3(n-3) (7.5%) and 18:4(n-3) (7.5%) were largely synthesized by flagellates (>80.7% of FA mass), while 18:1(n-9) (1.8%) and 18:2(n-6) (1.3%) were exclusively found in flagellates. On average, 5533 µg C lipid L^{-1} was made available to the copepods at each feeding event.

The uptake of ^{13}C in the algae was rapid and averaged 15.3 ± 0.9 atom% after 5 days. It remained stable throughout the entire copepod feeding experiments ($P > 0.05$). Maximum enrichment occurred in 16:3(n-3) with 23.3 atom%, the minimum was detected in 18:0, with 2.3 atom% labeled.

Carbon uptake by *P. minutus*

Total lipid mass of *P. minutus* CV did not vary significantly over time ($P > 0.05$), averaging 2.8 ± 0.6 µg C ind^{-1}.

Carbon assimilation in total lipid occurred throughout the experiment, and the amount of assimilated carbon reached 1.4 ± 0.1 µg C_{assi} ind^{-1}, which represented 54.6 ± 0.1% of the total lipid carbon, when the experiment was terminated. Thus, the assimilation rate of labeled carbon into copepod total lipid carbon was 2.6% day^{-1}.

Major FAs of *P. minutus* were 16:0, 16:1(n-7), 18:0, 18:1(n-9), 18:3(n-3), 20:5(n-3) and 22:6(n-3), together contributing 74% of the FAs (Table II). Most FAs had stable masses during the experiment (Supplementary Table SII). Only the relative and absolute concentrations of 16:1(n-7) and 16:3(n-4) increased significantly ($P < 0.01$). Main fatty alcohols were 14:0 and 16:0 (69%

*Table I: Absolute and relative FA compositions of the algae cultures used as food for the copepods. Contribution of diatoms (*Chaetoceros debilis *and* Contricribra weissflogii*) and flagellates (*Rhodomonas salina *and* Dunaliella salina*) to the overall FA compositions. Labeling expressed as atom% (AT%) during the experimental time. The presented values are calculated means ± standard deviation from single algae cultures (*n = *8)*

			Contributions to FA (%)		
	Mass (µg C L^{-1})	Relative mass (%TL)	Diatoms	Flagellates	AT%
FA					
14:0	165.8 ± 15.6	3.0 ± 5.1	54.7	45.3	13.4 ± 4.1
15:0	24.8 ± 17.6	0.4 ± 0.4	100.0	0.0	16.6 ± 1.1
16:0	757.2 ± 313.3	13.7 ± 4.7	73.6	26.4	13.5 ± 4.8
16:1(n-7)	896.4 ± 621.3	16.2 ± 10.7	99.0	1.0	14.3 ± 3.2
16:2(n-4)	198.7 ± 140.5	3.6 ± 7.9	100.0	0.0	16.9 ± 2.6
16:3(n-3)	1.1 ± 0.8	0.1 ± 0.3	0.0	100.0	23.3 ± 2.2
16:3(n-4)	399.4 ± 282.4	7.2 ± 5.0	100.0	0.0	17.1 ± 3.0
16:4(n-1)	54.5 ± 33.4	1.0 ± 6.5	93.4	6.6	13.5 ± 0.4
16:4(n-3)	8.1 ± 5.7	0.1 ± 1.8	0.0	100.0	23.0 ± 1.3
18:0	185.9 ± 52.8	3.4 ± 2.3	64.6	35.4	2.3 ± 0.8
18:1(n-7)	58.9 ± 41.7	1.1 ± 1.4	0.0	100.0	13.8 ± 6.3
18:1(n-9)	100.6 ± 71.1	1.8 ± 2.4	0.0	100.0	13.1 ± 6.2
18:2(n-6)	73.6 ± 52.0	1.3 ± 2.5	0.0	100.0	13.4 ± 6.3
18:3(n-3)	416.3 ± 182.7	7.5 ± 17.8	12.3	87.7	16.5 ± 6.6
18:3(n-6)	4.0 ± 2.8	0.1 ± 0.9	0.0	100.0	22.9 ± 1.8
18:4(n-3)	413.5 ± 277.7	7.5 ± 12.3	1.5	98.5	10.9 ± 4.2
18:5(n-3)	23.7 ± 16.7	0.4 ± 0.9	0.0	100.0	10.3 ± 4.1
20:5(n-3)	1167.4 ± 504.5	21.1 ± 10.0	74.6	25.4	14.4 ± 4.5
22:6(n-3)	571.0 ± 190.3	10.3 ± 5.4	63.6	36.4	14.5 ± 6.4

AT%: ^{13}C atom percent; FA, fatty acid; TL, total lipid.

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Table II: P. minutus *and* O. similis. *Changes in FA and alcohol compositions (mean of mass% ± standard deviation of total FAs and fatty alcohols, respectively) and in wax ester content (% of total lipid) at the beginning (t = 0 d) and at the end (t = 21 d) of the feeding experiment (n = 3 each)*

	Relative mass (% FA and % FAlc)			
	P. minutus		*O. similis*	
	t = 0	*t* = 21	*t* = 0	*t* = 21
FA				
14:0	1.3 ± 0.3	1.2 ± 0.1	2.6 ± 0.6	2.3 ± 0.7
15:0	0.9 ± 1.5	–	1.1 ± 0.2	–
16:0	6.7 ± 2.2	8.9 ± 4.2	20.5 ± 1.7	18.0 ± 0.9
16:1(n-5)	–	–	0.5 ± 0.4	0.6 ± 0.5
16:1(n-7)	5.7 ± 1.4	12.3 ± 1.1	1.8 ± 0.7	0.9 ± 0.2
16:2(n-4)	3.4 ± 2.4	1.8 ± 0.2	–	–
16:3(n-4)	0.4 ± 0.4	3.8 ± 0.8	0.2 ± 0.3	–
16:4(n-1)	2.8 ± 3.9	0.9 ± 0.6	–	–
17:0	0.5 ± 0.9	–	1.0 ± 0.1	0.5 ± 0.4
18:0	7.1 ± 1.8	9.2 ± 1.2	28.3 ± 15.2	33.7 ± 14.3
18:1(n-5)	0.6 ± 0.0	0.8 ± 0.1	0.2 ± 0.4	0.6 ± 0.5
18:1(n-7)	1.3 ± 0.5	1.8 ± 0.1	0.9 ± 0.8	1.5 ± 1.3
18:1(n-9)	25.1 ± 11.6	24.5 ± 3.1	14.1 ± 3.7	12.7 ± 4.7
18:2(n-6)	5.0 ± 1.8	3.6 ± 1.9	3.3 ± 1.6	2.7 ± 2.1
18:3(n-3)	7.7 ± 1.2	3.1 ± 1.8	0.8 ± 0.8	0.6 ± 0.5
18:3(n-6)	0.5 ± 0.9	–	–	–
18:4(n-3)	1.5 ± 0.8	3.0 ± 1.7	0.5 ± 0.5	0.8 ± 0.1
20:0	1.1 ± 0.6	0.6 ± 0.7	0.5 ± 0.4	0.6 ± 0.5
20:1(n-7)	0.6 ± 0.8	–	–	–
20:1(n-9)	0.7 ± 1.0	0.4 ± 0.4	0.8 ± 0.7	3.2 ± 1.9
20:1(n-11)	0.4 ± 0.4	0.2 ± 0.3	0.2 ± 0.4	0.2 ± 0.4
20:3(n-6)	1.8 ± 3.2	–	–	–
20:4(n-3)	3.9 ± 1.2	1.7 ± 0.2	1.3 ± 0.4	0.9 ± 0.8
20:4(n-6)	0.5 ± 0.2	–	–	–
20:5(n-3)	10.3 ± 3.3	12.5 ± 0.3	6.7 ± 1.4	6.0 ± 2.3
22:1(n-7)	0.3 ± 0.6	–	–	0.2 ± 0.4
22:1(n-9)	0.3 ± 0.4	0.4 ± 0.4	0.2 ± 0.3	1.2 ± 0.4
22:1(n-11)	0.9 ± 1.1	–	–	0.2 ± 0.4
22:5(n-3)	0.7 ± 0.3	0.7 ± 0.1	1.0 ± 1.0	1.7 ± 0.7
22:6(n-3)	11.3 ± 3.1	7.9 ± 1.0	11.8 ± 2.4	8.4 ± 3.0
FAlc				
14:0	21.3 ± 7.1	51.7 ± 30.1	16.4 ± 2.6	9.3 ± 9.1
16:0	48.0 ± 5.9	63.4 ± 16.3	57.8 ± 11.0	36.2 ± 17.7
18:1(n-7)	2.5 ± 1.6	1.8 ± 2.0	–	–
18:1(n-9)	19.5 ± 2.8	8.8 ± 4.6	11.3 ± 1.3	4.8 ± 4.6
20:1 (both isomers)	6.9 ± 10.0	0.9 ± 1.0	14.5 ± 13.6	49.7 ± 30.7
22:1 (both isomers)	1.9 ± 1.2	0.8 ± 0.8	–	–
WE (% TL)	61.3 ± 3.5	57.9 ± 19.2	27.3 ± 7.7	32.6 ± 15.4

FA, fatty acid; FAlc, fatty alcohol; WE, wax ester; –, below detection limit.

of the fatty alcohols). All fatty alcohols showed stable portions during the course of the experiment ($P > 0.05$). The wax ester content was also stable with an average of 60%.

The assimilation of FAs and alcohols, expressed as µg C_{assi} per individual, is presented in Table III. Maximum

Table III: P. minutus *and* O. similis. *Carbon assimilation into main copepod FAs and alcohols (mean ± standard deviation expressed as 10^{-3} µg C_{assi} ind-^{1}) at the end of the feeding experiment.*

	P. minutus		*O. similis*	
	n	C assi (10^{-3} µg C ind^{-1})	*n*	C assi (10^{-3} µg C ind^{-1})
FA				
16:0	3	71.1 ± 33.9	3	0.5 ± 0.4
16:1(n-7)	3	202.4 ± 50.7	1	0.2
16:2(n-4)	3	34.9 ± 7.7		–
16:3(n-4)	3	81.1 ± 24.3		–
18:0	3	6.9 ± 2.2	3	0.5 ± 0.4
18:1(n-9)	3	242.8 ± 84.3	3	1.6 ± 1.8
18:2(n-6)	3	5.2 ± 1.2	3	0.1 ± 0.1
18:3(n-3)	3	14.2 ± 9.2	1	0.4
18:4(n-3)	3	12.9 ± 9.0	2	0.2 ± 0.1
20:5(n-3)	3	180.1 ± 38.5	3	3.2 ± 2.3
22:6(n-3)	3	70.3 ± 9.9	3	1.6 ± 1.2
FAlc				
14:0	3	190.4 ± 75.8	1	0.3
16:0	3	248.9 ± 129.4	2	1.1 ± 0.7
18:1(n-7)	1	8.4	–	–
20:1 (both isomers)	2	9.8 ± 5.0	2	0.1 ± 0.0

FA, fatty acid; FAlc, fatty alcohol; –, below detection limit.

carbon assimilation was determined in 18:1(n-9) with $242.8 \times 10^{-3} \pm 84.3 \times 10^{-3}$ µg C_{assi} ind^{-1} after 21 days ($P < 0.001$). 16:1(n-7) and 20:5(n-3) had the second highest carbon assimilation, with $202.4 \times 10^{-3} \pm 50.7 \times 10^{-3}$ µg C_{assi} ind^{-1} and $180.1 \times 10^{-3} \pm 38.5 \times 10^{-3}$ µg C_{assi} ind^{-1}, respectively ($P < 0.001$). In fatty alcohols, maximum carbon assimilation was determined in 16:0, with $248.9 \times 10^{-3} \pm 129.4 \times 10^{-3}$ µg C_{assi} ind^{-1} ($P < 0.01$), followed by 14:0 with $190.4 \times 10^{-3} \pm 75.8 \times 10^{-3}$ µg C_{assi} ind^{-1} ($P < 0.01$). Main FAs 16:0, 18:0, 20:5(n-3) and 22:6(n-3) were assimilated at a linear rate (Fig. 1). By Day 21, around 75.6 ± 6.9% of carbon was renewed in 20:5(n-3), and this exchange reached 45.7 ± 4.7% in 16:0 and 46.9 ± 6.9% in 22:6(n-3) ($P < 0.001$). In 18:0, 4.2 ± 1.2% of carbon was assimilated ($P < 0.05$). Diatom FATM were assimilated at a very high rate (Fig. 2), as after 21 days almost all carbon was renewed in 16:1(n-7), 16:2(n-4) and 16:3(n-4) ($P < 0.001$). Flagellate markers did not show such intense carbon turnover rates, despite a linear increase ($P < 0.01$). At the end of the feeding experiment 54.3 ± 14.9% were assimilated in 18:1(n-9), and in the C_{18} PUFA assimilation ranged between 9.5 ± 4.5% and 22.9 ± 3.4%. In the fatty alcohols (Fig. 1), a linear carbon exchange occurred, reaching between 62.6 ± 11.7% and 76.7 ± 9.6% after 21 days ($P < 0.001$).

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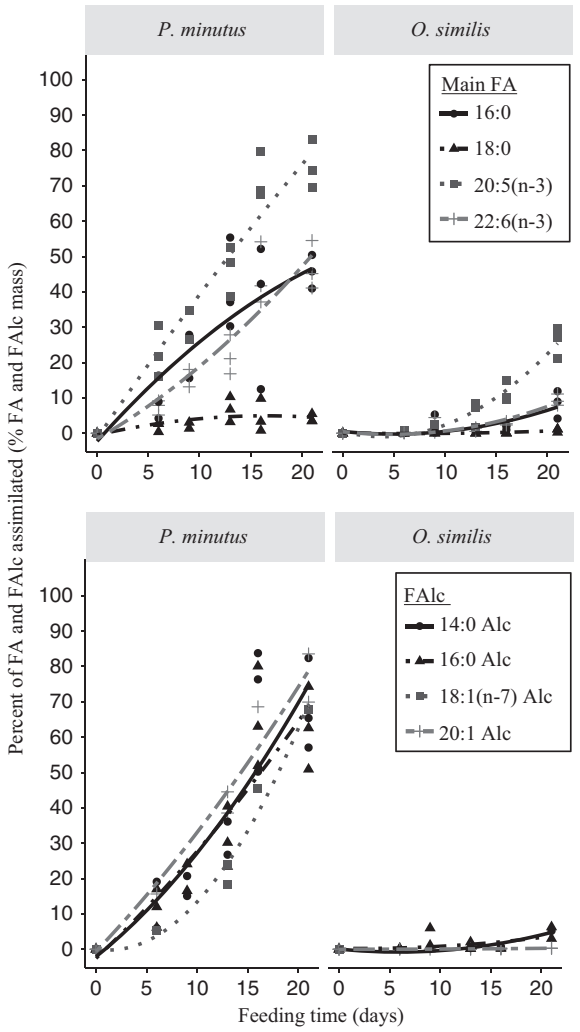

Fig. 1. *P. minutus* and *O. similis*. Proportion of carbon assimilated into main FAs and alcohols (expressed as % of FA and alcohol carbon mass) during the experiment (21 days of feeding).

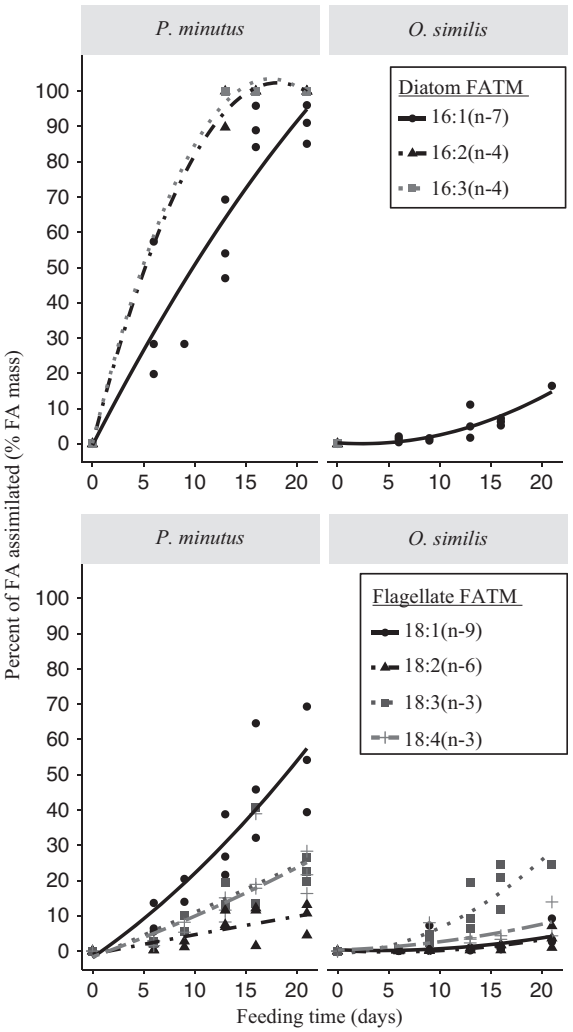

Fig. 2. *P. minutus* and *O. similis*. Proportion of carbon assimilated into FATM (expressed as % of FA carbon mass) during the experiment (21 days of feeding).

Carbon uptake by *O. similis*

The total lipid content of *O. similis* females increased from $0.8 \times 10^{-1} \pm 0.03 \times 10^{-1}$ to $2.2 \times 10^{-1} \pm 0.1 \times 10^{-1}$ µg C ind^{-1} ($P < 0.01$). By Day 21, the amount of assimilated total lipid carbon, calculated from the labeling results, reached $0.2 \times 10^{-1} \pm 0.1 \times 10^{-1}$ µg C ind^{-1} ($P < 0.01$), which corresponded to 9.4 ± 0.2% of the total lipid mass. Overall, the daily carbon turnover rate of total lipid was 0.9×10^{-3} µg C day^{-1}, that is 0.5% of total lipid per day.

Major FAs of *O. similis* comprised 16:0, 18:0, 18:1(n-9) and 22:6(n-3), together contributing 80% to the FA mass (Table II). Most FA masses were constant during the experiment ($P > 0.5$) (Table IIs), except for 18:0 and 18:4(n-3), which increased in absolute but not in relative masses ($P < 0.01$). Dominant fatty alcohols were 14:0, 16:0 and 20:1 (both isomers), corresponding to 92% of the fatty alcohols. All fatty alcohols had constant masses over time ($P > 0.05$), together with a stable wax ester content (average of 30%).

The assimilation of carbon in FAs is presented in Table III. Maximum values occurred in 20:5(n-3) with $3.2 \times 10^{-3} \pm 2.3 \times 10^{-3}$ µg C_{assi} ind^{-1} ($P < 0.01$). It was followed by 18:1(n-9) ($1.6 \times 10^{-3} \pm 1.5 \times 10^{-3}$) and 22:6(n-3) ($1.6 \times 10^{-3} \pm 1.2 \times 10^{-3}$ µg C_{assi} ind^{-1}). Fatty alcohol assimilation was in the same range compared to that of the FAs and the increase of assimilated carbon

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was significant ($P < 0.05$). At the end of the experiment, $1.1 \times 10^{-3} \pm 0.7 \times 10^{-3}$ µg C_{assi} ind^{-1} were assimilated in 16:0 and 0.3×10^{-3} µg C_{assi} ind^{-1} ($n = 1$) in 14:0. Initial assimilation in FAs was not yet detected at Day 6 (Fig. 1). By Day 21, around 25.9 ± 4.5% of carbon was assimilated in 20:5(n-3). The portion of assimilated carbon reached 8.3 ± 3.9% in 16:0 and 9.3 ± 1.6% in 22:6(n-3) ($P < 0.01$). In 18:0, only negligible amounts of carbon were assimilated (0.8 ± 0.5%, $P > 0.05$). The portion of carbon assimilated into the diatom FATM 16:1(n-7) reached 16.6% ($n = 1$) after 21 days ($P < 0.001$) (Fig. 2), whereas the other diatom FATM 16:2 (n-4) and 16:3(n-4) had masses below the detection limit. Regarding the flagellate FATM at the end of the feeding experiment, 24.6% ($n = 1$) were assimilated in 18:3 (n-3) and 9.1 ± 6.8% in 18:4(n-3). In 18:1(n-9), only 5.0 ± 3.7% were assimilated, and 3.9 ± 3.1% in 18:2(n-6). Carbon exchange in the fatty alcohols was very low (Fig. 1), reaching only 5.2% ($n = 1$) in 14:0 and 4.7 ± 2.3% in 16:0 ($P < 0.05$).

DISCUSSION

To study carbon transfer in lipid-driven food chains, Lee *et al.* (1971b) introduced the concept of FAs as trophic biomarkers being transferred from phytoplankton origin to higher trophic levels. Investigations using gas chromatography allow changes in the FA masses to be quantified. This analytical approach can be applied in either long-term dietary studies or feeding experiments with animals that rapidly accumulate extensive amounts of lipids, such as the large *Calanus* species (Graeve *et al.*, 2005). In the present study on smaller copepod species, processes of lipid carbon exchange did not involve major mass changes and were therefore not elucidated by the above method alone. To overcome this limitation, the food items were labeled with ^{13}C and their transfer into copepods was followed by compound specific isotope analysis (CSIA). This method was introduced to marine science in the early 1990s (Meier-Augenstein, 1999; Boschker and Middelburg, 2002). Combined with experiments using labeled food, e.g. phytoplankton, this approach allows tracking the dietary carbon assimilation in specific compounds, e.g. proteins or lipids (Graeve *et al.*, 2005). Such biochemical processes and pathways can only be revealed by experiments with labeled material.

The two copepod species were offered the same phytoplankton food, supplied in excess to ensure that the copepods were not food-limited (Sakshaug *et al.*, 2009). Since the copepods were fed a mixed diet only, food selectivity could not be studied in detail. Even though diatoms and flagellates were offered in similar concentrations, the copepods could have eaten the two taxa in different proportions. A higher assimilation rate of a specific FATM could therefore be the result of preferred ingestion and/or more efficient assimilation.

In the algal cultures 16:1(n-7) and C_{16} PUFA were mainly synthesized by the diatoms, whereas flagellates accumulated high amounts of C_{18} PUFA, which is in agreement with previous studies (Ackman *et al.*, 1968; Graeve *et al.*, 1994a; Falk-Petersen *et al.*, 1998). The monounsaturated 18:1(n-9), a major product of the FA biosynthesis, was an important component in flagellates and hence referred to as a flagellate marker in this study. At higher trophic levels, this FA is usually considered as a marker for carnivory, since it is a major FA of most marine animal lipids (Falk-Petersen *et al.*, 1990). All algal FAs were sufficiently labeled (15 atom%). Only 18:0 showed very low concentrations of ^{13}C label (two atom%) in all algal cultures and throughout the experiment, resulting in an underestimation of the uptake of this FA by the copepods. Low labeling of 18:0 has previously been observed in other studies (B. Lebreton, La Rochelle, personal communication), and may be due to physiological processes, possibly related to very high turnover rates. This may result in 18:0 being rapidly desaturated or elongated and therefore not stored unchanged (Li *et al.*, 2014).

Calculations of carbon assimilation were based on the average atom percentage in algal FAs, since even though one dietary FA in the copepods mainly originates from the same algal compound, smaller amounts may be elongated and/or desaturated from other FAs (Dalsgaard *et al.*, 2003). The CSIA method does not provide information about the exact biosynthetic pathway of each FA. Hence, depending on the respective pathway, the assimilation of some FAs may have been slightly over- or underestimated in *P. minutus* and *O. similis*.

Turnover rates of total lipid

After 6 days of feeding, assimilation of labeled lipids was already detected in *P. minutus* but not yet in *O. similis*. The uptake of label by *O. similis* in the initial phase (until Day 9) was probably below the detection limit of the GC-IRMS. The fact that mortality, probably caused by handling, was high during the first 9 days for *O. similis* may also explain the low assimilation in the beginning. Another explanation of the low initial lipid assimilation may be that *O. similis* needed some time to adapt to new food conditions.

At the end of the feeding experiment (21 days), more than half of the total lipid carbon in *P. minutus* was

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derived from ingested algal carbon, whereas only 10% of total lipid carbon was assimilated by *O. similis*. Overall, the daily rate of carbon assimilation was five times higher in *P. minutus* than in *O similis*.

The lipid carbon assimilation rate of *P. minutus* revealed by this study (2.6% day^{-1}) is comparable with those of *Calanus* species. *Calanus glacialis*, the main grazer in Arctic shelf seas, has a daily assimilation rate of 3.0%, compared to a rate of 2.7% day^{-1} for *C. finmarchicus* (Graeve *et al.*, 2005). This pronounced efficiency in lipid assimilation seems to be a typical adaptive mechanism of herbivorous species in high-latitude environments to make full use of the productive season. In winter, when phytoplankton food becomes scarce, the large *Calanus* species hibernate at depth in a resting stage (diapause) with very limited utilization of their lipid reserves, as these are needed to fuel reproductive processes in early spring (Sargent and Falk-Petersen, 1988; Hagen, 1999; Hagen and Auel, 2001; Lee *et al.*, 2006). In contrast, *P. minutus* do not overwinter in a true diapause. Thus, they rely partially on their lipid deposits for metabolic maintenance that they supplement by opportunistic feeding (Kwaniewski, 1990; Lischka *et al.*, 2007). Considering this more flexible feeding mode, their efficiency to assimilate dietary lipids is quite high and suggests that lipid reserves play an important role in their life strategy.

The slow turnover rate of total lipid carbon in *O. similis* (0.5% day^{-1}) may be explained by their omnivorous feeding mode. The species maintains its metabolic activity throughout the year, feeding on a wide variety of organisms from small flagellates to copepod nauplii and faecal pellets (Franz, 1988; Kattner *et al.*, 2003; Lischka and Hagen, 2007). Hence, extensive lipid accumulation does not play a crucial role in their life-cycle strategy and therefore, a high turnover efficiency of lipid reserves is probably not of major importance. Assuming that *O. similis* accumulates limited stores of wax esters and triacylglycerols, the turnover rate of 0.5% day^{-1} may approximately correspond to the turnover of polar lipids. However, the increase of total lipid mass in *O. similis* was surprisingly high, considering the limited assimilation of FAs, alcohols and therefore total lipid. This may be due to a natural high variability in the lipid content among these copepods, as indicated by the high standard deviation of the FA contents. June and August/September are considered the two main reproduction periods for *O. similis* in Svalbard (Lischka and Hagen, 2005). Narcy *et al.* (2009) did an individual-based lipid study on *O. similis* in Kongsfjorden and found huge individual differences in lipid contents among the females in September. The high variability may be associated with the overlap of two cohorts: newly molted and lipid-rich females that did not start to reproduce yet and the older less lipid-rich females that are actively reproducing and thus invest energy in eggs. The apparent lipid increase in our study could therefore potentially be caused by a non-homogeneous division of the two cohorts of *O. similis* in the experimental bottles.

Assimilation of dietary FAs

This study showed that consistent amounts of carbon were assimilated in the copepods' main FAs, with *O. similis* exhibiting a much less intense assimilation than *P. minutus*. In both species, carbon turnover was substantial in 16:0, 20:5(n-3) and 22:6(n-3). Less carbon exchange was detected in 18:0, but this was probably an underestimation due to poor labeling in the algae. The FAs 16:0, 18:0, 20:5(n-3) and 22:6(n-3) are major elements of phospholipids, which are structural components of all biomembranes and essential for copepods (Lee *et al.*, 1971b; Albers *et al.*, 1996). Rapid assimilation of these FAs is not surprising, as phospholipid turnover occurs at a high rate in most animal cells, almost half of them is exchanged every one or two cell divisions (Van den Bosch, 1980; Dawidowicz, 1987). Earlier studies suggested that this intense turnover is related to the maintenance of cellular viability (Dawson ,1973).

A comparison of carbon assimilation in FATM between the two copepod species revealed clear differences in their lipid biosynthetic capacities. *Pseudocalanus minutus* assimilated diatom FATM at a much higher rate than flagellate FATM. At the end of the experiment, the entire original carbon pool in 16:1(n-7) and C_{16} PUFA was replaced by dietary carbon from the experiment. Two processes may explain these differences. Firstly, *P. minutus* is a selective particle filter feeder (as many copepods) and seems to prefer diatoms over flagellates, due to, for example, size range selection (Poulet, 1974). Secondly, *P. minutus* fed equally on both sources, but FA assimilation was more efficient from diatoms than from flagellates. It is suggested that *P. minutus* is particularly adapted to feed efficiently on diatoms and to accumulate energy reserves from them (Norrbin *et al.*, 1991). On the contrary, *O. similis* in this study appeared to be equally efficient in ingesting and/or assimilating FAs from diatoms and flagellates, but generally at a much lower rate than *P. minutus*. The feeding preferences of *O. similis* are still under discussion. Some studies indicate that *O. similis* prefers motile prey (Drits and Semenova, 1984; Svensen and Kiørboe, 2000) while other studies suggest that *O. similis* could feed on diatoms (Kattner *et al.*, 2003; Lischka and Hagen, 2007). The experimental design of our study does not allow distinguishing between the two possibilities, and we therefore

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encourage additional studies, maybe via high-speed cinematography, to solve this aspect of selective feeding versus preferential accumulation of specific FAs.

Wax ester biosynthesis

It is well established that wax ester biosynthesis in copepod species usually relies on dietary input for the FA moieties, whereas the fatty alcohols are biosynthesized *de novo* (Sargent *et al.*, 1977; Lee *et al.*, 2006). In *P. minutus*, more than half of the lipids were deposited as wax esters (59%), which is consistent with the observations of Lischka and Hagen (2007). Unlike *O. similis*, *P. minutus* synthesized large amounts of fatty alcohols (respectively wax esters). *P. minutus* uses wax esters mainly to support winter survival, which contrasts with other herbivorous copepods that use their wax ester deposits primarily for reproductive processes (Scott *et al.*, 2000; Lee *et al.*, 2006). *P. minutus* is known to synthesize shorter-chain fatty alcohols (Fraser *et al.* 1989; Lischka and Hagen 2007). This was reflected in high portions of renewed carbon (63%) in 16:0 and 18:1(n-7) alcohols. However, 20:1 fatty alcohols were also assimilated. It has been suggested that small copepod species are not able to produce these long-chain FAs *de novo* (Kattner *et al.*, 2003). Their presence in small copepods would therefore originate from potential feeding on *Calanus* or fish faecal pellets that contain high amounts of long-chain monounsaturated fatty alcohols (Prahl *et al.*, 1984; 1985; Harvey *et al.*, 1987; Lischka and Hagen, 2007). As our experiment was only based on algal food, *P. minutus* must be able to biosynthesize these fatty alcohols *de novo* or via elongation of shorter-chain saturated precursors (e.g. 14:0 and 16:0).

In *O. similis*, the very low assimilation of labeled carbon into fatty alcohols combined with a steady lipid carbon mass confirms that the fatty alcohols (respectively wax esters) were not used for metabolism nor replaced by dietary input. Apart from buoyancy aspects, utilization of wax esters may be an additional energetic strategy for *O. similis* to buffer the poor food supply in winter and to fuel reproductive processes (Lischka and Hagen, 2007). In our study, the lipids of *O. similis* comprised on average 30% of wax esters (max. 59%), which is a rather high portion for a non-diapausing omnivorous species (Graeve *et al.*, 1994a). In the Southern Ocean, *O. similis* females accumulated on average 15% wax esters (max. 23%) in autumn (Kattner *et al.*, 2003), while in Kongsfjorden females had an average of 18% (max. 37%) in September (Narcy *et al.*, 2009). Only a few of the *O. similis* females used for our experiment were carrying eggs, suggesting that reproduction was about to happen (Lauris Boissonnot, Longyearbyen, personal observation). Therefore, the low fatty alcohol biosynthesis may be explained by the fact that wax esters had already reached their maximum levels (Lischka and Hagen, 2007). This energy may, at least partially, be required for the reproduction peak in August/September.

CONCLUSIONS

The contrasting life strategies of *P. minutus* and *O. similis* are clearly reflected by differences in their capacities to ingest and/or assimilate lipids from dietary sources. *Pseudocalanus minutus* is a herbivorous species that relies on lipid depots, possibly to fuel its metabolism during overwintering. The species exhibited a very high lipid turnover rate, comparable to that of the biomass-dominating herbivorous *Calanus* species. Also, *P. minutus* is able to feed more intensively and/or assimilates FAs more efficiently from diatoms than from flagellates. Short-chain as well as long-chain fatty alcohols are synthesized *de novo*, combined with dietary FAs and stored as wax esters in relatively high proportions, indicating that they play a major role for maintenance during overwintering and for reproductive processes. In contrast, *O. similis*, as an omnivorous species, does not depend to such a large extent on the accumulation of lipid reserves. However it may use its lipid stores during periods of major metabolic demands such as reproduction or as an energy buffer during short periods of poor food supply. *O. similis* assimilates dietary lipids at a lower rate than *P. minutus*. It is able to synthesize FAs from diverse food sources such as diatoms and flagellates, with no apparent preference between the two algal taxa. *O. similis* does not synthesize fatty alcohols at high rates, but contained almost 30% wax esters, suggesting that energy depots for important metabolic processes were already replenished.

In conclusion, *P. minutus* is much more efficient than *O. similis* to channel lipids through the Arctic food web, regardless of whether the food source is dominated by diatoms or flagellates. This study highlights the importance of small copepods in the Arctic lipid-driven pelagic food web. We recommend further work on the lipid metabolism and energetic strategies of these particular copepods, especially in view of the rapidly changing Arctic environment.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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3.2 Manuscript II

Year-round population dynamics of *Limacina helicina* and *L. retroversa* in a high-Arctic fjord

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Year-round population dynamics of *Limacina helicina* and *L. retroversa* in a high-Arctic fjord

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Abstract

The thecosome pteropods *Limacina helicina* and *L. retroversa* are important contributors of the zooplankton community in high-latitude environments but little is known about their distribution and life cycle. We collected the small-size fraction of the population in 2012 and 2013 in Adventfjorden (78° N). The temporal distribution of these 2 species was investigated in parallel to physical and biological environmental parameters in order to understand their variability in abundance and size. Young stages of *L. helicina* were widespread in terms of time and space. Hatching occurred at 0.05 mm, in late summer/autumn. Growth occurred during all seasons, but was slow during the first year of development (0.02 mm day^{-1}) and accelerated in the second year of development (0.05 mm day^{-1}). Our study suggests that *L. helicina* overwinter the first year as a small (<1 mm) juvenile and that this species need at least 2 years to reach maturation. In comparison, *L. retroversa* was only found in low numbers with a heterogeneous size distribution. This more temperate species was most likely advected with Atlantic water masses since our results did not support that *L. retroversa* reproduce in high-Arctic environments.

Key words. *Limacina helicina*, *L. retroversa*, juveniles, distribution, growth rate

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Introduction

Shelled pteropods (thecosomes) are significant components of polar marine ecosystems. They can dominate the zooplankton community at times and are key species in the pelagic food web. They effectively graze on phytoplankton and small particles (Perissinotto 1992; Noji et al 1997; Bernard and Froneman 2009) and are in turn preyed upon by large zooplankton, birds, fish and marine mammals (Hopkins and Torres 1989; Lalli and Gilmer, 1989; Lancraft et al. 1991; Hunt and al 2008). Thecosomes also have a significant role in the production and export of organic matter and calcium carbonate (Berner and Honjo 1981; Fabry 1990; Bathmann et al. 1991; Hunt et al. 2008). During productive periods, they may contribute up to 72% of the organic carbon export in the Southern Ocean (Manno et al. 2010). Part of this flux is related to the production of feacal pellets that sink to deeper layers (Gilmer and Harbison 1991; Accornero et al. 2003). Pseudo-faeces are also produced, as the result of the degradation of mucus nets that individuals use for grazing (Harbison and Gilmer 1986). Due to their aragonite shells that rapidly sink after death, thecosomes are also responsible for a large portion of calcium carbonate flux in polar waters (Byrne et al. 1984; Tsurumi et al. 2005).

In Arctic ecosystems, two species of thecosome pteropods are present, *Limacina helicina* and *L. retroversa* (Kattner et al. 1998; Hop et al. 2006; Bauerfeind et al. 2014). Both species are restricted by a narrow range of temperature and salinity, which makes them useful as biological indicators of water masses and environmental changes. *L. helicina* inhabits polar waters and is adapted to temperature between -1.6 and 4 °C. *L. retroversa* is a boreal species and is usually associated to Atlantic water masses. The species thrives at temperatures ranging from 2 to 7 °C (Chen and Bé 1964; van der Spoel 1967; Lalli 1970; Conover and Lalli 1972; Bé and Gilmer 1977; Hopkins 1985, 1987). *L. helicina* is a prominent member of the Arctic zooplankton community while *L. retroversa* occurs episodically when introduced to the system by Atlantic water masses (Hop et al. 2006; Walkusz et al. 2009). The last decade a shift from a dominance of *L. helicina* to *L. retroversa* has been observed in the Fram Strait as a consequence of climate warming due to increased of a warm Atlantic water inflow (Bauerfeind et al. 2014).

L. helicina reaches a maximum size of 8 mm (Gannefors et al. 2005). It develops to adults in summer and reproduces in late summer/autumn (Gannefors et al. 2005). Veligers/juveniles constitute the main overwintering stages. Whether or not they grow and develop during this period or during the following spring/summer remains unclear (Gannefors et al. 2005; Bednaršek et al. 2012; Lischka et al. 2012). The duration of *L. helicina*'s life cycle is still under debate. Some studies suggest that individuals reach maturation in 1 year and die after reproduction (Fabry 1989; Gannefors et al. 2005; Hunt et al. 2008) while others suggest a longer life span, with possibly more than one reproductive event (Kobayashi 1974; Bednaršek et al. 2012). The life cycle of *L. retroversa* (maximum size of 3 mm, Hsiao 1939) has been less studied than that of *L. helicina*. Some studies conducted in sub-polar environments suggested a one-year life cycle, with one reproductive event in spring (Hsiao 1939) or in autumn (Meinecke and Wefer 1990). However, constant reproductive activity throughout the year has been considered the most likely, with an intense event in spring and another in autumn (Lebour 1932; Dadon and De Cidre 1992). Whether or not *L. retroversa* is only an expatriate or able to complete its life cycle successfully in Arctic waters is still unknown (Lischka et al. 2012).

A growing body of literature focused on the impact of climate change on thecosomes due to the high sensitivity of their aragonite shell to acidification (e.g. Comeau et al. 2012; Bednaršek et al. 2012, 2014; Lischka et al. 2012; Lischka and Hagen 2016). The combination of temperature rise and pH decrease is expected to lead to a decline of the population in the next decades and might impact the entire Arctic pelagic food chain (Lischka et al. 2011). Although the response of *Limacina* spp. to climate change is now better understood, there is a major lack of knowledge regarding their life history. The population structure, the longevity of individuals and the growth rates are parameters that are poorly known and still under debate. One limitation to the understanding of the life cycle differences between *L. helicina* and *L. retroversa* has been that veligers and early juveniles are morphologically indiscernible (Lischka, pers. comm.). However, they can be clearly identified using molecular markers (Kohnert, unpublished data).

The goal of this study was to fill the gap in knowledge concerning the life history of thecosomes in Arctic fjords by (1) applying barcoding methods for the identification of *Limacina* spp. early

stages, (2) investigating the occurrence of *L. helicina* and *L. retroversa* in relation to environmental parameters and (3) investigating the annual growth of veligers and juveniles.

Material and methods

Weather and oceanographic data

The study was conducted from January 2012 to December 2013 in Adventfjorden, a small side-fjord of Isfjorden, on the west coast of Svalbard (78 °N) (Fig.1). The fjord is 8.3 km long, 3.4 km wide and less than 100 m deep. It is exposed to inflow from the Atlantic-derived, warm West Spitsbergen Current and the Arctic-derived, cold East Spitsbergen Current (Węsławski et al. 1999; Zajączkowski et al. 2010). Two larger and several smaller rivers discharge freshwater and sediments. Thus, Adventfjorden is strongly influenced by brackish water and fine sediment particles during the main melting season in summer (Leikvin and Evenset 2009).

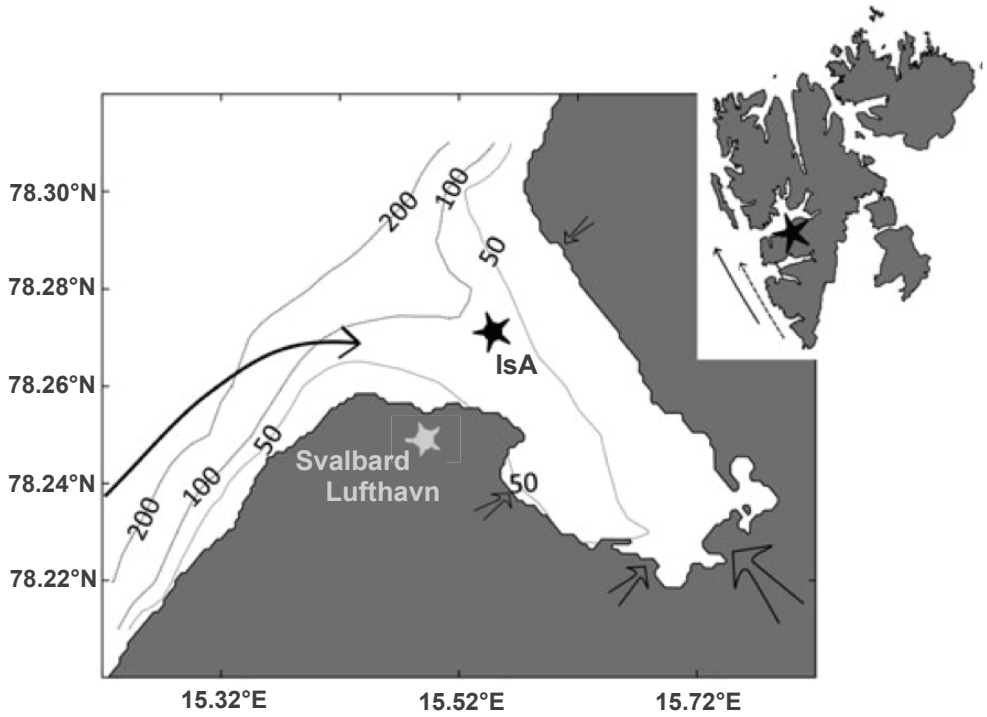

Figure 1. Map of the sampling station IsA (78.261 °N, 15.542 °E), and the meteorological station Svalbard Lufthavn (78.242 °N, 15.502 °E). The dashed arrow represents the influence of the Arctic water masses and the plain arrow represents the general influences of Atlantic water

masses. Open arrows indicate fresh water influences from rivers into Adventfjorden (from Stübner et al. 2016).

The hydrography (salinity, temperature, and density) and fluorescence were measured vertically at each sampling event at IsA station (78.261 °N, 15.542 °E, Fig.1), from bottom to surface using a hand-held CTD with a fluorometer attached (CTD, SAIV A/S).

The data of air temperature, precipitation, wind direction and wind speed were provided by the Norwegian Meteorological Institute. The data set includes measurements conducted every 6 hours from January 2012 to December 2013 at the station Svalbard Lufthavn, (78.242 °N, 15.502 °E, Fig.1). The data was analyzed using the free software Ocean Data View (Schlitzer 2005).

Sampling

The zooplankton community was sampled at IsA station (Fig.1), (78.261 °N, 15.542 °E), on board a small boat (PolarCirkel, Akva Group) from UNIS (University Centre in Svalbard) in 2012 and 2013. Sampling was carried out on a monthly basis when the weather conditions allowed it. Animals were collected by vertical hauls from 65 to 25 m (deep layer) and 25 to 0 m (surface layer) using a WP11 net (63 µm mesh size, 0.25 m^2 net opening). The use of these nets only allows capturing *Limacina* spp. of small size. Individuals with a shell diameter larger than 1-2 mm seem to be able escape from them (Harris et al. 2000; pers. obs.).

Live specimens were immediately fixed in a seawater/formaldehyde (4%) solution for later determination of the species and size composition. Additional samples were fixed in an ethanol (70%) solution and frozen at -80 °C for subsequent molecular analyses.

Limacina spp. specimens were measured (shell diameter) and counted under a Leica MZ12 Stereomicroscope. Identification to the species level was done in parallel to counting for specimens larger than 0.5 mm, considering that *L. retroversa* has a pointed spiral shell while *L. helicina* has a flat shell (van der Spoel and Dadon 1999). Morphological identification was not possible for specimens smaller than 0.5 mm since first life stages of both *L. retroversa* and *L. helicina* have a flat shell (Lischka, pers. comm).

Molecular analyses

To determine the smallest individuals to species level, 52 veliger (0.11 - 0.41 mm) were randomly picked from the samples fixed in 70% ethanol (Tab.1). Molecular analyses were performed at the Bavarian State Collection of Zoology (ZSM Munich).

Table 1. Samples collected for the molecular identification of *L. helicina* and *L. retroversa.*

Date	# individuals	size range (mm)	Depth
6 September 2012	6	0.11 - 0.34	25-0 m
6 September 2012	6	0.15 - 0.24	65-25 m
19 September 2012	6	0.13 - 0.33	25-0 m
19 September 2012	6	0.23 - 0.34	65-25 m
18 October 2012	6	0.24 - 0.42	25-0 m
18 October 2012	6	0.20 - 0.50	65-25 m
12 December 2012	6	0.14 - 0.38	25-0 m
12 December 2012	10	0.15 - 0.38	65-25 m

Due to the small size of investigated specimens, whole individuals were used to extract genomic DNA. We followed the CTAB extraction method (Knebelsberger and Stöger 2012) with a modified collection of dissolved DNA in a spin column from a NucleoSpin Tissue set (Macherey-Nagel GmbH & Co) to assure maximum DNA recovery (Kohnert unpublished data). Nuclear Histone 3 marker (H3) contains a diagnostic nucleotide to distinguish between *L. helicina* and *L. retroversa*, as base 307 is G in *L. helicina* and T in *L. retroversa* (Kohnert, unpublished data). H3 was amplified in 0,2 ml illustra^{TM} PuReTaq^{TM} Ready-To-Go^{TM} PCR tubes (GE Healthcare) with 23 µl molecular water, 1 µl of template DNA and 0.5 µl of forward and reverse primer (10 pm/µl), respectively. We used the primers H3aF: 5'-ATG GCT CGT ACC AAG CAG ACV GC-3' and H3aR: 5'-ATA TCC TTR GGC ATR ATR GTG AC-3' (Colgan et al. 2000) with following PCR settings: initial denaturation for 5 min. at 94 °C followed by 36 cycles of denaturation for 45 s at 94 °C, annealing for 50 s at 45 °C, elongation and extension at 72 °C for 200 s and a final elongation step at 72 °C for 10 min. Successful amplicons were purified using a DNA Clean & Concentrator Kit (ZYMO Research) according to the manufacturer's manual with a final elution volume of 15 µl.

Sequencing was performed at the Genomic Service Unit of Department of Biology, Ludwigs-Maximilians-Universität Munich, using Big Dye 3.1 with 5µl diluted (2 pm/µl) amplification primers and 2 µl of purified PCR-product. Sequences were edited in Geneious R8 (8.1.7.) (www.geneious.com, Kearse et al. 2012) and aligned with the implemented Mafft plugin (Katoh et al. 2009). As a reference, *Limacina* spp. sequences generated from clearly identifiable adult specimens of the respective species were included. Latter samples were collected in Svalbard (*L. helicina*) and Bergen (*L. retroversa*). Genetic vouchers are stored at the ZSM Munich.

A 336 bp long sequence was successfully amplified for 39 specimens. 11 samples failed in PCR or resulted in sequences that could not be assembled/aligned, rendering a success rate of 78%. Among all samples, only 1 individual was identified as *L. retroversa* (6 September 2012, deep layer), indicating that the community was composed at 98% of *L. helicina.*

Analyses of distribution patterns

Statistical analyses were performed using the free software R (team RDC 2010). To assess the environmental forcing on the thecosomes abundances in the entire water column, 2 principal component analyses (PCA) were performed using the R package FactoMineR (Lê et al. 2008). The environmental data (water properties and meteorology) as well as time (expressed as day of the year) were computed as active variables. Biological data (thecosomes abundances) were added as supplementary variables. Seawater densities were not included in the PCA because they were computed from T and S, and the strong correlation among these variables could artificially alter PCA results. Missing values were estimated with the package missMDA (Husson and Josse 2010) using the relations between all variables, from 2 dimensions of the PCA.

Identification of cohorts and growth rates

To examine the size-distribution patterns of the thecosome populations, the shell diameters of *L. helicina* and *L. retroversa* were combined in size-frequency histograms for each sampling date. Replicates from deep (65-25 m) and surface (25-0 m) layers were pooled, since no size difference was detectable (t-test, $p>0.05$).

The package mixdist (Macdonald and Du 2012) was used to fit mixture distribution models to the shell diameter distributions of *L. helicina* and *L. retroversa*. Sizes were separated in 20 classes, from 0.1 to 1 mm. Initial values (frequency of each size class) were implemented, and parameters (mean values, and standard deviations of sub-distributions) were estimated by the Kernel density estimation.

For further investigation of the growth rates, it was necessary at this stage to identify the different cohorts that possibly co-occurred at all sampling dates. From the Kernel density estimation, and in the size-range of individuals captured by our nets, three cohorts of *L. helicina* were distinguished throughout the study period. At each sampling date, one or two cohorts were occurring. In 2012, the first cohort of *L. helicina*, named H_0 was present alone from 27 January until 19 September. At this date, the cohort H_1 appeared. H_0 and H_1 were both found until 15 November 2012. Thereafter, H_0 disappeared from the samples, possibly due to the capacity of grown individuals to escape from the nets. H_1 was then the only observed cohort until 13 August 2013 when the cohort H_2 appeared. Both cohorts co-occurred until the end of our study.

The shell size distribution of *L. retroversa* was heterogeneous. Therefore, no cohorts could be identified.

Growth rates of *L. helicina* were calculated based on the estimated cohorts and calculated as (1), according to Bednaršek et al. (2012):

(1) $G_{H_{n,t1\to2}} = \frac{L_{t_2} - L_{t_1}}{t_2 - t_1}$

where H_n is the cohort of interest, L is the mean shell diameter in mm (estimated by the Kernel density estimation), and t is the time (in days).

The statistical significance of growth rates was tested with a Fisher test (ANOVA), using linear regressions.

Results

Environmental conditions

Water masses

Adventfjorden was influenced by local water (LW) and transformed Atlantic water (TAW), in similar temporal patterns in 2012 and 2013 (Fig.2).

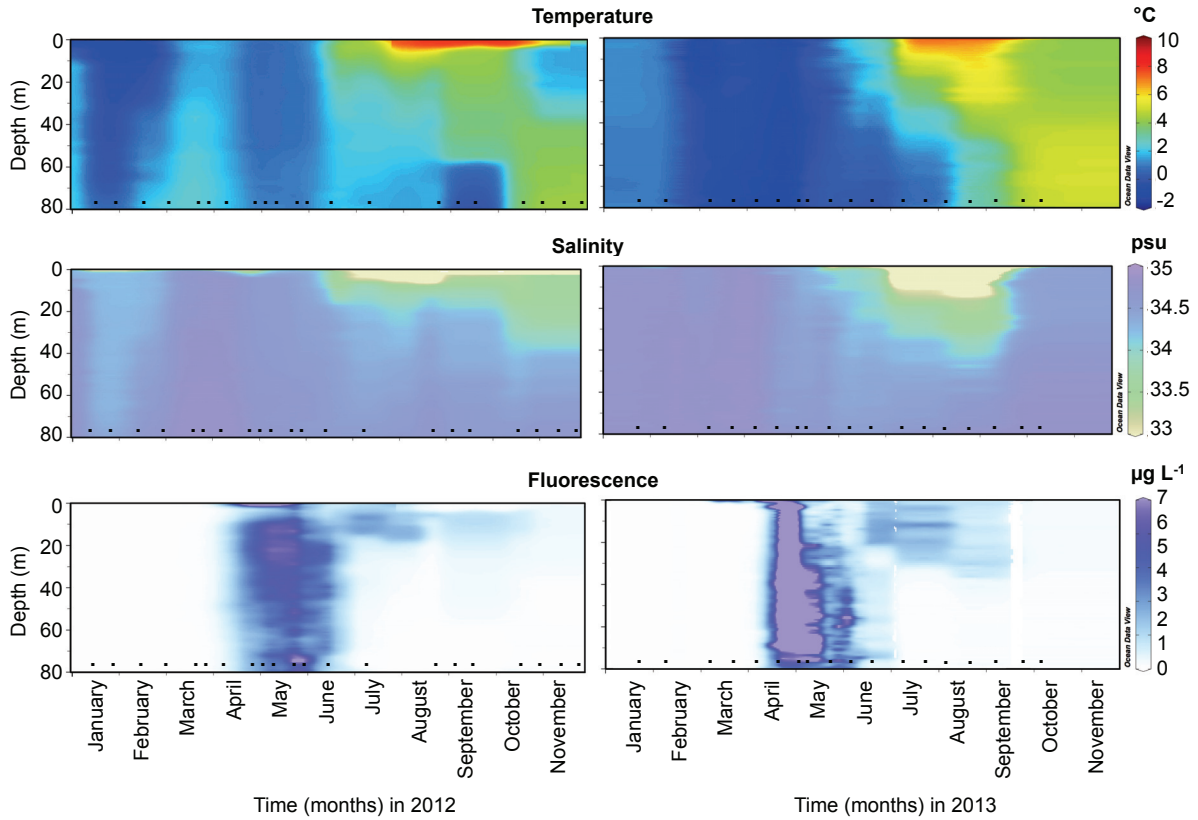

Figure 2. Contour plots of environmental variables. Temperature (upper panel), salinity (middle panel) and fluorescence as a proxy for Chl *a* (lower panel) were linearly interpolated between measurements. Black dots indicate dates for *Limacina* spp. sampling.

At the start of the year, until April, the fjord was mainly characterized by an inflow of transformed Atlantic water (T>1 °C, S>34.7 psu). During this period, a cooling of TAW occurred, while salinity remained high. From May to August, TAW inflow became limited while LW (T<1 °C, S<34.5 psu) prevailed in the fjord. Between June to September, river runoff led to

stratification of the water column, with a freshwater layer at the surface, characterized by high temperature (>8 °C) and low salinity (<33 psu). The deep layer was still characterized by the colder LW. In mid-august/September, a new inflow of warmer and more saline TAW occurred at depth. In parallel, mixing of the water column started, leading to the formation of an intermediate layer (IW) that remained until the end of the year (34<S<34.7 psu).

Phytoplankton

Chl *a* concentrations were negligible from January to mid-April. In late April, the concentrations increased, to reach maximum values in mid-April/May with ca. 4 µg L^{-1} in 2012 and ca. 7 µg L^{-1} in 2013. Chl *a* concentrations remained high in the entire water column until June. Between July and September Chl *a* values decreased and were mainly restricted to surface layers. From September/October on, low winter concentrations were reached again. Outside the peak bloom period, cells of the <10 µm fraction dominated the phytoplankton community (Marquardt et al. 2016).

Population structure

Abundances

L. helicina was found in the water column throughout the entire sampling period, from January 2012 to October 2013 (Fig.3a). In 2012, 95 individuals m^{-3} were found in January and February. In spring and summer, the abundances did not exceed 50 ind. m^{-3}, except for July when >300 ind. m^{-3} were found. In September 2012, there was a sudden increase in abundance to >1500 ind. m^{-3} whereas in October-December less than 250 ind. m^{-3} were counted. In 2013, the abundances were generally lower, varying between 0.4 and 4 ind. m^{-3} from January to June. In July, the numbers increased and the maximum abundance of 1700 ind. m^{-3} was found again in September. As in 2012, the numbers decreased in October to 270 ind. m^{-3}. Throughout the sampling period, twice as many individuals were found in the surface layer (25-0m) as compared to the deeper layer (65-25m). In September, individuals were >20 times more abundant in surface than deep waters. Mortality seemed high between September and October in both years (50 ind. d^{-1} in 2012 and 46 ind. d^{-1} in 2013) whereas it was negligible during the rest of the sampling period.

L. retroversa was only observed from 19 September 2012 to 11 February 2013, with an average of 14 ind m^{-3} (Fig.3b). Individuals were 2 times more abundant in deep waters as compared to surface.

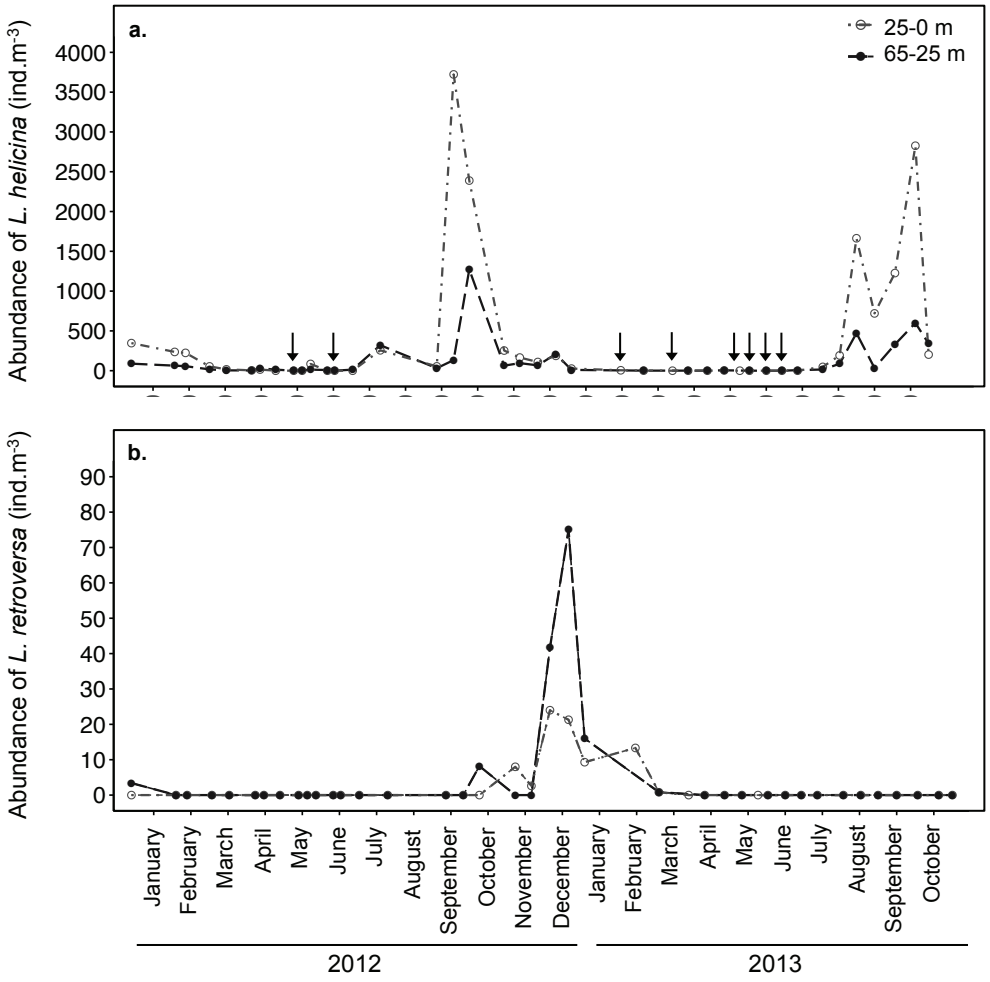

Figure 3. Abundances of (**a.**) *L. helicina* and (**b.**) *L. retroversa* in the surface (25-0 m) and deep (65-25 m) layers in Adventfjorden, in 2012 and 2013. Arrows represent the dates when no *L. helicina* was found in the samples. *L. retroversa* was only found between 19 September 2012 and 11 February 2013.

The first and second principal components (PC) of environmental variables explained 34% and 16% of the variance in the dataset, respectively. All variables except salinity had positive loadings on PC1 (Fig.4). Salinity, fluorescence and meteorological variables (precipitation, air

temperature, and wind direction) were positively correlated with PC2, while water temperature and wind speed, as well as the time factor were negatively correlated with PC2.

L. helicina abundances were positively placed with respect to PC1, while *L. retroversa* abundances were negatively correlated. Both thecosome groups were negatively correlated with PC2. *L. retroversa* had no close correlation with any environmental variables.

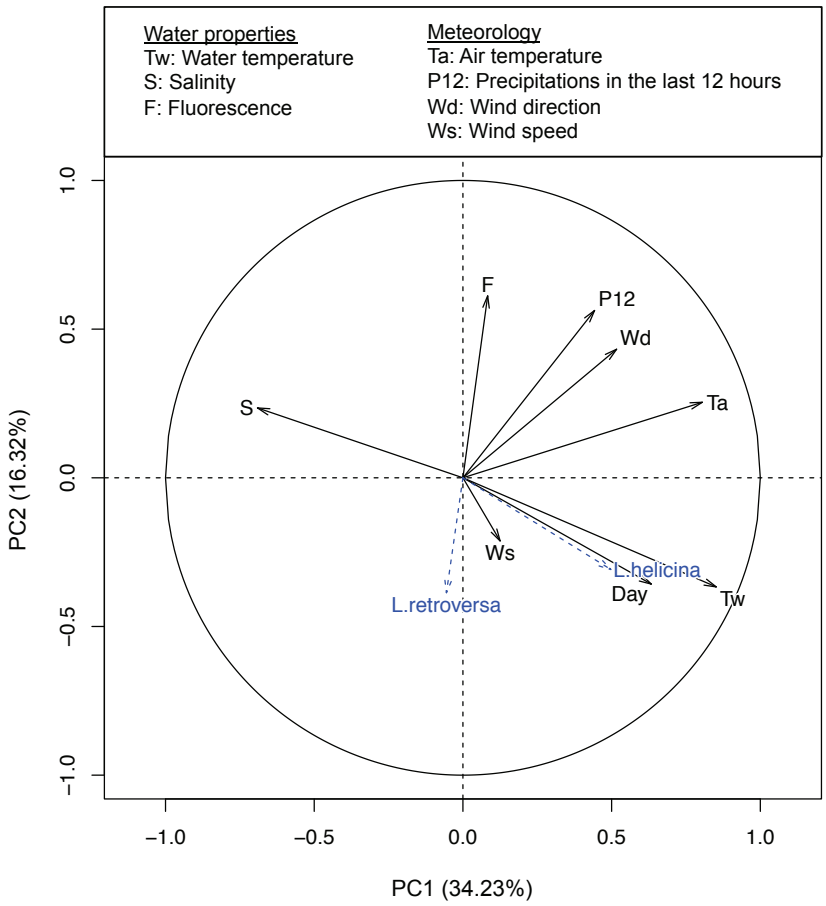

Figure 4. Principal component analysis of environmental variables in Adventfjorden. Biological data (abundances of *L. helicina* and *L. retroversa*) are imputed as supplementary variables.

Size distribution and growth

The mixed distribution model applied to the data on *L. helicina* shows that the population may be composed by either 1 or 2 cohorts (Fig.5). From January to September 2012, the population was characterized by a single normal distribution of size, corresponding to a cohort named H_0. Between September and December, a bimodal distribution was observable, corresponding to the

cohort H_0 and the new H_1, characterized by smaller sizes. From January to August/September 2013, the size structure reflected the presence of only one cohort, H_1. In August, the H_2 cohort appeared.

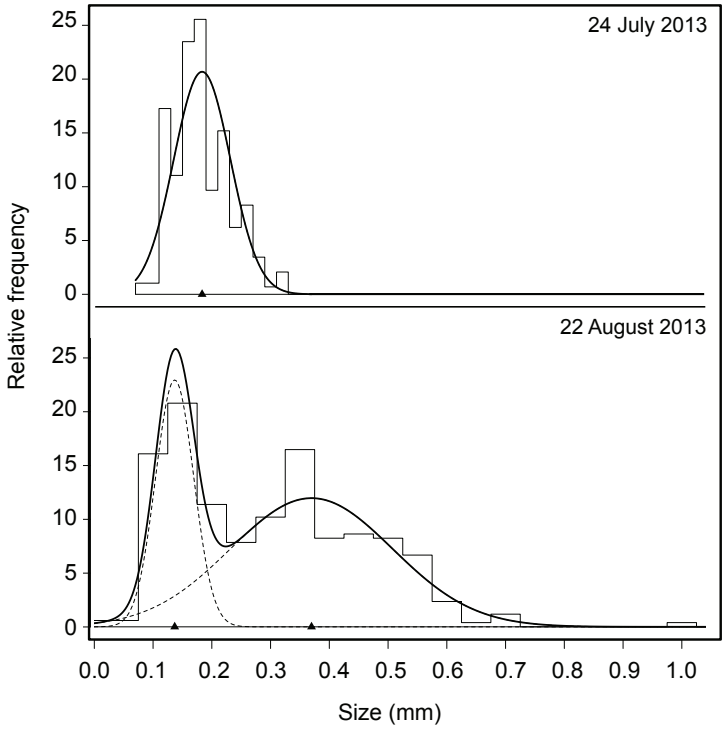

Figure 5. Size-frequency histograms of *L. helicina* caught on 24 July 2013 and 22 August 2013 as an example of the population structure reflected by the samples. This configuration is the same in 2012 and 2013, with 1 cohort present in late winter to summer and 2 cohorts in autumn/early winter. Overlaying solid lines are best fitting mixture models. Mean shell diameter values are represented by triangles. Dotted curved lines represent the different cohorts.

For the H_0 cohort, the modal peak of size was of 0.14 mm on 27 January, with minimum shell diameter of 0.11 mm (Fig.6). The size range was narrow (sd = 0.05 mm). In the winter following hatching (27 January to 29 March 2012), the growth rate of H_0 was of 0.0002 mm d^{-1} ($p<0.05$) (Fig.10). By the end of winter, individuals reached sizes of 0.15 mm. The growth was still slow in spring/early summer (29 March to 6 July), with a rate of 0.0004 mm d^{-1} ($p<0.05$). By the end of spring, individuals reached sized of 0.19 mm. In summer, (6 July to 19 September), individuals grew at a rate of 0.0011 mm d^{-1} ($p<0.001$), reflecting an increase of shell size by a

factor 1.4, reaching a shell diameter of 0.24 mm. In autumn (19 September to 15 November), individuals showed maximum growth with a rate of 0.0066 mm d^{-1} ($p<0.001$). During this season, sizes were multiplied by a factor 2.5 and reached 0.80 mm in November (sd = 0.12 mm). Shell diameters were 6 times larger in November as compared to January.

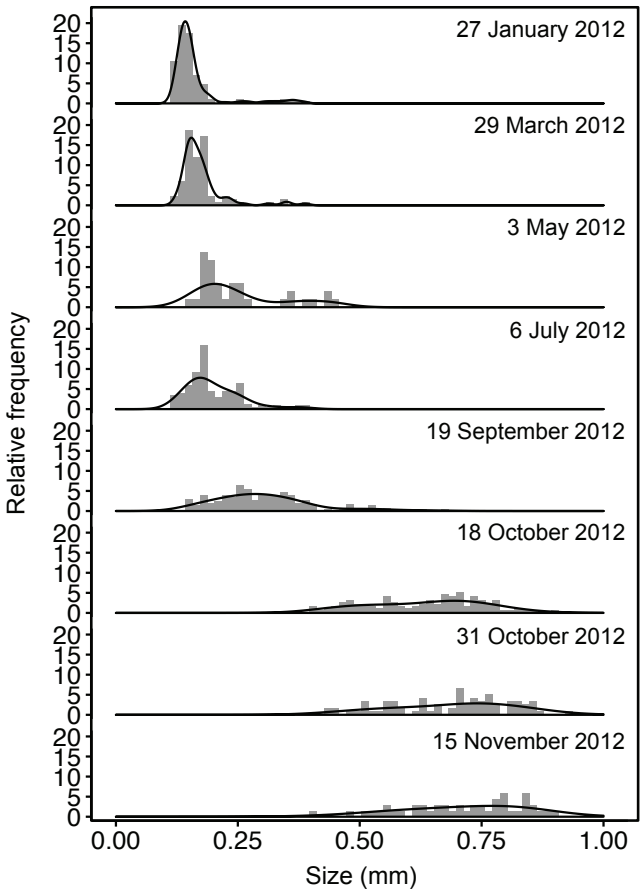

Figure 6. Size-frequency distribution of the H_0 cohort of *L. helicina*, divided into different months between January and December 2012. The modal curve fitted to the sample plot represents the probability of density of each date.

For the H_1 cohort, the modal peak of size was of 0.13 mm on 19 September 2012, with minimum shell diameter of 0.05 mm (sd = 0.01 mm) (Fig.7). In the first autumn following hatching (19 September to 15 November), H_1 had a growth rate of 0.0012 mm d^{-1} ($p<0.05$) (Fig.10). In winter (31 October 2012 to 5 April 2013) and spring (5 April to 24 July), the growth rate was of 0.0003

mm d^{-1} ($p<0.05$). At the end of summer (8 September), animals reached 0.60 mm, i.e. 3 times their size at the beginning of the season (24 July), reflecting a growth rate of 0.0088 mm d^{-1} ($p<0.001$). One year after hatching, in autumn 2013, growth rate was of 0.0044 mm d^{-1} ($p<0.001$), i.e 4 times faster than during the first autumn. In October, individuals measured 0.73 mm (sd = 0.19 mm), they were 6 times bigger than when newly hatched.

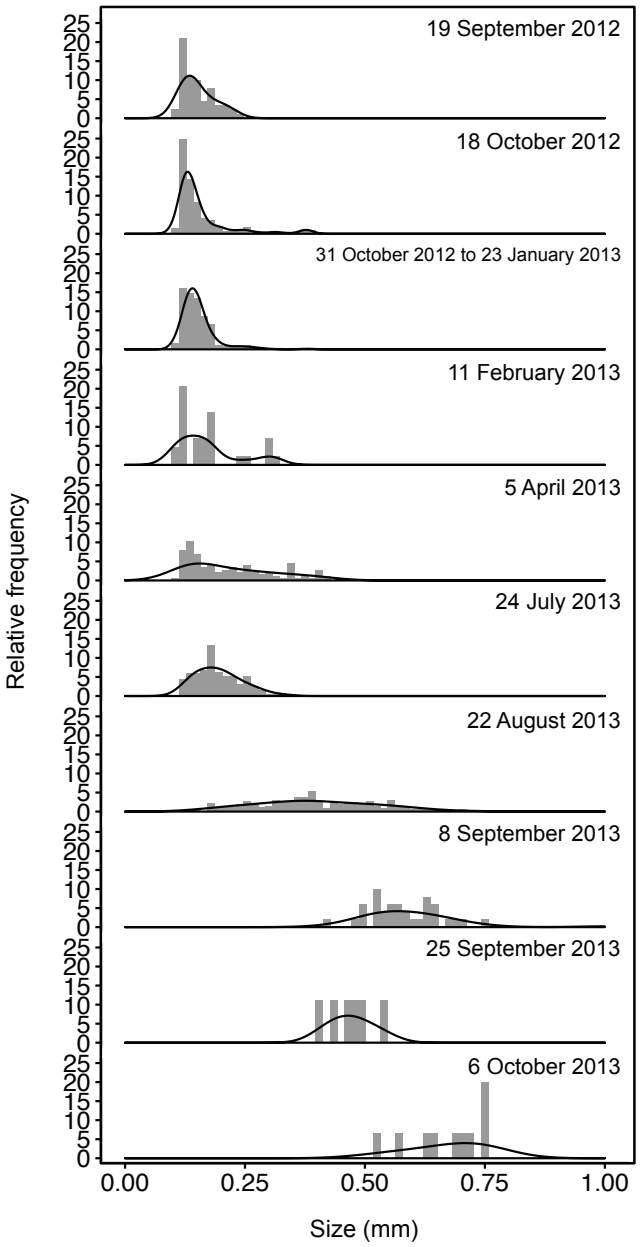

Figure 7. Size-frequency distribution of the H_1 cohort of *L. helicina*, divided into different months between September 2012 and December 2013. The modal curve fitted to the sample plot represents the probability of density of each date.

The cohort H_2, hatched in August-September 2013, had a constant size range, with a modal peak at 0.16 mm (Fig.8). The growth rate was negligible from 22 August to 13 October 2013 (0.0002 mm d^{-1}, $p>0.05$) (Fig.10).

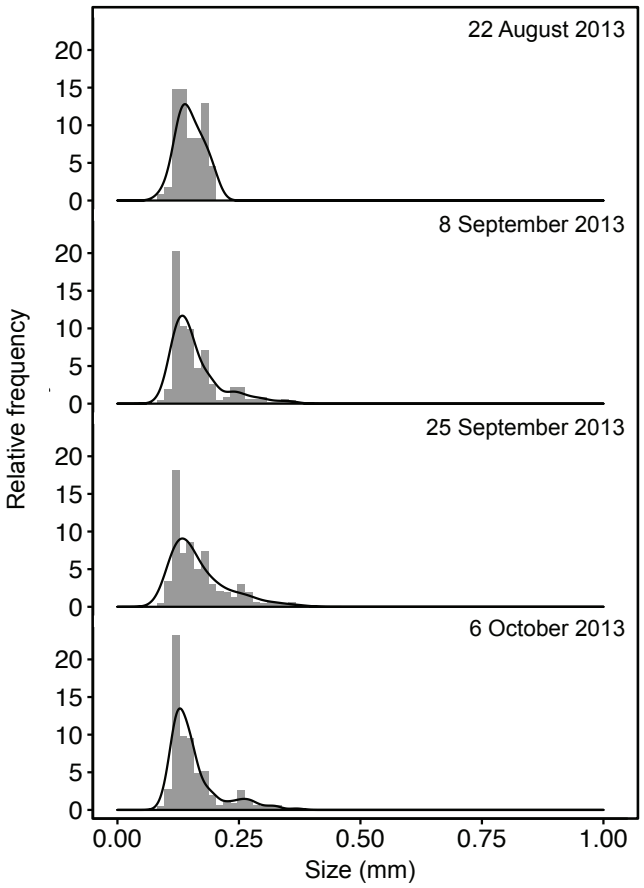

Figure 8. Size-frequency distribution of the H_2 cohort of *L. helicina*, divided into different months between September 2012 and December 2013. The modal curve fitted to the sample plot represents the probability of density of each date.

L. retroversa showed a patchy distribution of size classes for all sampling dates, no modal distribution could be applied for this species (Fig.9). Sizes ranged between 0.48 and 1.60 mm.

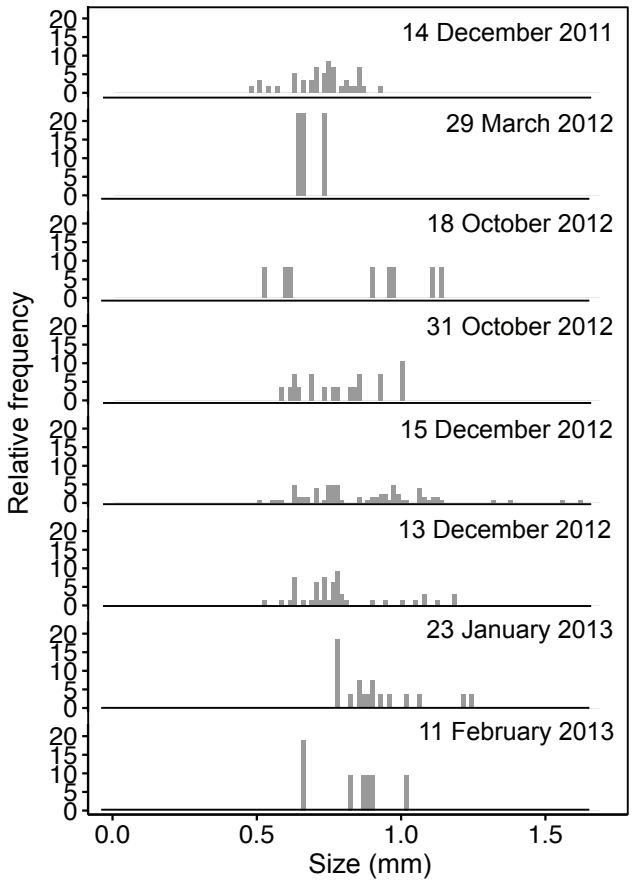

Figure 9. Size-frequency distribution of *L. retroversa*, divided into different months between December 2011 and February 2013.

Discussion

Life cycle of *L. helicina*

L. helicina has been commonly observed in Arctic waters (Kobayashi 1974; Gilmer and Harbison 1991; Gannefors et al. 2005). The species is known to have a patchy distribution, making it difficult to catch animals (Kattner et al. 1998; Gannefors et al. 2005; Howes et al. 2015). However, these results are only based on the larger individuals (Wiborg 1954; Percy and Fife 1985; Østvedt 1995; pers. obs.). We observed that young stages were present throughout the year. Our results also suggested that individuals were evenly distributed in the fjord. A more patchy distribution would most likely not result in the continuous presence of young individuals

at this one unique sampling location. Our present study is hence the first which clearly demonstrates that veligers and juveniles are widespread, both in spatial and temporal aspects. The difference in distribution patterns between early life stages and adults may be related to biological processes as *L. helicina* adults trend to gather in large swarms to reproduce (Dadon 1990; Dadon et al. 1992; Noji et al. 1997). This may be an adaption for successful reproduction, as it facilitates mating (Folt and Burns 1999).

According to Gannefors et al. (2005), *L. helicina* reproduces in late summer/autumn, with a peak of abundance of veligers in early September. Our results confirm this timing of reproduction since we observed maximum abundances in parallel with the smallest size ranges of individuals from August to October, and considering that veligers hatch 2-6 days after spawning (Lalli and Wells 1978). In addition, *L. helicina* abundances were strongly correlated with time, as revealed by the PCA.

It has been suggested that the timing of reproduction of *Limacina* spp. depends on the feeding conditions of adults in spring (Böer et al. 2006; Bernard and Froneman 2009). This may explain the earlier onset of reproduction in 2013 as compared to 2012 (August vs. September), since Chl *a* concentrations were 1.8 times higher in 2013 than in 2012. The accumulation of sufficient levels of lipid storage may be implicated in the timing of reproduction (Maas et al. 2011). In a situation of low primary production, females would need to feed longer on the available food in order to accumulate enough fatty acids to fuel reproduction processes (Boissonnot et al. submitted).

Since veligers/juveniles start feeding immediately after hatching (Gannefors et al. 2005), they may find advantage in being present in the euphotic zone of the water column (Kobayashi 1974) and hence in the depth range of highest food concentration during productive months. In our study, maximum abundances of *L. helicina* in late summer were characterized by a very high concentration of individuals in surface layers. This confirms the results of Kobayashi (1974), which suggested that the veligers/juveniles concentrate in surface layers (50-0 m). However, our study has to be considered in the perspective of Adventfjorden being a shallow fjord (less than 100 m deep). *L. helicina* is assumed to be able to seasonally migrate towards waters deeper than

1500 m (Kobayashi 1974). Our results therefore refer to a relatively small layer of the potential vertical distribution of *L. helicina*. This concentration of young stages in surface layers may suggest that reproduction occurs in surface waters and/or that hatching occurs in surface since spawns, which consist of free-floating eggs ribbons (Lalli and Gilmer 1989), have positive buoyancy due to high lipid contents (Gannefors et al. 2005; Tsurumi et al. 2005).

Some studies assume that *L. helicina* population is composed by only one cohort at a time (Kobayashi 1974; Gannefors et al. 2005) while newer studies argue for an overlap of more than 2 cohorts (Hunt et al. 2008, Bednaršek et al. 2012). In our study, we collected only small individuals (<1 mm) that reflected an overlap of 2-3 cohorts. In addition, mature females (>5.0 mm, Lalli and Wells 1978) must be present for reproduction, at least in late summer/ autumn. Therefore, we consider our study to reflect the co-existence of at least 3 cohorts. In an extremely variable environment such as the Arctic ecosystem, having a one-year life cycle with no overlap of cohorts would be a hazardous life-strategy (Bednaršek et al. 2012). An overlap of 3 cohorts guarantees a certain level of stability at the population level and allows facing the high variability in physical conditions.

The growth rates of *L. helicina* are still under debate. Some studies suggest that this species constantly grows throughout the year (Bednaršek et al. 2012) while others suggest that growth depends on season. Kobayashi (1974) reported maximum growth in winter whereas Lischka et al (2012) found that growth ceased during this period. Our data suggest that individuals increase in size throughout the entire year, but with varying growth rates depending on seasons and developmental stages (Fig.10). Veligers started to grow directly after hatching, but at a low rate during overwintering. Since there is low food availability during this season, growth may be fueled, at least partially, by lipid reserves that individuals accumulated by feeding after hatching (Boissonnot et al. submitted). Maximal growth of H_0 occurred in autumn 2012 while the growth of H_1 was maximum in summer 2013 when the individuals tripled in size. Previous studies hypothesized that an important factor contributing to differences in growth rates is food availability, with a higher food supply allowing a faster development (Hunt et al. 2008; Bernard and Froneman 2009; Maas et al. 2011). In our study, the phytoplankton bloom reached higher concentrations in 2013 than in 2012, possibly fueling a faster and earlier growth of *L. helicina*.

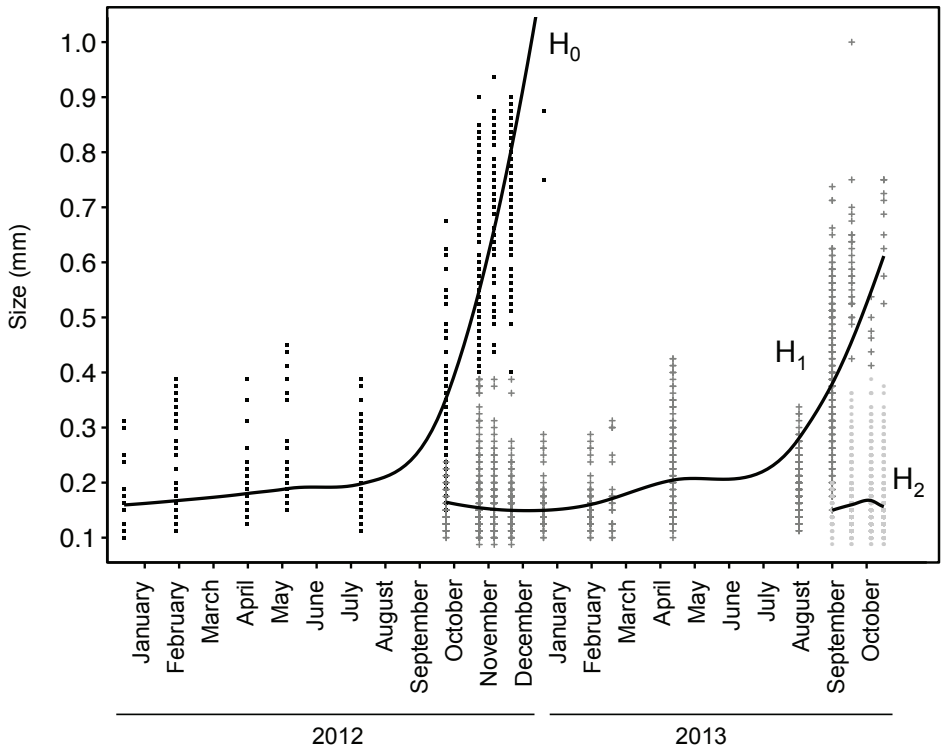

Figure 10. Individual sizes of the 3 cohorts of *L. helicina* present during our study. Points represent measured data. Trend lines were added to visualize better the growth of each cohort. They represent locally weighted regressions of the size.

One year after hatching, juveniles reached a size of 0.7-0.8 mm, which was 6 to 10 times their initial size. Our results further indicate that growth accelerates in the second year of *L. helicina* life cycle, with a growth rate 4 times higher in the second autumn than in the first following hatching.

Given the size reached by *L. helicina* one year after hatching, and considering that adults reach an average size of 5 mm (Gannefors et al. 2005), we propose a life span of at least 3 years. This conclusion is in line with Bednaršek et al. (2012) who suggested that the species needs at least 2 overwintering periods before reaching maturity. We further believe that one additional year is spent as veliger stage.

We propose an annual growth rate of 0.002 mm day^{-1} within the first year of development of *L. helicina*, followed by a faster rate of at least 0.005 mm day^{-1}. In contrast, Bednaršek et al (2012) estimated an annual growth rate of 0.006 mm day^{-1}, but individuals <1 mm were considered as a unique cohort while we suggest that they reflect 2 cohorts. Kobayashi et al. (1974) observed the same low growth rate as we did within the first year of development, but suggested a much faster growth within the second year, with individuals growing from 1 mm in September to 2.8 mm in November. This was not reflected by our results, since individuals reached 0.75 to 1 mm in October-November. However, since we did not collect individuals larger than 1 mm, growth may be faster at larger sizes, later in their development. Gannefors et al (2005) suggested a growth of 0.03 mm day^{-1} but this result was derived from the assumption that individuals grow to 5 mm in only one year, which our long term and high frequency data does not support.

Presence of *L. retroversa*

In Svalbard waters, the sub-polar species *L. retroversa* has been reported to occur episodically and at low densities (Lalli and Gilmer 1989, Kattner et al. 1998). The species is considered as a marker of Atlantic waters (Lebour 1932, Morton 1954), and thrives in waters with a temperature range of 2 to 7 °C (Lalli 1970; Bé and Gilmer 1977). In our study, *L. retroversa* was observed in autumn 2012, when Isfjorden was mainly influenced by transformed Atlantic waters. This warm and saline Atlantic inflow is mainly transported in the deep layer (Svendsen et al. 2002; Marquardt et al. 2016). The species showed highest abundances in the 65-25 m layer, suggesting that the population had been advected to the fjord with inflowing Atlantic water. However, *L. retroversa* was not found in autumn 2013 despite that the fjord at this time also was influenced by transformed Atlantic waters. When *L. retroversa* was abundant in 2012, the water column temperature averaged 2.5 °C, which is at the lowest edge of *L. retroversa* temperature optimum. In autumn 2013, the water temperature averaged 4.6 °C which is close to the temperature optimum of *L. retroversa* but no animals were found. The patchy distribution of *L. retroversa* in its area of origin (Meinecke and Wefer 1990) is very likely to explain its irregular occurrence in Svalbard waters.

In the early 20th century, *L. retroversa* was regarded as a sub-polar species that only occurred south of 65 °N (Lebour 1932; Redfield 1939). However recently, *L. retroversa* expanded northwards and can now be found at up to 79 °N in Fram Strait and in the Barents Sea (van der Spoel and Heyman 1983; Meinecke and Wefer 1990; Bathmann et al. 1991; Bauerfeind et al. 2009). A long-term study based on sediment traps in the Fram Strait even suggested a shift of the thecosome community, since 2005/2006, pointing towards a dominance of *L. retroversa* (Bauerfeind et al. 2014). This change would be associated with a warming of the water since 2000 (Schauer et al. 2008, Beszczynska-Möller et al. 2012; Bauerfeind et al. 2014). Our study does, however, not confirm this trend. While we also observed a warm Atlantic inflow, *L. retroversa* were present only in autumn 2012 and on average 4 times less abundant than *L. helicina*.

Whether or not *L. retroversa* reproduces in polar latitudes is unclear (Lischka et al. 2012). To our knowledge, no study elucidated this question. We observed a miscellaneous size distribution of this species. According to the size classification of Hsiao (1939) both juveniles and adults were present in Isfjorden during our study. No veligers were observed while previous studies indicated that *L. retroversa* has a peak of reproduction in autumn, in sub-polar areas (Lebour 1932; Meinecke and Wefer 1990; Dadon and De Cidre 1992). We therefore suggest that *L. retroversa* does not reproduce in Arctic waters. It has been reported that pteropods enter eddies as adults or juveniles and are subsequently retained (Tsurumi et al. 2005). *L. retroversa* would therefore be advected with Atlantic water masses, without being able to fulfill its life cycle in these high latitudes.

Conclusion

The distribution of *L. helicina* in Arctic waters has always been described as patchy. Our study reveals that this observation is only valid for adults. Veligers and juveniles are more evenly distributed both in time and space. We propose that the life-cycle of *L. helicina* lasts at least 3 years. Hence the population reflects a co-occurrence of, at times, 3 cohorts, with 2 cohorts of non-mature individuals. We further suggest that juveniles grow 4 times faster in their second year of development as compared to their first year. Having 2 cohorts of juveniles with different

growth rates and therefore different metabolism has implications for physiological studies conducted with small-stages. It is crucial to stop considering all individuals < 1 mm as one single cohort. We suggest delimitation between the 2 cohorts of juveniles at 0.50 mm.

In association with the warming of Svalbard water masses, a shift of the thecosome community to a dominance of *L. retroversa* is expected. Our study confirms that the species is advected in Svalbard fjords with Atlantic water masses. However the low numbers of individuals observed combined with the fact that the species does not fulfill its life cycle in high-latitudes suggest that *L. retroversa* is not taking over the thecosome community in Svalbard waters.

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3.3 Manuscript III

Lipid and fatty acid turnover of the pteropods *Limacina helicina*, *L. retroversa* and *Clione limacina* from Svalbard waters

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Abstract

This study aimed at a better understanding of the fatty acid turnover in Arctic pteropods. Thecosome pteropods *Limacina helicina* (adults and juveniles) and *L. retroversa* (adults) were collected in summer/autumn in Kongsfjorden and Isfjorden (Svalbard, 78 °N) and fed with ^{13}C labeled flagellates and diatoms for 6 days. Gymnosome pteropods *Clione limacina* were sampled in summer in northern Svalbard and fed with ^{13}C labeled *L. retroversa* for 23 days. Fatty acid compositions were determined by gas chromatography and ^{13}C enrichment of fatty acids was analyzed by compound-specific isotope analysis, using isotope ratio mass spectrometry. Among the thecosomes, maximum lipid turnover occurred in *L. retroversa* adults (1.3% day^{-1}). This species exhibited physiological adaptations typical of sub-Arctic species, with high lipid assimilation rates and the ability to integrate fatty acids more rapidly from flagellates than from diatoms. *L. helicina* showed a lower lipid turnover than *L. retroversa*, and adults of *L. helicina* were slower than juveniles to renew their fatty acids (0.1% day^{-1} and 0.2% day^{-1}, resp.). *L. helicina* adults integrated lipids from flagellates and diatoms with the same intensity, whereas juveniles showed a clear preference for diatoms, suggesting that feeding strategies depend also on the developmental stage. The lipid turnover rate of *Clione limacina* averaged at only 0.07% day^{-1}, which is significantly lower than previously reported for this species at the same latitude. This suggests that during our experiment *C. limacina* individuals utilized dietary energy primarily to maintain their basic metabolism rather than investing it into lipid storage.

Key words. Pteropods; ^{13}C labeling; lipid turnover; fatty acid assimilation

Introduction

The Arctic environment is characterized by extreme seasonality in light conditions, with continuous sunlight in summer and complete darkness during the winter period. Therefore, strong pulses of primary production are followed by periods of food scarcity, especially for herbivorous species. Abundant zooplankton species such as copepods and euphausiids are adapted to the seasonality of food availability by storing large amounts of lipids, as triacylglycerols (TAG) and/or wax esters (WE) (Lee 197, Sargent and Falk-Petersen 1988, Falk-Petersen et al. 2000). This strategy allows them to survive during food scarce periods while reducing their metabolism (Hirche 1996, Hagen & Auel, 2001, Lee et al. 2006, Huenerlage et al. 2014, Freese et al. 2015, 2016). Also pteropods have the capacity of storing lipids as diacylglycerol esters (DAGE) and/or TAG to cope with the strong seasonality in food supply (Lalli & Gilmer 1989, Gilmer & Harbison 1991, Böer et al. 2006), They are however, less studied than copepods and euphausiids although they can contribute more than 20% of the zooplankton biomass in Arctic waters (Gannefors et al. 2005, Blachowiak-Samolyk et al. 2008).

In Arctic waters, *Limacina helicina* is the main representative of thecosome pteropods, while *L. retroversa* dominates in North Atlantic waters (Van der Spoel 1967, Lalli 1970, Conover & Lalli 1972, Hopkins 1985, 1987). *Limacina* spp. reach a maximum size of 13 mm (Gilmer & Harbison 1991, Gannefors et al. 2005). They are omnivorous and feed by excreting a mucous web, in which food particles become entangled (Gilmer 1972, 1990, Harbison & Gilmer 1992). Their diet consists of diatoms and flagellates, but also of detritus and small zooplankton (Gilmer & Harbison 1991, Gannefors et al. 2005). *Limacina* spp. are preyed upon by larger carnivorous zooplankton, for examples ctenophores (Larson & Harbison 1989) and gymnosome pteropods (Conover & Lalli 1972), as well as seabirds, and several species of fish and baleen whales (Meisenheimer 1905, Gilmer and Harbison 1991, Falk-Petersen et al. 2001, Karnovsky et al. 2008).

L. helicina develops to adults in summer and reproduces in late summer/autumn (Gannefors et al. 2005). Veligers/ accumulate lipids from phytoplankton diet and probably utilize them during the dark period, in addition to relying on omnivorous feeding (Gannefors et al. 2005). It is, however,

still under debate whether their metabolism is reduced during overwintering (Hunt et al. 2008, Bednaršek et al. 2012). Some studies suggest that individuals reach maturation in 1 year and die after reproduction (Gannefors et al. 2005)), while others hypothesize that *Limacina* spp. needs at least two years to reach maturity (Kobayashi 1974, Bednaršek et al. 2012). The life cycle of *L. retroversa* has been less studied than that of *L. helicina*. Some studies conducted in sub-polar environments suggested a one-year life cycle, with one reproductive event in spring (Hsiao 1939) or in autumn (Meinecke et al. 1990). However, constant reproductive activity throughout the year has been considered the most likely, with an intense event in spring and another in autumn (Lebour 1932, Dadon and De Cidre 1992).

L. helicina and *L. retroversa* have moderate total lipid levels (<20% of the dry mass) (Falk-Petersen et al. 2001, Gannefors et al. 2005). Storage lipids are deposited as TAG, while wax WE or DAGE have not been detected (Kattner et al. 1998). Major fatty acids are the saturated 16:0 and 18:0, and the polyunsaturated fatty acids 20:5(n-3) (EPA) and 22:6(n-3) (DHA), typical components of membrane phospholipids. Some fatty acids can be used as trophic markers (FATM) and thus capture changes in feeding behavior (Dalsgaard et al. 2003 and references therein). Correspondingly, they portion of 16:1(n-7) is highest in spring, while the amount of 18:4(n-3) increases in summer and autumn, reflecting a dietary change from diatoms in spring to primarily flagellates in summer and autumn (Gannefors et al. 2005).

Many studies on *Limacina* spp. focus on the impact of climate change, due to the high sensitivity of their aragonite shell to acidification (e.g. Comeau et al. 2009, Lischka & Riebesell 2012, Bednaršek et al. 2012, 2014). The combination of temperature rise and pH decrease is expected to result in a strong decline in the population, with a cascading negative impact on the entire Arctic pelagic food chain (Lischka et al. 2010). The role of *Limacina* spp. with regard to lipid carbon turnover in the Arctic is largely unknown. So far, no study has focused on the differences in lipid turnover rates among thecosome species and developmental stages. The contribution of pteropods to the carbon flux in the marine food web may thus be underestimated (Lalande et al. 2013). In the context of global change, it is therefore of great interest to understand their capacities in terms of fatty acid biosynthesis and energy transfer to higher trophic levels.

Clione limacina is the only gymnosome pteropod in Arctic waters, with a maximum size of 70-80 mm (Conover & Lalli 1972). This species is an important food source for baleen whales, seabirds and planktivorous fish (Lebour 1931, Lalli 1970). It has long been considered to feed exclusively on *Limacina* spp. (Meisenheimer 1905, Lalli 1970, Conover & Lalli 1972, Hopkins 1985). Such monophagous feeding strategy, with a strong dependency on one species with a patchy distribution, implies a high feeding efficiency and the need of energy reserves to survive long periods of food paucity (Böer et al. 2005). *C. limacina* is able to assimilate carbon from its prey at more than 90% efficiency and nitrogen at almost 100% efficiency (Conover & Lalli, 1972, 1974). A recent molecular study, however, suggests that this species may also feed on copepods and amphipods (Kallevik 2013). *C. limacina* is characterized by high lipid contents of up to 50% dry mass (Ikeda 1972, Lee 1974, Phleger et al. 1997, Böer et al 2005), mainly deposited as TAG and DAGE (Kattner et al. 1998, Falk-Petersen et al. 2001). DAGE are used as long-term energy reserves, whereas TAG serve as short and medium-term energy store (Kattner et al. 1998). Both lipid classes are utilized to fuel growth and reproduction processes. It is under debate to what extent these lipids also serve as buoyancy aids (Phleger et al. 1997, Böer et al. 2005).

Major fatty acids of *C. limacina* are 16:0, 18:0, 20:5(n-3), and 22:6(n-3), which are key constituents of phospholipids (Falk-Petersen et al. 2001). An exceptional feature of *C. limacina* is the significant occurrence of odd-chain fatty acids, such as 17:0 and 17:1(n-8), which together with 15:0 and 16:0 alkyl moieties dominate the composition of DAGE lipids (Böer et al. 2006). Previous studies suggested that the *de novo* synthesis of odd-chain fatty acids is initiated with propionate (3 carbon atoms) and that this precursor may originate from dimethylsulfoniopropionate (DMSP), which is accumulated in *Limacina spp.* from its phytoplankton food (Kattner et al. 1998, Falk-Petersen et al. 2001, Böer et al. 2005). Propionate would subsequently be elongated and desaturated (Kattner et al. 1998). Adult *C. limacina* can survive without food for almost a year (Böer et al. 2006). This exceptional long-term starvation ability is facilitated by a combination of very low metabolic activity, body shrinkage and utilization of lipid components (Böer et al. 2007). *C. limacina* also utilizes the lipid reserves to fuel maturation, gonad production and spawning during summer. The lipids stores are replenished during late summer and autumn, and they may serve as energy for metabolic

maintenance during winter, although it is not clear, if *C. limacina* ceases to feed during the dark period (Böer et al. 2005). The efficiency of gymnosomes to synthesize lipid components from their diet has not been well studied. Most studies focused on their capacity to synthesize odd-chain fatty acids (Kattner et al. 1998, Böer et al. 2005), but the metabolic turnover of fatty acids remains poorly understood.

This study aimed at examining the turnover of total lipid and of single fatty acids of pteropods, in order to better understand their role in the transfer of lipid carbon through the Arctic marine food web. Three major questions have been addressed: (1) How high are the total lipid turnover rates of the pteropods *Limacina* spp. and *C. limacina*? (2) Do *L. helicina* and *L. retroversa* differ with respect to dietary uptake of fatty acids? (3) To which extent does *C. limacina* incorporate fatty acids from *L. retroversa*? Feeding experiments were conducted with *L. helicina* juveniles and adults, *L. retroversa* adults, and *C. limacina* adults. To monitor the assimilation of dietary fatty acids as well as the turnover rates of carbon lipid, *Limacina* spp. were fed with ^{13}C labeled algae. Accordingly, labeled *L. retroversa* were offered to *C. limacina*. We analyzed the ^{13}C enrichment of fatty acids through compound specific isotope analysis (CSIA) following the method described in Graeve et al. (2005). To our knowledge, this is the first time that labeled feeding experiments have been successfully conducted with *L. helicina, L. retroversa*, and *C. limacina.*

Material and methods

Sampling and experimental work

Limacina retroversa adults were sampled on 23 September 2014 in Kongsfjorden, on the west coast of Spitsbergen (Norway), on board RV *Helmer Hanssen* by vertical hauls from 100 m to 0 m with a WP3 net (1000 μm mesh size, 1.0 m^2 net opening). *L. retroversa* adults that were used as food for *Clione limacina* were sampled following the same procedure, in Isfjorden, a fjord south of Kongsfjorden, every 3-4 days from 20 September to 10 October 2014. *L. helicina* adults were sampled on 28 July 2015 in Isfjorden. They were collected in surface waters, using a scoop net while snorkeling because sampling from a boat with WP2 or WP3 nets damaged their shells and caused rapid death after collection (pers. obs.). *L. helicina* juveniles were sampled by vertical net hauls from 50 m to 0 m with a WP2 net (200 μm mesh size, 0.25m^2 net opening) on

29 September 2015 in Isfjorden on board a small boat (*PolarCirkel*) from UNIS (University Centre in Svalbard).

C. limacina were sampled in the Arctic Ocean, north of Svalbard, in July 2014 on board RV *Lance*, by vertical hauls from bottom to surface with a MIK net (method Isaac Kid; opening 3.14m^2, mesh size 1.5 mm and net bag 7 m long). The organisms were kept in 20 L buckets filled with seawater, which was exchanged twice a week.

All individuals were transferred to containers filled with 0.7 µm filtered seawater of 4°C and transported to the laboratories at UNIS in thermo boxes within 5 h after capture, except for adult *L. retroversa*, which were kept on board for experiments.

Set up of feeding experiments

Live pteropods are extremely fragile. We therefore handled all individuals with great care and disturbed them as little as possible. Previous studies advised to use antibiotics to prevent bacterial infections (reviewed by Howes et al. 2014), but even small concentrations induced high mortalities of the thecosomes in our study, hence this method was abandoned for both *Limacina* spp. and *C. limacina*.

Feeding of *Limacina helicina* and *L. retroversa*

Immediately after arrival at UNIS, clearly active *Limacina* spp. specimens were sorted with glass pipettes. Due to their large size, adult *L. helicina* were identified by eye, whereas the smaller *L. helicina* juveniles and adults of *L. retroversa* were sorted under a dissecting microscope. The identification of the two species was based on shape and size, as described by Lalli & Wells (1978). *L. retroversa* dominated the thecosome community in 2014. They were easily recognizable by their pointy spiral shell and adults measured more than 1.0 mm in diameter. *L. helicina* prevailed in 2015. They were distinguishable from *L. retroversa* by their flat-coiled shell. The diameter of the shells of females ranged between 5.0-7.0 mm and juveniles had a shell diameter of 0.2-0.4 mm. All individuals were transferred to glass bottles containing 1 L of 0.7 µm filtered seawater. Three *in situ* replicates (t_0) of each species/stage were immediately frozen at -80 °C (Table 1).

The thecosomes were kept at continuous light to imitate ambient conditions, i.e. polar day. Light intensity was approximately 50 μmol m^{-2} s^{-1} (incident radiation measured with surface reference sensor in air LI-190, LI-COR). Two thirds of the water in all bottles were changed every second day to feed the thecosomes and maintain high oxygen concentrations. In parallel, three bottles were emptied and the animals deep-frozen (-80 °C) in triplicates for lipid analyses (Table 1). Mortality rates were low during the first four days. They increased by day 5-6 and were very high after day 6, therefore, the experiments were stopped after six days.

Table 1. Samples of *Limacina helicina* and *L. retroversa* (juveniles, adults) analyzed from the feeding experiments in 2014 and 2015. Number of specimens per replicate for each date (expressed in number of days from the beginning of the experiment).

Time	*L. helicina* juveniles	*L. helicina* adults	*L. retroversa* adults
Day 0	40,40,40	1,1,1	5,5,5
Day 2	45,50,52	1,1,1	8,8,9
Day 3	-	1,1,1	-
Day 4	49,50,56	1,1,1	10,10
Day 6	46,50,56	1,1,1	5,5,5

The thecosomes were fed with a mixture of diatoms (*Contricribra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*). Total concentrations were higher than 10,000 cells mL^{-1} (>> 200 µg Chl *a* L^{-1}), exceeding those of a typical spring bloom and ensuring excess of food for *Limacina* spp. (Howes et al. 2014). The algae were grown in a medium composed of 0.7 µm-filtered seawater enriched with f/2 Guillard medium (15 mL L^{-1}) and ^{13}C sodium bicarbonate (15 mg L^{-1} in 2014 and 1.5 mg L^{-1} in 2015). We used lower concentration of the ^{13}C labeled bicarbonate in 2015 than 2014 to adjust the labeling method. The concentration of ^{13}C in the algae and subsequently in the pteropods only needs to be slightly higher than the natural concentration to avoid an overload of the IRMS detector (Faraday cups). Therefore, we gradually decreased the quantity of ^{13}C for the algae labeling since 2005 (Graeve et al. 2005), and the atom% reached in the algae fatty acids was 3-4 times lower in 2015 than in 2014 (see results section).

Silicate was added to the medium for diatoms (1.5 mL L^{-1} of 7.5 µmol $Si(OH)_4$ L^{-1}) to ensure optimal growth. *R. salina* were kept at 4°C, *C. weissflogii* and *D. salina* were maintained at 15°C, since they were growing faster than at 4°C. All cultures were grown at a 12h light:12h dark day cycle. Before feeding the thecosomes, algal cells were counted with a haemacytometer (Schoen 1988) and Chl *a* concentrations were measured by fluorometry using methanol as the extracting solvent (Holm-Hansen & Riemann 1978). On average, the Chl *a* values reached 40 µg L^{-1}, confirming that food was provided in excess in the incubation bottles. Samples for algal lipid analyses were taken by filtering 2 x 5 mL of each algal culture on 0.7 µm GF/F filters (Whatman).

Ingestion rates of *L. helicina* juveniles were determined immediately after sampling on 22 September 2015. Live animals were sorted and transferred into to 1 L bottles filled with ambient water and sieved over 60 µm mesh to exclude larger zooplankton. Bottles contained 50, 100, and 200 individuals (2 replicates for each density). In addition, three bottles without pteropods served as controls. The bottles were attached to a slowly rotating plankton wheel and incubated for 24 h in the dark to avoid algal growth and at the ambient temperature of approximately 5° C. At the end of the experiment, the juveniles were retrieved from the bottles, and mortality rates were very low. Chl *a* concentrations were determined in two subsamples of 200 mL per bottle at the beginning and at the end of the experiment, according to the protocol described above in three subsamples of 200 mL per bottle. Ingestion rate was calculated according to Frost (1972) with slight modifications as Chl *a* removal rate: (Chl *a* initial - Chl *a* final) / (number of *L. helicina*). An ingestion rate of 3.4 x 10^{-4} µg Chl *a* ind^{-1} d^{-1} (n = 2) was found for *L. helicina* juveniles.

These ingestion rates expressed as µg Chl *a* ind^{-1} d^{-1} were converted to µg C lipid ind^{-1} d^{-1} using the measured content of Chl *a* per algal cell and the measured lipid content per cell. To calculate the lipid ingestion rates of adult *L. helicina*, we used 1.4 µg Chl *a* ind^{-1} d^{-1} as determined by Bernard et al. (2012). For adult *L. retroversa* we used 0.6 x 10^{-1} µg Chl *a* ind^{-1} d^{-1} as determined by Noji et al. (1997)

Feeding of *Clione limacina*

C. limacina, which were sampled in July 2014, were kept without food for ten weeks in 110 L aquaria filled with 0.7 µm filtered seawater. This starvation period corresponded to the time necessary to successfully culture and label their prey *L. retroversa*, once they had appeared in the fjord and were sampled. Half of the water was renewed twice a week to maintain sufficient levels of oxygen. Pumps were installed in the aquaria to generate a slow circular flow (JBL ProFlow 300, water circulation set at 80 L h^{-1}).

Feeding started on 23 September 2014 with *L. retroversa* that had been fed with ^{13}C labeled algae for three days. To keep track of the feeding success, *C. limacina* were individually kept in glass bottles containing 1 L of 0.7 μm filtered seawater. Five (on 23 and 25 September) or three (on 8, 10 and 13 October) active *L. retroversa* were transferred to the bottles. Feeding rates were determined by counting the number of emptied shells after 24 h and 48 h. Non-swimming *L. retroversa* specimens were removed, since *C. limacina* apparently feeds only on active *Limacina* spp. (Lalli 1970). The feeding experiment lasted for 23 days. Individuals were frozen three days after their last feeding episode (Table 2). Ingestion rates were calculated from the number of *L. retroversa*, and converted to lipid carbon, considering that *L. retroversa* contained on average 3.04 µg lipid C ind^{-1} (see results section).

Table 2. Samples of *Clione limacina* from the 2014 feeding experiment, duration of feeding and number of adult *Limacina retroversa* successfully ingested. n indicates the number of replicates (1 individual per replicate).

Time	**Number of adult *L. retroversa* ingested**
Day 0	0 (n=3)
Day 11	9 (n=3), 10 (n=2)
Day 17	5 (n=1), 9 (n=1), 10 (n=1)
Day 20	8 (n=2), 9 (n=1)
Day 23	7 (n=1), 9 (n=1), 10 (n=3), 17 (n=1), 18 (n=2)

Fatty acid analysis

Prior to lipid extraction, we removed the gut of *C. limacina* to exclude non-assimilated fatty acids from our calculations, since *L. retroversa* were clearly visible in the stomachs. The size ratio *Limacina* spp./algae is much larger than that of *C. limacina*/*Limacina* spp. therefore the guts were not removed from *Limacina* spp.

Total fatty acids of algae and pteropods were extracted according to Folch et al. (1957) with slight modifications, and separated using a gas chromatograph (HP 6890N, Agilent Technologies Deutschland GmbH & Co. KG) (see Boissonnot et al. 2016). The chromatograms were evaluated using ChemStation software (Agilent Technologies Deutschland GmbH & Co. KG). Total lipid mass per individual was calculated by summing up single fatty acid masses.

Carbon isotopic ratios

The ^{13}C isotopic enrichment in fatty acids was measured using a Thermo GC-c-IRMS (gas chromatography-combustion-isotope-ratio mass spectrometry) system (Thermo Scientific Corporation, Bremen, Germany) (see Boissonnot et al. 2016). For each analytical run, two reference gas pulses were used for data calibration at the start and at the end, together with the internal 23:0 FAME (δ -32.50 vs. PDB). The chromatographic peak areas and carbon isotope ratios were obtained with the instrument-specific software (Isodat 3.0) and the reference standards 14:0 and 18:0 FAME (Iowa University) were used with known δ-values for further calculations.

Isotopic ratios of each fatty acid are normally expressed in δ notation according to formula (1).

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

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

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

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

Results include the atom percent of enriched samples as well as their natural background (Brenna et al. 1997).

To only consider the enrichment that resulted from the assimilation of labeled food, the atom percent excess (ATE) was calculated according to (3).

(3) $$ATE\ (\%) = AT_{t=i} - AT_{t=0}$$

where *t=i* is the number of days since the beginning of the ^{13}C feeding experiment and *t=0*, the starting day of the experiment. $AT_{t=0}$ is therefore an average of the AT in all fatty acids *in situ*.

Since the dietary fatty acids did not only contain ^{13}C but also ^{12}C, to calculate the percentage of carbon assimilated in the pteropods fatty acids (PA), the atom percent excess (*ATE*) was divided by the total average labeling in the fatty acids of the food-source. For thecosome pteropods, average fatty acid atom percent in the algae mixture was used in the calculation ($AT_{algae,\ 2014}$ = 13.7%, $AT_{algae,\ 2015}$ = 3.7%). Average AT of *L. retroversa* fatty acids at Day 3 was used to estimate the portion of carbon assimilated by *C. limacina* ($AT_{L.\ retroversa,\ Day3}$ = 2.2%) (4).

(4) $$PA = \frac{ATE}{AT(food)}$$

To estimate the assimilation of carbon (C_{assi}) as mass (µg C_{assi} ind^{-1}) (5), the portion of carbon assimilated (*PA*) was multiplied by the mass of each fatty acid (*B*) expressed as carbon mass (in µg C ind^{-1}). The carbon mass was derived from the number of moles of fatty acid in the pteropods. The molecular mass of each labeled fatty acid was calibrated by its carbon atom percentage to incorporate the carbon mass variation according to the $^{13}C/^{12}C$ ratio (De Troch et al. 2012).

(5) $$C_{assi}(\mu g\ C\ ind^{-1}) = B \times PA = B \times \frac{ATE}{L}$$

Finally, the relative assimilation of fatty acids was calculated as (6).

(6) $$Relative_{assi} = \frac{C_{assi}}{TL_{assi}} \times 100$$

Statistical analyses

Statistical analyses were performed using the free software R 3.2.1 (team RDC 2010). Normal distribution of the total lipid and fatty acid turnover rates was tested with Shapiro-Wilk test. One-way ANOVA followed by Tukey HSD tests were performed on polynomial regressions (degree 2). Portions were arcsin transformed prior to tests.

Principal component analyses (PCA) were performed to explore the thecosomes fatty acid compositions and to determine their similarity. Variables were arcsin($\sqrt{x}$) transformed for better separation of observations when analyzing portions. Fatty acids comprising <1% were removed and the rest recalculated to 100%.

Results

Fatty acid composition and ^{13}C uptake of algal cultures

In all experiments (2014 and 2015), major fatty acids of the algae mixture (diatoms and flagellates) were 16:0, 16:1(n-7), 20:5(n-3) and 22:6(n-3), reflecting the contribution of both diatoms and flagellates to the fatty acid masses at a ratio of about 60% diatoms vs. 40% flagellates in 2014 and 50% vs. 50% in 2015 (Table 3). The mono-unsaturated fatty acid 16:1(n-7) and the PUFA 16:2(n-4) and 16:3(n-4) were mostly provided by diatoms (>89%). The PUFA 18:3(n-3) and 18:4(n-3) were largely synthesized by flagellates (>88%). The fatty acid 18:2(n-6) was found exclusively in flagellates.

On average, the algal lipid carbon added to the incubation bottles at each feeding event was 6005 µg C L^{-1} in 2014 and 9863 µg C L^{-1} in 2015. Throughout the experiment, the ^{13}C enrichment in the algae averaged at 13.7 atom% in 2014 and at 3.7 atom% in 2015 (Table 3). It remained constant throughout the feeding experiments with pteropods ($p>0.05$). In 2014, maximum enrichment occurred in 16:3(n-4) with 18.9 atom%. The minimum enrichment was detected in 18:0, with 2.4 atom% being labeled. In 2015, maximum enrichment was detected in 16:2(n-4) (5.3 atom%) and the minimum in 18:0 (1.2 atom%).

Table 3. Absolute and relative fatty acid (FA) compositions of the algal food from the 2014 and 2015 experiments. Contribution of diatoms (*C. weissflogii*) and flagellates (*R. salina* and *D. salina*) to the fatty acid masses in the mixture. The ^{13}C enrichment is expressed as atom% (AT%). The presented values are calculated from single algae cultures and averaged for the mixture, for the entire culture period (n = 6 at each date, 30 days of culture).

FA	Mass (µg C L^{-1})	Relative composition (% total lipid)	Contributions to FA mass (%)		AT%
			diatoms	flagellates	
2014					
14:0	114 ± 62	1.9 ± 3.9	42.9	57.1	13.0 ± 4.4
15:0	22 ± 19	0.4 ± 8.0	100.0	0.0	16.0 ± 0.7
16:0	780 ± 252	12.9 ± 5.5	70.3	29.7	13.9 ± 5.4
16:1(n-7)	1005 ± 355	16.7 ± 7.9	99.4	0.6	14.0 ± 3.6
16:2(n-4)	262 ± 101	4.4 ± 6.3	89.6	10.4	17.4 ± 0.5
16:3(n-4)	464 ± 299	7.7 ± 7.4	95.2	4.8	18.9 ± 0.5
18:0	168 ± 117	2.8 ± 4.7	64.8	35.2	2.4 ± 0.8
18:1(n-7)	75 ± 41	1.2 ± 6.0	0.0	100.0	15.0 ± 7.0
18:1(n-9)	93 ± 63	1.5 ± 6.8	0.0	100.0	12.9 ± 6.4
18:2(n-6)	78 ± 49	1.3 ± 6.2	0.0	100.0	13.0 ± 6.3
18:3(n-3)	467 ± 171	7.8 ± 5.0	11.1	88.9	17.2 ± 6.7
18:4(n-3)	475 ± 166	7.9 ± 7.1	2.5	97.5	11.7 ± 5.2
18:5(n-3)	37 ± 8	0.6 ± 8.0	0.0	100.0	11.6 ± 5.1
20:5(n-3)	1272 ± 484	21.1 ± 5.8	71.1	28.9	14.1 ± 4.8
22:6(n-3)	693 ± 232	11.5 ± 5.3	56.3	43.7	14.5 ± 5.9
2015					
14:0	215 ± 54	2.1 ± 4.5	32.1	67.9	4.2 ± 0.7
15:0	37 ± 26	0.4 ± 8.0	100.0	0.0	5.0 ± 0.3
16:0	1294 ± 90	12.9 ± 3.8	54.9	45.1	3.6 ± 0.4
16:1(n-7)	531 ± 305	5.3 ± 6.9	90.6	9.4	4.0 ± 1.3
16:2(n-4)	234 ± 165	2.3 ± 8.0	100.0	0.0	5.3 ± 0.4
16:3(n-4)	577 ± 363	5.8 ± 7.3	94.4	5.6	4.5
18:0	858 ± 65	8.6 ± 3.8	55.4	44.6	1.2 ± 0.1
18:1(n-7)	258 ± 126	2.6 ± 6.2	0.0	100.0	3.0 ± 0.7
18:1(n-9)	223 ± 86	2.2 ± 5.4	0.0	100.0	4.0 ± 0.8
18:2(n-6)	333 ± 150	3.3 ± 5.9	0.0	100.0	4.1 ± 0.8
18:3(n-3)	1218 ± 825	12.2 ± 7.7	2.1	97.9	4.2 ± 0.6
18:4(n-3)	1563 ± 1008	15.6 ± 7.5	4.4	95.6	3.4 ± 1.4
18:5(n-3)	7 ± 5	0.1 ± 8.0	0.0	100.0	1.5
20:5(n-3)	1649 ± 206	16.5 ± 3.9	58.8	41.2	3.8 ± 1.1
22:6(n-3)	868 ± 229	8.7 ± 4.6	31.3	68.7	3.1 ± 1.2

Total lipid and fatty acid composition of pteropods

During the feeding experiments the total lipid mass as well as the fatty acid masses did not change significantly in *Limacina* spp. (juveniles and adults) and adult *Clione limacina* specimens ($p>0.05$).

The total lipid mass of *L. helicina* juveniles averaged at 0.04 ± 0.01 µg C ind^{-1} (Table 4). Major fatty acids were 16:0, 18:0, 20:5(n-3) and 22:6(n-3), together contributing 57.4% to the total lipid mass. *L. helicina* juveniles also contained substantial quantities of 18:4(n-3) and of the long chain mono-unsaturated fatty acids (MUFA) 22:1(n-9) and 22:1(n-7) (>3.7%). The total lipid mass of *L. helicina* adults averaged at 203 ± 72 µg C ind^{-1}. The major fatty acids 16:0, 20:5(n-3) and 22:6(n-3) together accounted for 65.2% of the total lipid mass. In addition, the saturated fatty acid 14:0 and the mono-unsaturated fatty acids 16:1(n-7) and 20:1(n-7) were present in considerable concentrations in the individuals (>3.0%). Total lipid mass of *L. retroversa* adults averaged at 3.0 ± 1.1 µg C ind^{-1}. Major fatty acids were 16:0, 20:5(n-3) and 22:6(n-3), contributing 68.1% to the total lipid mass. Also, substantial amounts of 18:0, 20:1(n-9) and 20:1(n-7) were detected (>2.6%). *C. limacina* exhibited the highest body size variability, reflected by total lipid masses, which ranged from 29.2 µg C ind^{-1} to 1234.5 µg C ind^{-1} with an average of 148 ± 255 µg C ind^{-1}. The major fatty acids 16:0, 18:0, 20:5(n-3) and 22:6(n-3) accounted for 63.5% of the total lipid mass. The fatty acid composition of *C. limacina* differed from that of the thecosome pteropods due to significant amounts of 18:1(n-9), 18:2(n-6) and 20:4(n-6) (>2.8%). Moreover, odd-chain fatty acids such as 17:0 (4.8%), and less so 17:1(n-8), 19:0, and 19:1 (together 1.7% of total lipid mass) contributed to the total lipids of *C. limacina*.

Table 4. Fatty acid compositions of *Limacina helicina* juveniles, adults, *L. retroversa* adults and *Clione limacina* adults (% total lipid mass) during the entire experiment. The number of replicates is given in brackets.

Fatty acid	*L. helicina* juveniles (11)	*L.helicina* adults (10)	*L. retroversa* adults (10)	*C. limacina* adults (23)
14:0	1.6 ± 0.4	3.1 ± 0.7	1.5 ± 0.6	0.8 ± 0.8
15:0	1.0 ± 0.2	0.5 ± 0.1	1.0 ± 1.1	1.7 ± 1.1
16:0	25.0 ± 3.9	12.6 ± 3.3	12.2 ± 1.9	14.3 ± 3.3
16:1(n-9)	--	--	--	0.3 ± 0.2
16:1(n-7)	2.0 ± 1.2	3.0 ± 1.3	1.5 ± 0.7	1.6 ± 1.1
16:2(n-4)	2.2 ± 0.3	0.4 ± 0.1	0.8 ± 0.3	0.3 ± 0.4
16:3(n-4)	1.8 ± 1.2	0.2 ± 0.1	0.6 ± 0.1	1.6 ± 1.5
17:0	--	--	--	4.8 ± 1.3
17:1(n-8)	--	--	--	1.1 ± 0.6
18:0	13.3 ± 4.0	3.0 ± 0.8	3.4 ± 1.0	14.0 ± 8.1
18:1(n-9)	1.1 ± 0.6	2.0 ± 0.6	0.6 ± 0.1	4.1 ± 3.8
18:1(n-7)	1.1 ± 0.7	1.0 ± 0.3	0.8 ± 0.2	1.4 ± 0.6
18:1(n-5)	--	0.1 ± 0.0	--	0.6 ± 0.4
18:2(n-6)	0.9 ± 0.3	1.4 ± 0.4	0.6 ± 0.2	2.8 ± 2.2
18:3(n-6)	--	--	0.1 ± 0.1	0.0 ± 0.0
18:3(n-3)	1.0 ± 0.5	1.8 ± 0.4	1.2 ± 0.3	0.5 ± 0.4
18:4(n-3)	4.4 ± 1.4	2.7 ± 0.6	0.7 ± 0.4	0.8 ± 0.8
19:0	--	--	--	0.3 ± 0.3
19:1	--	--	--	0.3 ± 0.4
20:0	3.1 ± 0.8	0.5 ± 0.2	1.2 ± 0.2	1.0 ± 0.4
20:1(n-11)	0.8 ± 0.3	0.6 ± 0.3	1.1 ± 0.5	1.0 ± 0.4
20:1(n-9)	2.1 ± 0.7	2.9 ± 1.1	2.6 ± 0.5	1.7 ± 0.6
20:1(n-7)	3.1 ± 1.8	4.1 ± 1.1	3.6 ± 0.5	2.6 ± 1.0
20:2(n-6)	--	--	--	1.7 ± 0.9
20:3(n-6)	1.4 ± 1.0	0.9 ± 0.2	2.3 ± 0.5	--
20:3(n-3)	0.7 ± 0.5	2.1 ± 0.6	1.6 ± 0.6	--
20:4(n-6)	0.9 ± 0.5	0.8 ± 0.2	0.9 ± 0.3	3.5 ± 2.2
20:4(n-3)	1.2 ± 0.8	1.1 ± 0.3	1.4 ± 0.6	0.7 ± 1.2
20:5(n-3)	10.2 ± 4.5	23.7 ± 5.9	25.9 ± 4.7	12.2 ± 5.5
22:1(n-11)	2.1 ± 1.8	0.6 ± 0.5	0.3 ± 0.1	0.1 ± 0.3
22:1(n-9)	4.7 ± 4.1	0.5 ± 0.2	0.8 ± 0.4	0.6 ± 0.5
22:1(n-7)	3.7 ± 2.6	--	0.2 ± 0.1	--
22:5(n-3)	1.6 ± 0.8	1.1 ± 0.3	2.3 ± 1.6	0.6 ± 0.9
22:6(n-3)	9.0 ± 4.0	28.9 ± 7.2	30.0 ± 7.1	23.0 ± 10.2
Main FA	57.4 ± 8.2	68.2 ± 9.9	71.5 ± 8.8	63.5 ± 14.6
Diat. FATM	6.0 ± 1.7	3.5 ± 1.3	2.9 ± 0.8	3.5 ± 1.9
Flag. FATM	6.3 ± 1.5	5.9 ± 0.8	2.6 ± 0.6	4.1 ± 2.4
LC-MUFA	16.5 ± 5.5	8.7 ± 1.7	8.7 ± 1.0	6.1 ± 1.4
Odd-chain FA	1.0 ± 0.2	0.5 ± 0.1	1.0 ± 1.1	8.2 ± 1.9

To visualize the differences of fatty acid compositions related to species and developmental stages, a PCA was performed on *L. helicina (*adults, juveniles) and *L. retroversa* (adults) (Fig. 1). Two principal components explained 55.7% (PC1: 40.9%; PC2: 14.8%) of the total variance in the data set. Three groups, which correspond to the thecosome species and stages, were identified by PCA analysis. Adults of *L. helicina* and *L. retroversa* had similar compositions, mostly driven by the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3). *L. helicina* juveniles formed a well-differentiated group, with high concentrations of the saturated FA 16:0, 18:0 and 20:0, as well as C_{16} PUFA and the long chain mono-unsaturated fatty acid 22:1 (both isomers: n-11 and n-9).

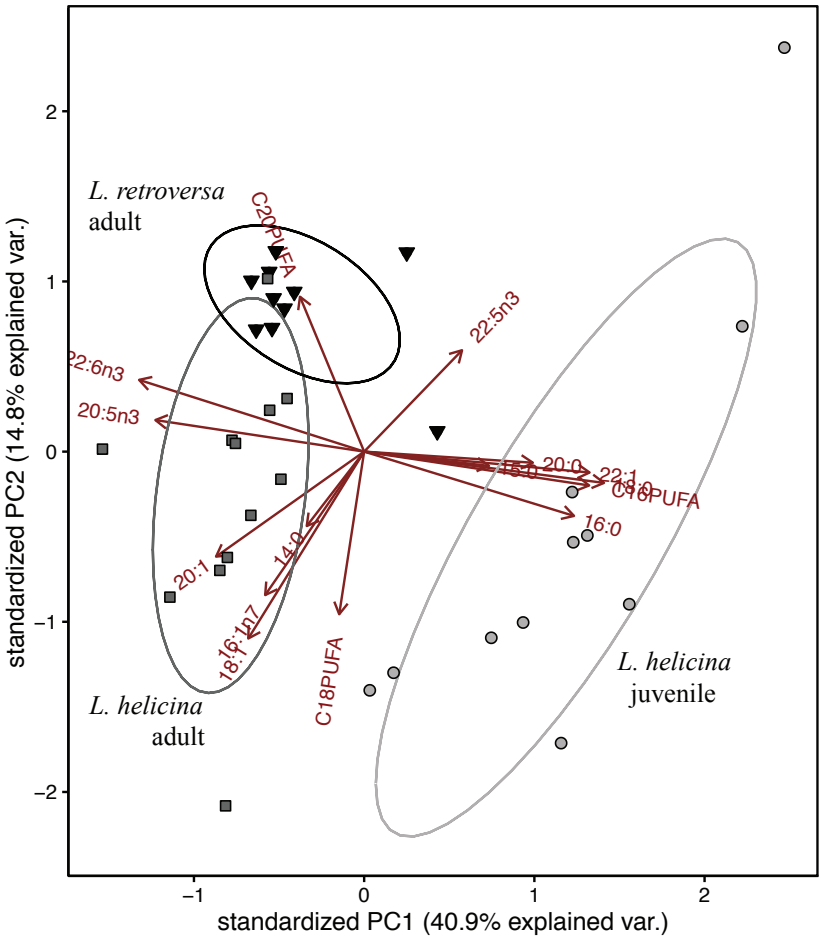

Figure 1. Biplot of principal component analysis of the fatty acid compositions of *Limacina helicina* juveniles, adults and *L. retroversa* adults. Variables are arcsin(sqrt) transformed. The circles indicate confidence intervals.

Carbon assimilation

Total lipid

The ingestion rate of *L. helicina* juveniles averaged 9.0 10^{-4} µg lipid C ind^{-1} d^{-1}. Individuals assimilated 13.2% of the total lipids of the ingested food. The increase of labeled lipid carbon was significant ($p<0.001$), reaching 1.4 ± 0.03% of the total lipid mass within six days (Fig. 2).

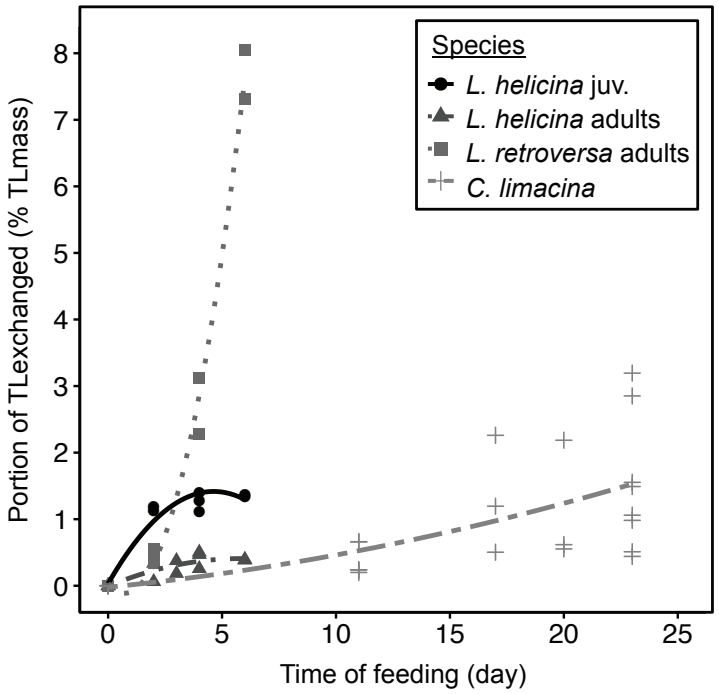

Figure 2. Portion of total lipid (TL) exchanged, expressed as % of total lipid mass, in *Limacina helicina* juveniles, adults, *L. retroversa* adults and *Clione limacina* adults during the feeding experiment (6 days for *Limacina* spp., 23 days for *C. limacina*).

The daily total lipid turnover rate was of 1.2 x 10^{-4} µg C ind^{-1} day^{-1}, which corresponds to 0.2 ± 0.05 % of total lipid carbon day^{-1}. The ingestion rate of *L. helicina* adults, calculated from Bernard et al. (2012) was 3.6 µg lipid C ind^{-1} d^{-1}. Individuals assimilated 1.1% of the total lipids of the ingested food. They had a lower carbon turnover rate than the juveniles, with only 0.3 ± 0.0006% exchanged by Day 6 (significant increase: $p<0.01$). The daily rate of total lipid turnover was of 0.04 µg C ind^{-1} day^{-1}, equaling 0.1 ± 0.001% of total lipid carbon day^{-1}. The ingestion rate of *L. retroversa* adults, calculated from Noji et al. (1997), was 0.2 µg lipid C ind^{-1} d^{-1}. They

assimilated 15.0% of the ingested lipids, 7.7 ± 0.5% of the total lipid carbon was replaced by Day 6. The portion of exchanged total lipid strongly increased during the 6 days of feeding ($p<0.001$). An average of 0.02 µg C ind^{-1} day^{-1} was assimilated as lipids, which corresponds to a daily rate of 1.3 ± 0.1% total lipid carbon day^{-1}. *C. limacina* ingested 0.6 *L. retroversa* ind^{-1} d^{-1}, which corresponded to an ingestion rate of 1.8 µg lipid C ind^{-1} d^{-1}. They assimilated 1.4% of the ingested lipids. Only 1.5 ± 1.0% of the total lipid carbon was replaced after 23 days. The daily total lipid assimilation rate was 0.03 µg C ind^{-1} day^{-1}, which corresponds to 0.07 ± 0.04% of total lipid day^{-1}.

Fatty acids

In *L. helicina* juveniles, maximum carbon assimilation was found in the dominating fatty acids 16:1(n-7), 20:5(n3) and 22:6(n-3) (51.0 ± 2.9 % of the total lipid carbon assimilated, Table 5). The turnover of structural fatty acids 16:0, 18:0, 20:5(n-3) and 22:6(n-3) ranged between 0.4 ± 0.01% and 2.2 ± 0.4% by Day 6 ($p<0.01$) (Fig. 3). Diatom FATM were assimilated at high rates, especially 16:1(n-7) with 6.2 ± 1.3% by Day 6 ($p<0.001$). Flagellate FATM were exchanged at a lower rate with 0.4 ± 0.2% to 1.1 ± 0.6% by Day 6 ($p<0.001$). Long-chain MUFA 20:1(n-7) and 22:1 (both isomers) were exchanged between 0.8 ± 0.03% and 4.9 ± 1.9% by Day 6 ($p<0.001$), while only traces of 20:1(n-9) were exchanged ($p>0.05$). The odd-chain fatty acid 15:0 was renewed at 1.6 ± 0.2% by Day 6 ($p<0.01$).

Table 5. Relative composition of fatty acids assimilated by *Limacina helicina* juveniles, adults, *L. retroversa* adults and *Clione limacina* adults (% total fatty acids assimilated). Only fatty acids that are shown in the figures of assimilation are presented in this table. Averages over Day 4 and 6 were calculated for *Limacina* spp. since both days reflected the same assimilation pattern. Day 23 was used for *C. limacina*. Number of replicates is shown in brackets.

Fatty acid	***L. helicina* juv. (4)**	***L. helicina* adult (4)**	***L. retroversa* adult (4)**	***C. limacina* (8)**
15:0	0.9 ± 0.3	9.0 ± 1.3	0.1 ± 0.1	1.1 ± 0.9
16:0	37.0 ± 0.4	20.2 ± 8.1	23.8 ± 1.2	26.6 ± 4.1
16:1(n-7)	11.7 ± 7.0	15.6 ± 4.3	1.0 ± 0.3	3.8 ± 2.8
16:2(n-4)	0.9 ± 0.1	3.0 ± 2.1	0.3 ± 0.1	0.0 ± 0.0
16:3(n-4)	1.2 ± 0.5	3.2 ± 3.6	0.5 ± 0.1	2.1 ± 2.5
17:0	-	-	-	4.8 ± 1.5
17:1(n-8)	-	-	-	0.9 ± 1.1
18:0	4.8 ± 1.4	13.9 ± 7.4	1.7 ± 0.3	6.5 ± 3.2
18:2(n-6)	0.7 ± 0.0	1.5 ± 2.5	4.8 ± 0.6	4.6 ± 4.4
18:3(n-3)	1.4 ± 0.4	7.3 ± 2.2	38.5 ± 0.7	19.2 ± 3.2
18:4(n-3)	1.8 ± 2.7	14.5 ± 8.5	7.7 ± 2.9	0.8 ± 1.1
19:1	-		-	0.9 ± 1.0
20:1(n-7)	2.4 ± 1.2	0.0 ± 0.0	2.1 ± 0.3	3.5 ± 1.0
20:1(n-9)	0.4 ± 0.3	1.1 ± 0.2	3.0 ± 0.0	7.2 ± 2.2
20:5(n-3)	6.4 ± 2.2	0.3 ± 0.1	9.1 ± 1.7	11.2 ± 3.9
22:1(n-11)	2.1 ± 0.2	-	-	0.3 ± 0.5
22:1(n-9)	25.4 ± 9.2	-	0.1 ± 0.0	0.3 ± 1.6
22:6(n-3)	2.8 ± 1.1	10.2 ± 2.9	7.4 ± 2.1	6.5 ± 4.9
Main FA	51.0 ± 2.9	44.7 ± 11.4	41.9 ± 3.0	50.7 ± 8.1
Diat. FATM	13.8 ± 7.0	21.8 ± 6.0	1.8 ± 0.3	5.9 ± 3.7
Flag. FATM	4.0 ± 2.7	23.3 ± 9.1	51.0 ± 3.0	24.5 ± 5.5
LC-MUFA	30.4 ± 1.2	1.1 ± 0.2	5.2 ± 0.3	11.3 ± 2.9
Odd-chain FA	0.9 ± 0.3	9.0 ± 1.3	0.1 ± 0.1	7.6 ± 2.3

Maximum carbon assimilation in *L. helicina* adults was determined in diatom FATM (55.4 ± 16.7% of total lipid assimilated, Table 5). The specimens exchanged small amounts of carbon in major fatty acids (0.3%, $p<0.05$) and in MUFA (traces, $p>0.05$) (Fig. 4). Diatom fatty acids were exchanged between 0.4 ± 0.1% ($p<0.05$) and 1.2 ± 0.4% ($p<0.01$) by Day 6. Flagellate markers were exchanged at similarly low rates as diatom markers (0.8 ± 0.4% in 18:3(n-3) and 0.5 ± 0.2% in 18:4(n-3) exchanged by Day 6, $p<0.05$). The fatty acid 18:2(n-6) was not exchanged significantly ($p>0.05$). The odd-chain 15:0 was exchanged at 1.5% by Day 6 ($p<0.01$, n = 1 at Day 6).

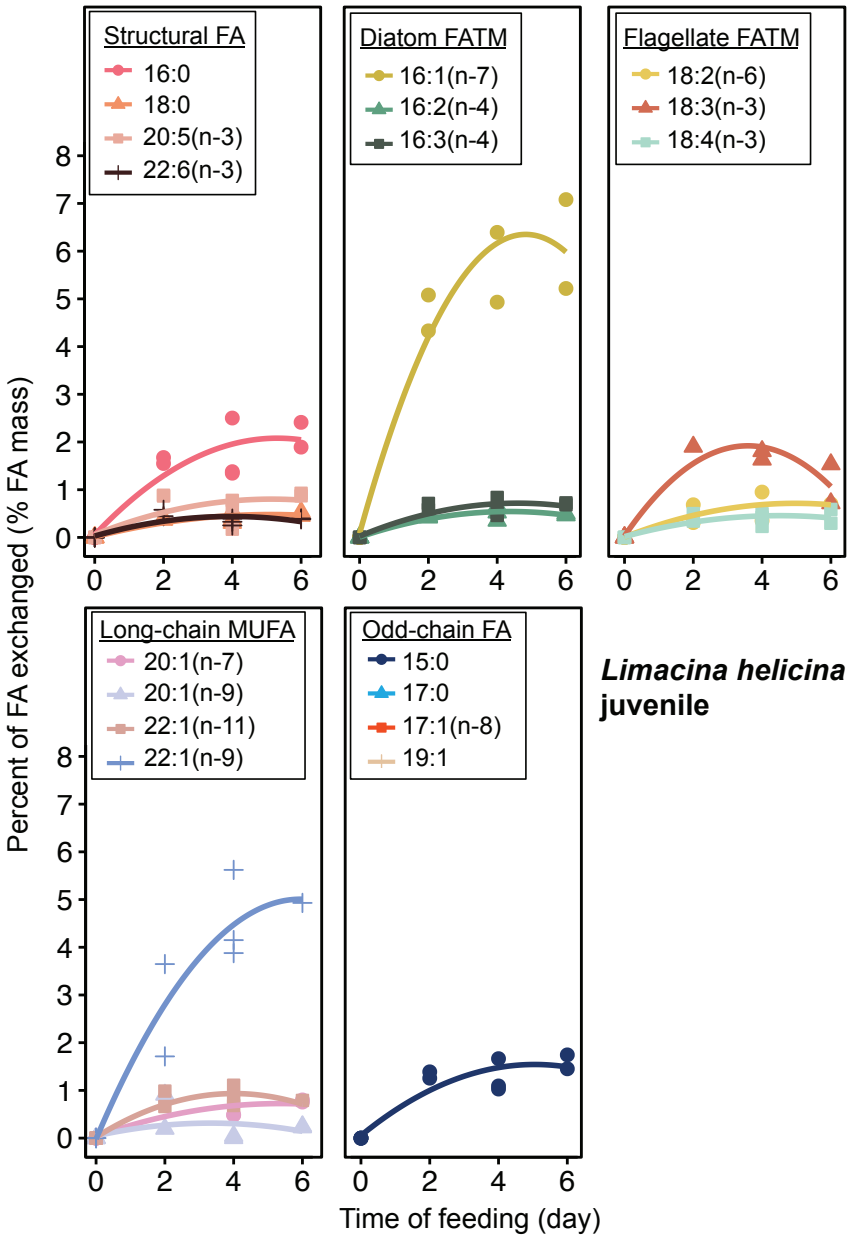

Figure 3. Portion of fatty acids (FA) exchanged by *Limacina helicina* juveniles (expressed as % of fatty acid mass) during the feeding experiment (6 days).

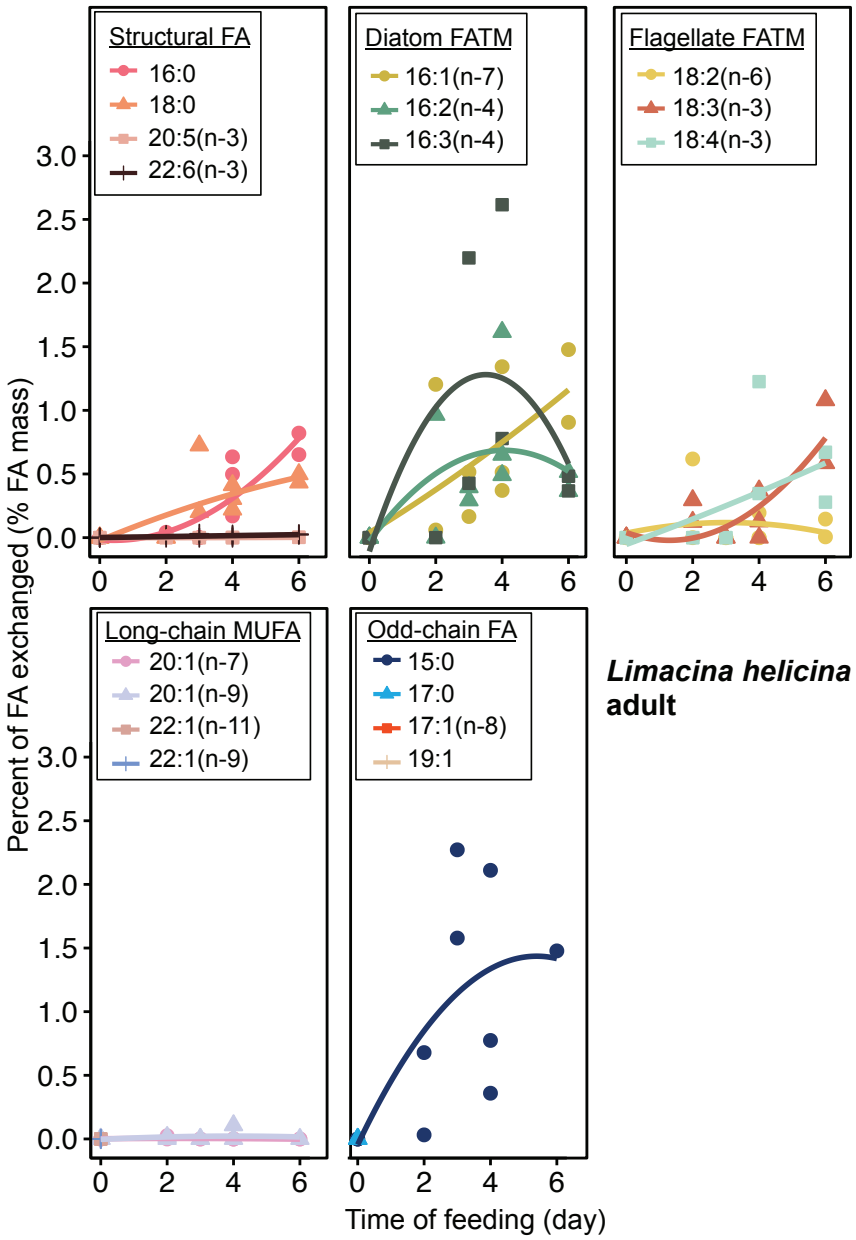

Figure 4. Portion of fatty acids (FA) exchanged by *Limacina helicina* adults (expressed as % of fatty acid mass) during the feeding experiment (6 days).

The highest carbon assimilation in *L. retroversa* adults occurred in flagellate FATM (51.0 ± 3.0% of total lipid assimilated) (Fig. 5). By Day 6, 1.2 ± 0.1% to 5.9 ± 0.7% ($p<0.001$) were renewed in the structural fatty acids 16:0, 18:0, 20:5(n-3) and 22:6(n-3). Between 1.6 ± 0.6 and 0.8 ± 0.7% of the diatom FATM were exchanged by Day 6 ($p<0.05$). Flagellate markers showed the highest assimilation, with 13.9 ± 0.9% ($p<0.001$) to 59.9 ± 0.3% ($p<0.001$) renewed by Day

6. Long-chain MUFA 20:1(n-7), 20:1(n-9) and 22:1(n-9) were exchanged between 0.4 ± 0.01% and 4.6 ± 2.6% ($p<0.001$). By Day 4, 15:0 reached 0.4 ± 0.2% carbon exchange ($p<0.01$) (below detection limit at Day 6).

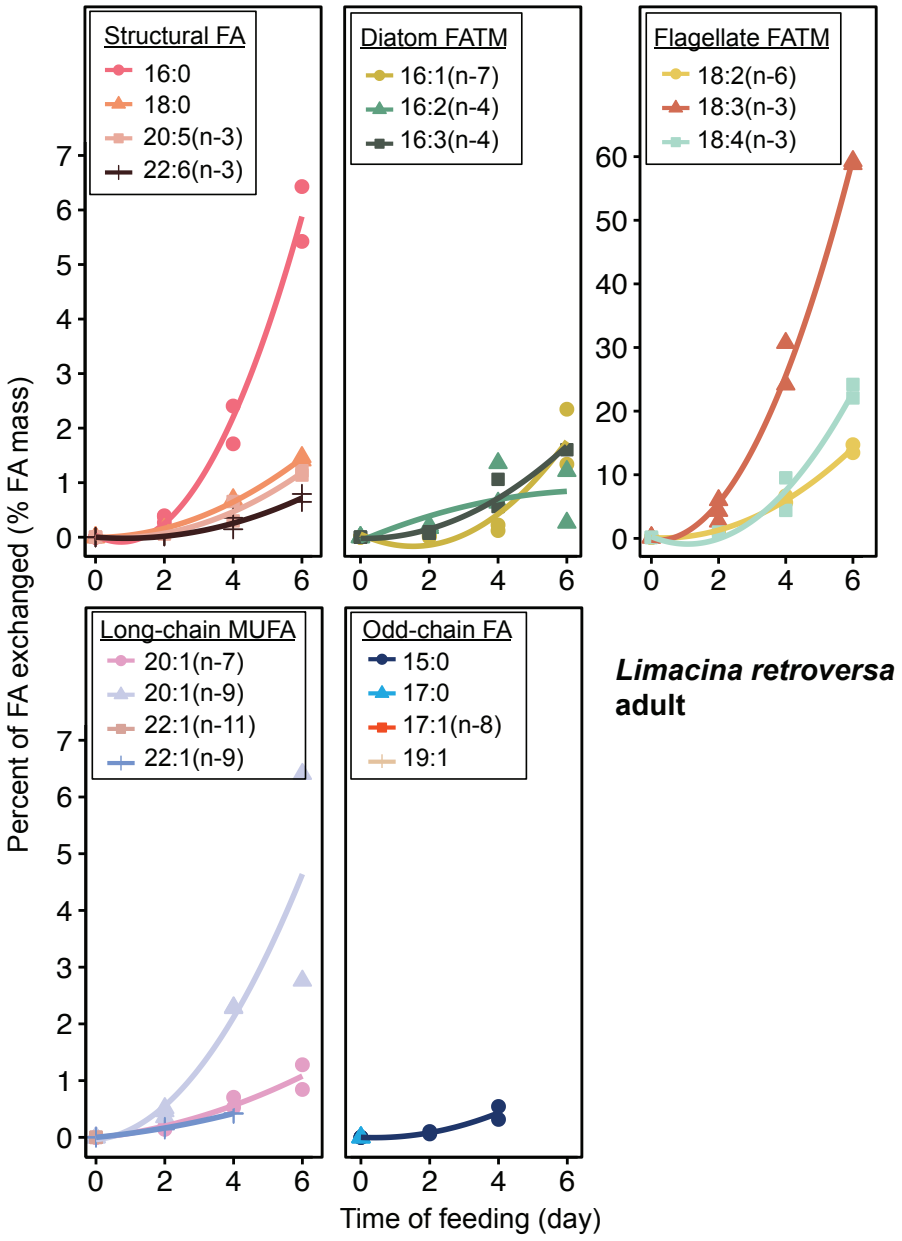

Figure 5. Portion of fatty acids (FA) exchanged by *Limacina retroversa* adults (expressed as % of fatty acid mass) during the feeding experiment (6 days). Please note the different scale used for flagellate FATM.

C. limacina showed the lowest carbon exchange rates of most fatty acids. However, carbon assimilation was detectable for most fatty acids (Fig. 6). Maximum carbon assimilation occurred in structural fatty acids 16:0, 18:0, 20:5(n-3) and 22:6(n-3) (50.7 ± 8.1% of total lipid assimilated). The exchanged carbon in these fatty acids reached between 0.1 ± 0.01% ($p<0.05$) and 1.3 ± 0.9% ($p<0.001$). Diatom FATM 16:1(n-7) reached 1.8 ± 1.4% ($p<0.01$), 16:3(n-4) had a lower assimilation (1.1 ± 0.9% at Day 23, $p<0.01$), and no assimilation was detectable in 16:2(n-4) ($p>0.05$). As in *L. retroversa*, flagellate markers were exchanged at a high rate, especially 18:3(n-3) (21.9 ± 18.9% at Day 23, $p<0.01$). Among long-chain MUFA, only 20:1(n-7) and 20:1(n-9) were exchanged at detectable amounts, with 2.2 ± 1.4% and 0.7 ± 0.3%, respectively, by Day 23 ($p<0.001$). Odd-chain fatty acids were also renewed, ranging between 0.9 ± 0.4% and 1.2 ± 0.7% by the end of the experiment ($p<0.01$).

Discussion

Limacina species have often been in the focus of ocean acidification research due to their calcified shell, including mesocosm and experimental approaches. It is still very difficult to keep these fragile and highly sensitive organisms in culture over an extended period of time, even in large water volumes (Lischka et al. 2010, Niehoff et al. 2013, Howes et al. 2014). In accordance, mortality rates of *Limacina* spp. increased after six day in our experiment. During the first days, however, the mortality rates were low and *Limacina* spp. exhibited normal swimming behavior. All species and stages showed an exchange of fatty acids, clearly indicating that the animals were feeding and incorporating at least part of the dietary components into lipids. Compared to Arctic copepods (Graeve et al. 2005, Boissonnot et al. 2016), the lipid exchange rates in *Limacina* spp. were low, which can at least partially be attributed to their very low ingestion rates, when being cultured (Maas et al. 2011, Howes et al. 2014, our study). The experiments with *L. retroversa* and *L. helicina* were performed in different years, but essentially under the same laboratory conditions and diets. Therefore we believe that we can compare the assimilation capacities among all species and stages.

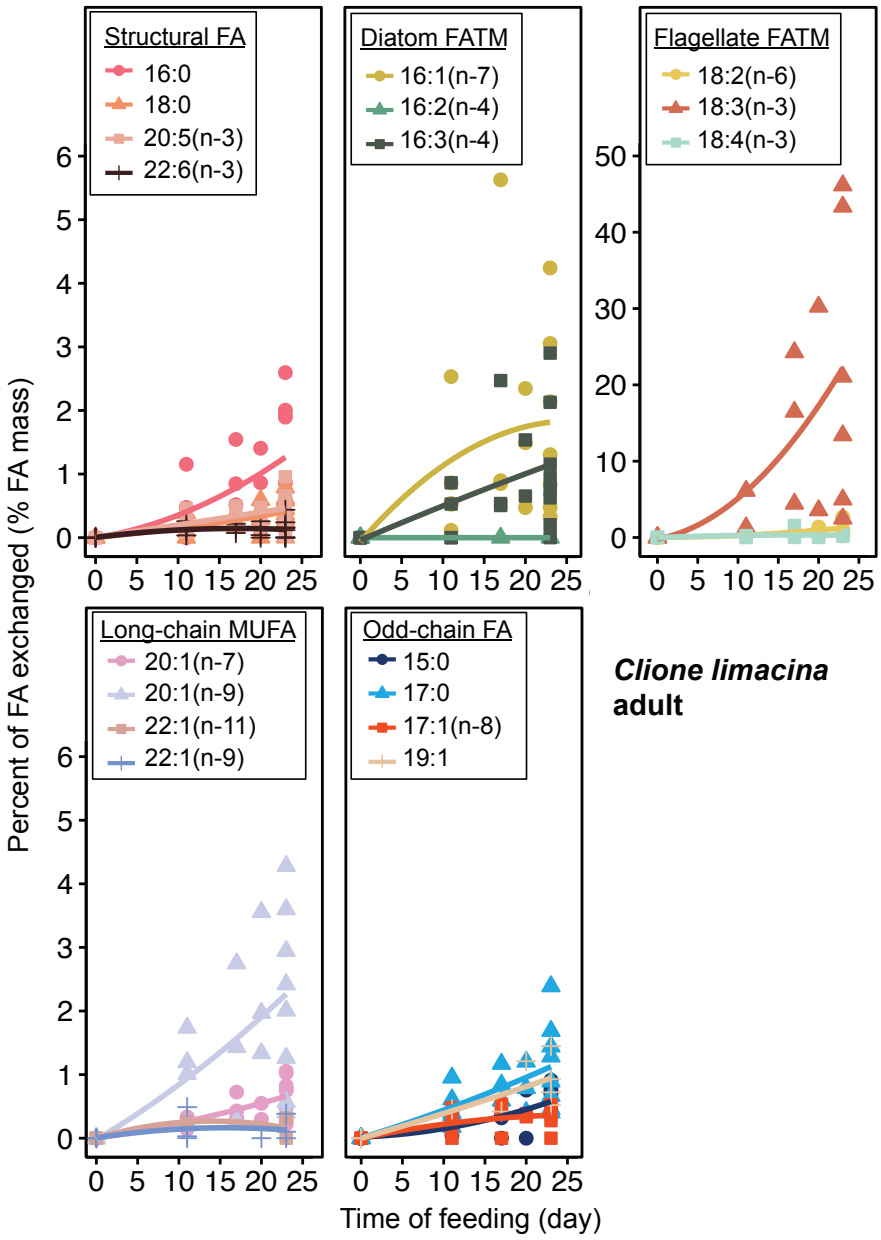

Figure 6. Portion of fatty acids (FA) exchanged by *Clione limacina* adults (expressed as % of fatty acid mass) during the feeding experiment (23 days). Please note the different scale used for flagellate FATM.

The thecosomes were fed with a mixture of flagellates and diatoms at a cell number ratio of 1:1 and at more than 800 µg C L^{-1}, which matches *ad libitum* food concentrations (Howes et al. 2014). Diatoms mainly provided the monounsaturated FA 16:1(n-7) and the C_{16} PUFA 16:2(n-4) and 16:3(n-4), while flagellates added high amounts of the C_{18} PUFA 18:2(n-6), 18:3(n-3) and

18:4(n-3), as previously reported (Ackman et al. 1968, Graeve et al. 1994, Falk-Petersen et al. 1998).

All fatty acids were sufficiently labeled, except for 18:0, which showed very low concentrations of ^{13}C label (2.4 atom% in 2014 and 1.2 atom% in 2015) in all algal cultures and throughout the experiment. Low labeling of 18:0 has been previously observed (Boissonnot et al. 2016) and might be due to physiological processes, resulting in varying turnover rates (Li et al. 2014).

Stage-related differences in fatty acid assimilation of *L. helicina*

Turnover of total lipid (considered in this study as the sum of fatty acids) and specific fatty acids varies with developmental stage. It depends on growth rates and stage-related needs in specific compounds (Brett et al. 2009). It has been suggested that *L. helicina* feeds opportunistically on any food particles collected by their mucous net (Gilmer 1972, Harbison and Gilmer 1992, Falk-Petersen et al. 2001, Gannefors et al. 2005). Our results confirm this for adults, since they assimilated FATM from both flagellates and diatoms in similar proportions, showing no preference for either algal group. In contrast, juveniles assimilated higher portions of diatom markers than those of flagellates (14% vs. 4% of assimilated fatty acids) even though they were sampled in September, when flagellates prevailed in the water column (Leu et al. 2006, Søreide et al. 2010, Hegseth and Tverberg 2013). This highlights the potential importance of diatom-derived fatty acids for the development of young thecosomes. In the light of decreasing sea ice cover and thus possibly declining diatom spring blooms (Li et al. 2009, Gao et al. 2012, Hegseth and Tverberg 2013), this issue deserves further attention in Arctic marine research.

Females were sampled in late July. We believe that the gonads of these females were mature because (1) *L. helicina* is known to reproduce in late summer/autumn in that area (Gannefors et al. 2005) and (2) veliger larvae were abundant in the water column from August on, indicating that spawning had occurred shortly after sampling the females. At capture, the females of *L. helicina* had significantly higher concentrations of the two omega-3 fatty acids EPA 20:5(n-3) and DHA 22:6(n-3) than the juveniles. In fact, their concentrations were the highest yet observed

in *L. helicina* in this season (Gannefors et al. 2005). These fatty acids are essential for gonad maturation processes and hatching success of copepods (Jónasdóttir et al. 2009) and it is very possible that they have a similar function in pteropods. Female and juvenile *L. helicina* also differed with respect to the carbon turnover of 18:4(n-3). In adults it reflected nearly 15% of the assimilated carbon, while it was only 2% in the juveniles. Gannefors et al. (2005) found high amounts of 18:4(n-3) in egg ribbons. Hence, this flagellate FATM also could be essential for reproductive processes.

Late in the season, *L. helicina* females are usually lipid-depleted, suggesting that they cease feeding or at least reduce feeding to only fuel basic metabolic needs, and it has been reported that most of them die shortly after reproduction (Fabry 1989, Gannefors et al. 2005, Hunt 2008). This could explain why we did not find any *L. helicina* females in the Kongsfjord/Isfjord by the end of August. During our experiment, however, the females were still feeding, as indicated by the continuous continued exchange of their fatty acids, and at least part of the diet-derived energy was invested in lipid biosynthesis, although at a very low rate. It is thus possible that the depletion of lipids in the natural habitat is due to low food availability.

Whether or whether not *L. helicina* juveniles grow during winter is under discussion. Lischka et al. (2012) suggested that growth ceases in winter and starts again in spring, at the onset of the phytoplankton bloom in the column water. Bednaršek et al. (2012) found that in the Southern Ocean *L. helicina antarctica* grows during winter, but at a lower rate than in spring/summer. The winter growth may be supported by omnivorous feeding and/or by lipid reserves (Paranjape 1968, Lalli & Gilmer 1989). In our study, *L. helicina* juveniles incorporated total lipids more intensively compared to adults. This suggested that this energy may be stored to prepare the sustainment of their growth throughout autumn and winter, in addition to feeding on the low levels of available food.

Zooplankton usually incorporates most dietary fatty acids without modifications, while other fatty acids are synthesized *de novo*. In copepods, for example, long-chain MUFA are produced from non-lipid dietary precursors. They serve as long-term energy reserves, usually stored as wax esters, and fuel winter metabolism and reproductive processes (Sargent & Falk-Petersen

1988, Kattner et al. 2007). Long-chain MUFA have also been found in considerable amounts in *L. helicina* from the Arctic and Southern Ocean and have been associated with feeding on copepods (Kattner et al. 1998, Falk-Petersen et al. 2001, Gannefors et al. 2005). We also detected long-chain MUFA in *L. helicina* and our results even show that carbon was assimilated into these compounds. The assimilation was statistically significant despite a high standard deviation of these fatty acids masses. In our incubation, copepods or copepod debris were not offered to the thecosomes and can thus be excluded as a source for these fatty acids. Hence, *L. helicina* may have the potential to synthesize long-chain MUFA. Such a capacity has been demonstrated for benthic gastropods (Ackman & Hooper 1973) and possible pathways may include the desaturation and elongation of dietary fatty acids. Further work is needed to address this question and clarify the origin of long-chain MUFA in *Limacina* spp.

Lipid assimilation processes in *Limacina helicina* and *L. retroversa* - a comparison

Sub-polar species usually have a higher metabolism compared to polar species, with faster developmental rates and shorter life cycles (Scott et al. 2000). Accordingly, in our study the North Atlantic species *L. retroversa* assimilated total lipids 13 times faster than the Arctic *L. helicina* in our study, indicating that they were feeding more intensely and/or assimilated lipids more rapidly. The difference in lipid assimilation between the two species may also be season-specific. High-latitude species, such as the copepod *Calanus hyperboreus*, are particularly efficient to accumulate lipids during a short period of primary production, whereas sub-polar species are better adapted to a longer productive period (Madsen et al. 2001, Ringuette et al. 2002, Søreide et al. 2008, Falk-Petersen et al. 2009). Therefore, *L. helicina* may be more efficient to build up lipid reserves in spring than in late summer, when the experiment was conducted. On the contrary, *L. retroversa* may be efficient to accumulate lipids during a longer period than *L. helicina*, which includes the time when the experiment was conducted.

The feeding biology of *L. retroversa* is poorly studied. The few studies that are available suggested that, in contrast to the omnivorous *L. helicina*, *L. retroversa* feeds primarily on flagellates and additionally on diatoms of ingestible size and shape (Morton 1954, Perissonotto

1992). Although *L. retroversa* was fed in equal proportions with diatoms and flagellates in our study, it assimilated FATM mainly from flagellates (51% of assimilated fatty acids vs. 2% from diatoms), corroborating that this species fed more and/or better assimilates fatty acids from flagellates than from diatoms. *L. helicina*, in contrast, showed no preference for one or the other algae taxa. The deviating feeding strategies of the two species may be due to evolutionary traits related to their different natural environments.

Also the life cycle of *L. retroversa* in the Arctic is not fully understood. Some studies suggest a one-year life span with one reproductive event in spring (Hsiao 1939) or in autumn, which would be similar to the life history of *L. helicina* (Meinecke et al. 1990). Other authors suggest that reproduction takes place throughout the year, with a peak in spring and in autumn (Lebour 1932, Dadon & De Cidre 1992). In our study, *L. retroversa* occurred in the fjord in mid-August and disappeared after mid-October. When we sampled the females for our experiments in mid September, they were reproducing as a parallel field study on the abundance and distribution of this species suggests (Boissonnot et al., unpublished data). The fatty acid compositions of adult *L. retroversa* and *L. helicina* were similar. *L. retroversa* also contained large amounts of EPA, DHA and the FATM 16:1(n-7), C_{16} PUFA and C_{18} PUFA (53% of the assimilated fatty acids). This suggests that *L. retroversa* females, like *L. helicina*, had mature gonads at capture and were using the FATM for egg production (Jónasdóttir et al. 2009). Another similarity between the two species was that *L. retroversa* adults disappeared from the water column in October while in. the experiments *L. retroversa* specimens continued to feed as indicated by exchanging their long-chain MUFA. Similarly to *L. helicina* juveniles, *L. retroversa* may thus also use these long-term energy reserves for overwintering.

Lipid transfer from *Limacina retroversa* to *Clione limacina*

Total lipid assimilation by *Clione limacina* (1.4% of the ingested lipids) was very low compared to the study conducted by Böer et al. (2006) on the same species, which report a complete assimilation of carbon within 1 week. The amount of carbon offered varied however, considerably among the experiments and this could at least partly explain the differences in lipid

assimilation rates: Böer et al. (2006) fed *C. limacina* with one *L. helicina* in five days (total lipid: 1.2 mg ind^{-1}, corresponds to app. 0.9 mg C ind^{-1}). In our study, *C. limacina* were fed with 3-4 of the much smaller *L. retroversa* within five days (total lipid: 0.01 mg C ind^{-1}). Therefore, the total lipid mass available for *C. limacina* was 10 times lower than in the experiment by Böer et al. (2006). The low lipid assimilation may also have been related to the physiological state of *C. limacina*. In our study, *C. limacina* were collected during the period of high reproductive activity (Mileikovsky 1970) and then starved for ten weeks. Body shrinkage associated with metabolic reduction can be expected during this period (Böer et al. 2005). The exchange rate of lipids increased throughout the our feeding experiment, suggesting the pteropods used the diet-derived energy first to fuel increasing metabolic activity and somatic growth to normal size, and only subsequently to deposit lipid. Somatic growth is also reflected by the highest turnover rates observed in structural fatty acids 16:0, 20:5(n-3) and 22:6(n-3), which are required to form biomembranes and have been shown to be essential for zooplankton growth (Müller-Navarra et al. 2000, Wacker & Elert 2001). Thirdly low lipid assimilation, as compared to that observed by Böer et al. (2006), may also be due to a different methodology. In their experimental study, Böer et al. (2006) did not remove the stomachs in *C. limacina*. Therefore, the lipid signal may have been masked by undigested food and did not truly reflect lipid accumulation.

It has been suggested that *C. limacina* has a large capacity of *de novo* fatty acid synthesis, in particular producing odd-chain fatty acids (Kattner et al. 1998, Böer et al. 2005). The biosynthetic pathway of the odd-chain fatty acids in *C. limacina* is still unclear, but the utilization of dimethylsulfoniopropionate (DMSP) has been suggested. DMSP is most likely provided by *Limacina* spp., which strongly accumulate this component, due to feeding on detritus and phytoplankton (Gilmer & Harbison 1991, Levasseur et al. 1994). After cleavage of DMSP to DMS and acrylic acid, the propionate moiety is formed, which is the starter molecule (3 carbon atoms) for the biosynthesis of odd chain fatty acids (Kattner et al. 1998). Odd-chain fatty acids are mainly incorporated into DAGE, which are long-term storage lipids (Kattner et al. 1998, Böer et al. 2005). Our results confirm that *C. limacina* produce these fatty acids *de novo*, since individuals assimilated substantial amounts of the odd-chain fatty acids 17:0,17:1(n-8) and 19:1 while none of these were detectable in *L. retroversa*. Assimilation rates in *C. limacina* were as high for odd-chain fatty acids as for even-chain fatty acids directly derived from the diet. The

reason for the formation of odd-chain fatty acids is not yet clear and the complete pathways of biosynthesis and incorporation of these unusual fatty acids into the storage lipid classes TAG and DAGE still need to be described.

Conclusions

The rates of lipid assimilation of thecosome pteropods are closely associated with the development stages and species. Juveniles of *Limacina helicina* assimilated dietary fatty acids significantly more rapidly than the adults and showed a predilection for diatom fatty acids. In contrast, adult *L. helicina* integrated fatty acids from diatoms and flagellates in similar portions, suggesting no preference for one of the algae taxa. The sub-Arctic *L. retroversa* exhibited higher fatty acid and total lipid turnover rates than the Arctic *L. helicina*. Also its feeding preferences diverge from those of *L. helicina*, since *L. retroversa* integrated fatty acids more rapidly from flagellates than from diatoms. *Clione limacina*, despite its high feeding efficiency on *L. retroversa* specimens, assimilated their lipids at a low rate, as compared to previous feeding studies with *L. helicina*. Our data thus suggest that *C. limacina* is better adapted to feeding on the larger and more lipid-rich *L. helicina*. In a context of global changes and the possible shift of the thecosome community towards a dominance of *L. retroversa* rather than *L. helicina*, this could have severe consequences on the performance of *C. limacina* in Arctic waters.

Pteropods seem to be less efficient than copepods in assimilating lipids, however, they contribute an important portion of the lipid transfer in the Arctic marine food web due to their larger size and seasonally high abundances. Pteropods reach maximum abundances in autumn, ensuring the continuity of energy supply for higher trophic levels during a critical period, when the major biomass species, *Calanus* spp., have left the upper water layers to enter dormancy at greater depth.

Acknowledgements

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4

RESULTS AND SYNOPTIC DISCUSSION

One of the most important features of Arctic ecosystems is the lipid-driven flow of energy through the food web, which determines the structure of communities and populations (Falk-Petersen et al. 2009). Lipids that are biosynthesized by ice-algae and phytoplankton are assimilated as high-energy stores by zooplankton (Lee 1975, Sargent and Henderson 1986, Falk-Petersen et al. 1987, Lee et al. 2006). Subsequently, these compounds are transferred across the entire food web, constituting the major source of energy for fish, birds and marine mammals in the Arctic (Falk-Petersen et al. 1990, 2004, Dahl et al. 2003).

This thesis aimed at better understanding the role of zooplankton in the transfer of lipids through the Arctic pelagic food web by:

1. Filling the gaps of knowledge in terms of abundance and life-cycle strategies of zooplankton species, particularly thecosome pteropods (manuscript II)

2. Estimating the lipid and fatty acid turnover capacities of main Arctic zooplankton species in relation to their life cycle strategies (manuscripts I and III)

The synoptic discussion first focuses on methodological advances with regard to feeding experiments using ^{13}C labeled prey items in combination with CSIA, and emphasizes new possibilities as well as their limitations (section 4.1). The discussion also highlights the high variability of

lipid and fatty acid turnover among zooplankton species as a result of their contrasting life strategies (section 4.2). The seasonal and stage-related variations of lipid turnover of main Arctic zooplankton species are examined in section 4.3. Section 4.4 combines knowledge on zooplankton distribution and experimental results on lipid and fatty acids turnover rates to discuss the role of the zooplankton community in the transfer of lipids and fatty acids throughout the Arctic pelagic food web. The last section of the discussion (section 4.5) frames the capacities of lipid transfer by Arctic zooplankton in the context of climate change and aims at evaluating possible future alterations in the lipid-driven food web.

4.1 Advantages of CSIA combined with experiments using labeled food

Studying the lipid carbon transfer within food webs has been a growing focus of ecosystem-based studies. A traditional approach is to evaluate changes in the lipid, fatty acid and fatty alcohol composition of organisms (e.g. Lee 1974, Graeve et al. 1994b, Kattner and Hagen 1995, Falk-Petersen et al. 2009). However this method exhibits certain limitations to adequately explain lipid deposition patterns (Graeve et al. 2005, Budge et al. 2011, manuscript I, III). The first problematic aspect is that the signal of dietary fatty acid incorporation of a consumer may be diluted by its initial fatty acid composition (Jobling 2003, 2004). An increase of the concentration of a dietary fatty acid is much easier to detect if its initial concentration in the consumer was low than if it was already high. Another aspect is that a change of a consumer's fatty acid profile can also be the result of the combination of dietary incorporation and mobilization of fatty acids for metabolism (Jobling 2003). Depending on the zooplankton species, development stage and feeding condition, one of the two relevant processes (incorporation versus mobilization) can dominate and change the fatty acid profile, or both contribute equally, leading to a stable profile (Graeve et al. 2005). Looking only at the fatty acid or lipid concentrations, the magnitudes of these two processes cannot be distinguished.

To overcome these limitations, radioactive ^{14}C labeling of fatty acids was used to follow the transfer of lipids and investigate their biosynthesis in consumers (Farkas et al. 1973, Sargent and Lee 1975, Lampert 1977, Dall et al. 1993). This method allowed for semi-quantitative monitoring of carbon lipid assimilation by examining the total radioactivity with a scintillation counter. In the recent past, ^{14}C was replaced by non-radioactive ^{13}C to estimate the biosynthe-

sis of fatty acids and alcohols in organisms (Graeve et al. 2005). The ^{13}C isotope is naturally present in marine systems, at an average abundance of 1.1% ^{13}C vs. 98.9% ^{12}C. In marine primary producers, this ratio is of 98.93% ^{12}C and 1.107% ^{13}C and slightly varies in organisms of higher trophic levels as ^{13}C undergoes little fractionation (<1 ‰) with trophic level (Minagawa and Wada 1984, Post 2002, Søreide et al. 2006).

The ratio of $^{13}C/^{12}C$ into individual compounds can be estimated by CSIA using a GC-IRMS that separates the target compounds with the GC unit and measure isotopic ratios with the IRMS unit (Graeve et al. 2005, section 2.4.3). CSIA were first developed in the early 1990s to evaluate natural isotope abundances (Meier-Augenstein 1999, Boschker and Middelburg 2002), but they can also be applied to feeding experiments based on ^{13}C labeled diet to detect processes of carbon assimilation into specific compounds such as fatty acids, even when the concentrations of the respective compounds remain unchanged (Graeve et al. 2005, manuscript I, III). The transfer of lipid carbon from a producer to one or several trophic levels of consumers can thus be accurately followed and its turnover rate quantified.

This method provides valuable functional information about lipid-related ecology, which can be useful in several research areas. A major application concerns the investigation of trophic relationships in ecosystems. One approach (1) is the use of mixing models that determine the ^{13}C isotopic signature of consumers' FATM to evaluate their trophic level and the origin of their diet. When applying this approach, it is crucial to know how long it takes for the consumer to turnover its fatty acids, which allows for evaluating whether or not the fatty acid profile reflects a recent diet. However, to date, only one study determined fatty acid assimilation rates, in Arctic *Calanus* spp. (Graeve et al. 2005). Current mixing isotope models are mainly based on the assumption that lipid turnover rates of organisms, for example zooplankton and fish, range between hours and days (i.e. Søreide et al. 2006, Kohlbach et al. 2016, 2017). With an accurate determination of the lipid turnover rates, these models could provide more precise results. Another approach in trophic relationships investigations (2) is the use of quantitative fatty acid signature analysis (QFASA) to determine the diet composition of a consumer (Iverson et al. 2004). The consumer's fatty acid signature (FAS) is compared to different weighted mixtures of the potential preys' FAS. The mixture that exhibits the smallest statistical distance from that of the consumer is considered to reflect the true diet. The proportional contribution of each prey type to the mixture, and hence to the consumer's diet is estimated from its FAS contribution. Calibration coefficients are necessary in these models to take into account the

fact that the FAS of the consumer does not reflect exactly that of its prey. These coefficients are estimated from controlled feeding experiments, where the consumer is fed a single diet until its FAS resembles that of its diet as closely as possible (e.g. Nordstrom et al. 2008, Thiemann et al. 2008, Budge et al. 2011). However the necessary duration of feeding to reach this steady state is so far empirical and in most cases inaccurate. Knowing lipid turnover rates would allow for adjusting these experiments and hence represent an important gain of precision. In both described approaches (1) and (2) used in food web interactions modeling, the use of experimentally quantified rates would represent a major improvement in the determination of trophic relationships.

When designing feeding experiments usinga ^{13}C labeled diet, it is of major importance to significantly increase the $^{13}C/^{12}C$ ratio of their diet to discard natural changes in ^{13}C abundance of the consumers. Another aspect is that the amount of label decreases with trophic level due to dilution (manuscript III). It is therefore crucial to reach a sufficient amount of label in the fatty acids of the first trophic level to ensure detectable changes in the top consumer. On the other hand, too high ^{13}C labeling can cause overloading of the IRMS detector. Labeling of the first trophic level (algae in this study), which is performed by adding ^{13}C sodium bicarbonate in the culture medium (see section 2.3.1), is therefore a crucial step. In the first feeding experiment using ^{13}C labeled diet conducted by Graeve et al. (2005) with copepods fed with diatoms, 200 mg L^{-1} of ^{13}C sodium bicarbonate were added. This resulted in a high labeling of the algae (37% of ^{13}C) and of the consumers (10-20%). Since then, I gradually decreased the quantity of ^{13}C added to the algal cultures to reach a concentration that does not cause technical problems, but is still significantly higher than the natural ratio (manuscript I, III). In 2014, 15 mg L^{-1} were added to the algal cultures, leading to a labeling of the algal diet of 14-15%. In 2015, the quantity of ^{13}C sodium bicarbonate was reduced to 1.5 mg L^{-1}, resulting in a labeling of the diet of 3.7%. The labeling success in consumers are summarized in Table 6. For feeding experiments with only one primary consumer, I suggest that a labeling of the first trophic level of 10-20% is ideal and can be achieved by adding 10-20 mg L^{-1} of ^{13}C sodium bicarbonate to the culture medium. For feeding experiments with two levels of consumers, I would recommend to label the first trophic level with 20-25% by adding 20-30 mg L^{-1} of ^{13}C sodium bicarbonate to the culture medium.

Regarding the calculation of assimilated carbon by a consumer (see section 2.4.3), it is worth underlining the importance of using the ^{13}C atom percent excess (ATE) instead of the atom

percent (AT). This is essential to correct for the natural ^{13}C background of the consumer and consider only the experimental enrichment (Coleman and Fry 1992, Brenna et al. 1997). Calculations of lipid assimilation based on AT, as performed by Graeve et al. (2005), lead to an overestimation of the consumer's efficiency. As an example, in the experiments conducted in the present study, the total lipids exchanged by *P. minutus* calculated from the ATE were of 55% total lipid after 21 days (manuscript III). If the AT had been used, the total lipids exchanged would have been estimated to 70% total lipid. Moreover, the overestimation of carbon assimilation tends to be higher for fatty acids with low enrichment than for fatty acids with high enrichment. For example, the turnover of 18:0 would be overestimated by factor 4 (15% vs. 4% total fatty acid) while 20:5(n-3) would be overestimated only by factor 1.2 (91% vs. 76% total fatty acid). These different levels of overestimation may have high consequences on the ecological interpretation of carbon assimilation into total lipids and fatty acids.

Table 6: Labeling success (atom%) of all trophic levels investigated in this study.

Year - manuscript	**Trophic level 1** (algae mixture)	**Trophic level 2**		**Trophic level 3** (*C. limacina*)
	AT	Species	AT	AT
2014	15.3%	*C. glacialis* CIV	4.8% (Day 21)	–
2014 - manuscript I	15.3%	*P. minutus* CV	8.1% (Day 21)	–
2014 - manuscript I	15.3%	*O. similis* female	2.3% (Day 21)	–
2015 - manuscript III	3.7%	*L. helicina* juv.	1.2% (Day 6)	–
2015 - manuscript III	3.7%	*L. helicina* adult	1.1% (Day 6)	–
2014 - manuscript III	13.7%	*L. retroversa* adult	2.2% (Day 3)	1.2% (Day 23)

Key-messages of section 4.1

• CSIA combined with feeding experiments using ^{13}C diet allow for a precise evaluation of the lipid and fatty acid turnover in organisms. The transfer of lipid carbon in a simple food web can be accurately quantified by this method.

• Experimentally determined lipid assimilation rates can be used in food web research, particularly to estimate trophic relationships.

• Labeling of the diet must be carefully adjusted. The ^{13}C label must be concentrated enough to ensure a clear monitoring of lipid assimilation by the consumer(s) but not over concentrated to avoid technical problems with the IRMS.

• It is crucial to offset natural ^{13}C content in the consumers to avoid an overestimation of their lipid turnover efficiency.

4.2 Lipid and fatty acid turnover reflects life strategies of Arctic zooplankton

The life cycle and feeding strategies of Arctic zooplankton organisms are highly adapted to the extreme conditions that characterize their environment (Lee and Hirota 1973, Clarke 1983, Falk-Petersen et al. 2009). These species accumulate different amounts of lipid reserves and store them under different lipid classes (Hagen and Auel 2001, Dalsgaard et al. 2003, Lee et al. 2006). However, the capacities of these organisms to turnover their total lipids and fatty acids are poorly understood. Only one study performed by Graeve et al. (2005) focused on this matter before. This thesis investigated to which extent the variations of lipid and fatty acid turnover rates among copepods (*P. minutus* and *O. similis*, manuscript I and *C. glacialis*, additional data) and pteropods (*L. helicina*, *L. retroversa* and *C. limacina*, manuscript III) are related to their life strategies. This section gathers the gained knowledge about these species lipid turnover capacities to distinguish general eco-physiological patterns among the Arctic zooplankton community.

Large differences in size and in storage capacities (Table 1, Table 2) result in large differences in total lipid masses among the species, as reflected in this study (Fig.13). *L. helicina* adult and *C. limacina* exhibited the highest total lipid masses, averaging at 203 μg C ind^{-1} and 148 μg C ind^{-1}, respectively. The total lipid mass of *C. glacialis* was lower but increased from 19 μg C ind^{-1} at Day 0 to 62 μg C ind^{-1} at Day 21, indicating that individuals accumulated lipids during the experiment. *P. minutus* and *L. retroversa* exhibited a constant total lipid mass averaging at 3 μg C ind^{-1}. *O. similis* exhibited an increase of its total lipid mass from 0.08 μg C ind^{-1} at Day 0 to 0.2 μg C ind^{-1} at Day 21. But this may be an artifact due to a high variability in the lipid content among individuals, probably associated with the overlap of two cohorts (Narcy et al. 2009, see manuscript III for more details). *L. helicina* juvenile had the lowest total lipid mass, averaging at 0.04 μg C ind^{-1}.

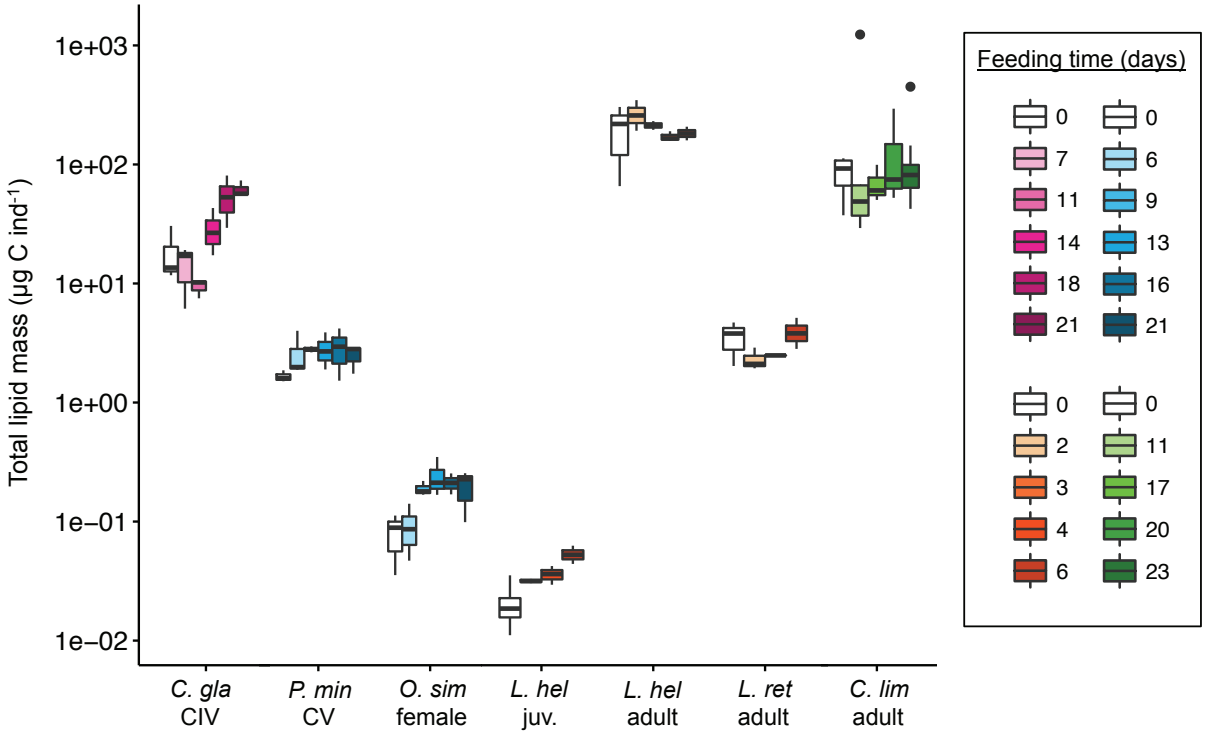

Figure 13: Total lipid mass (μg C ind^{-1}) of zooplankton species during the various feeding experiments.

To compare the efficiency of total lipid and fatty acid assimilation among species, (1) the high differences between their total lipid masses were discarded by considering assimilation rates expressed as a percent of total lipid mass; (2) all species of the second trophic position were offered the same phytoplankton food composed of a mixture of flagellates and diatoms at a cell number ratio of 1:1 (manuscript I, III).

The algae were supplied in excess to ensure that the consumers were not food-limited (Sakshaug et al. 2009, Howes et al. 2014). This mixed diet was chosen to match the *in situ* conditions in summer to early autumn, when a co-dominance of flagellates and diatoms is observed in Svalbard waters (Leu et al. 2006, Søreide et al. 2010, Hegseth and Tverberg 2013). Since the individuals were not fed with single algal cultures, food selectivity could not be studied in detail. Even though diatoms and flagellates were offered in similar concentrations, the consumers could have ingested the two taxa in different proportions. A higher assimilation rate of a specific FATM may therefore be the result of preferred ingestion and/or more efficient assimilation. Diatoms mainly provided the monounsaturated fatty acid 16:1(n-7) and the C_{16} PUFA 16:2(n-4) and 16:3(n-4), while flagellates produced high amounts of the C_{18} PUFA 18:2(n-6), 18:3(n-3) and 18:4(n-3) which is in line with previous studies (Ackman et al. 1968, Graeve et al. 1994b, Falk-Petersen et al. 1998) (see manuscripts I and III for the algae fatty acid composition). The monounsaturated 18:1(n-9), a major component of the fatty acid biosynthesis of copepods, was present in elevated amounts in flagellates and was hence referred to as a flagellate marker in the copepods diet.

4.2.1 Herbivorous feeding strategy

Among all studied species, the herbivorous copepods *C. glacialis* and *P. minutus* were able to turnover their total lipids at the highest rates, averaging 1.3 and 2.6% d^{-1}, respectively (Table 7). In addition, *C. glacialis de novo* synthesized fatty alcohols and long chain MUFA (1.2% and 1.4 d^{-1}, respectively) (Fig.14), which serve as long term energy reserves and are usually stored as wax esters. The results also suggested that *P. minutus* is able to *de novo* synthesize the long chain fatty alcohol 20:1 (manuscript I), which contradicts previous assumptions that the species can only *de novo* synthesize short chain alcohols (Kattner et al. 2003). This pronounced efficiency in lipid and fatty acid assimilation reflects the adaptive mechanism of Arctic herbivorous species, which need to rapidly accumulate energy during the short primary production bloom in spring/early summer (Lischka and Hagen 2005, Søreide et al. 2008, manuscript III). *C. glacialis* mainly uses its lipid reserves to fuel molting, gonad maturation and reproductive processes (Sargent and Falk-Petersen 1988, Hagen 1999, Lee et al. 2006, Falk-Petersen et al. 2009). Mainly between November and March, the species overwinters in true diapause at depth with very limited utilization of its lipid reserves (Hagen and Auel 2001, Freese et al. 2017). In contrast, *P. minutus* does not overwinter in a true diapause. It partially relies on lipid deposits for metabolism that it supplements by opportunistic feeding (Kwaniewski 1990, Lischka et al.

Table 7: Daily lipid assimilation and lipid turnover of zooplanton species.

Species	**Lipid assimilation (μg C ind^{-1} d^{-1})**	**Lipid turnover (% total lipids ind^{-1} d^{-1})**
C. glacialis CIV	0.8	1.3
P. minutus CV	0.07	2.6
O. similis female	0.001	0.5
L. helicina juvenile	0.0001	0.2
L. helicina adult	0.04	0.1
L. retroversa adult	0.02	1.3
C. limacina adult	0.03	0.07

2007). Considering this more flexible feeding mode, it is surprising that its efficiency to turnover its total lipids was higher than that of *C. glacialis* and this suggests that lipid reserves play an important role in its life strategy. An additional explanation for the higher lipid turnover of *P. minutus* as compared to *C. glacialis* may be related to season-specific strategies (see section 4.3.1).

The herbivorous *C. glacialis* and *P. minutus* assimilated diatom FATM (2.4 and 4.6% d^{-1} respectively) at a much higher rate than flagellate FATM (1.2 and 1.3% d^{-1} respectively) (Fig.15). At the end of the experiment (Day 21), the portion of exchanged diatom FATM was 2 times higher than that of flagellate FATM in *C. glacialis* and 3 times higher in *P. minutus*. Both species are selective particle filter feeders and have been reported to prefer diatoms over flagellates (Poulet 1974, Norrbin et al. 1991, Søreide et al. 2008). These copepods are also thought to have flexible feeding strategies by switching their diet to flagellates when diatoms are not abundant (Levinsen et al. 2000, Lischka and Hagen 2007). These results suggest that when both diatoms and flagellates are present, *C. glacialis* and *P. minutus* feed on both taxa but with a pronounced preference for diatoms. It is also possible that the individuals fed equally on both algae sources, but fatty acid assimilation was more efficient from diatom as compared to flagellate FATM.

4.2.2 Omnivorous feeding strategy

Species with an omnivorous feeding strategy, i.e. *O. similis* and *L. helicina*, exchanged their total lipids at the low rates of 0.1-0.5% d^{-1} (Table 7). These species maintain metabolic activity throughout the year, fueled by opportunistic feeding. *O. similis* is an ambush feeder that consumes a wide variety of organisms from small flagellates to copepod nauplii and faecal pellets (Franz 1988, Kattner et al. 2003, Lischka and Hagen 2007). *L. helicina* feeds on any food particles collected by its mucous net (Gilmer 1972, Harbison and Gilmer 1992, Falk-Petersen et

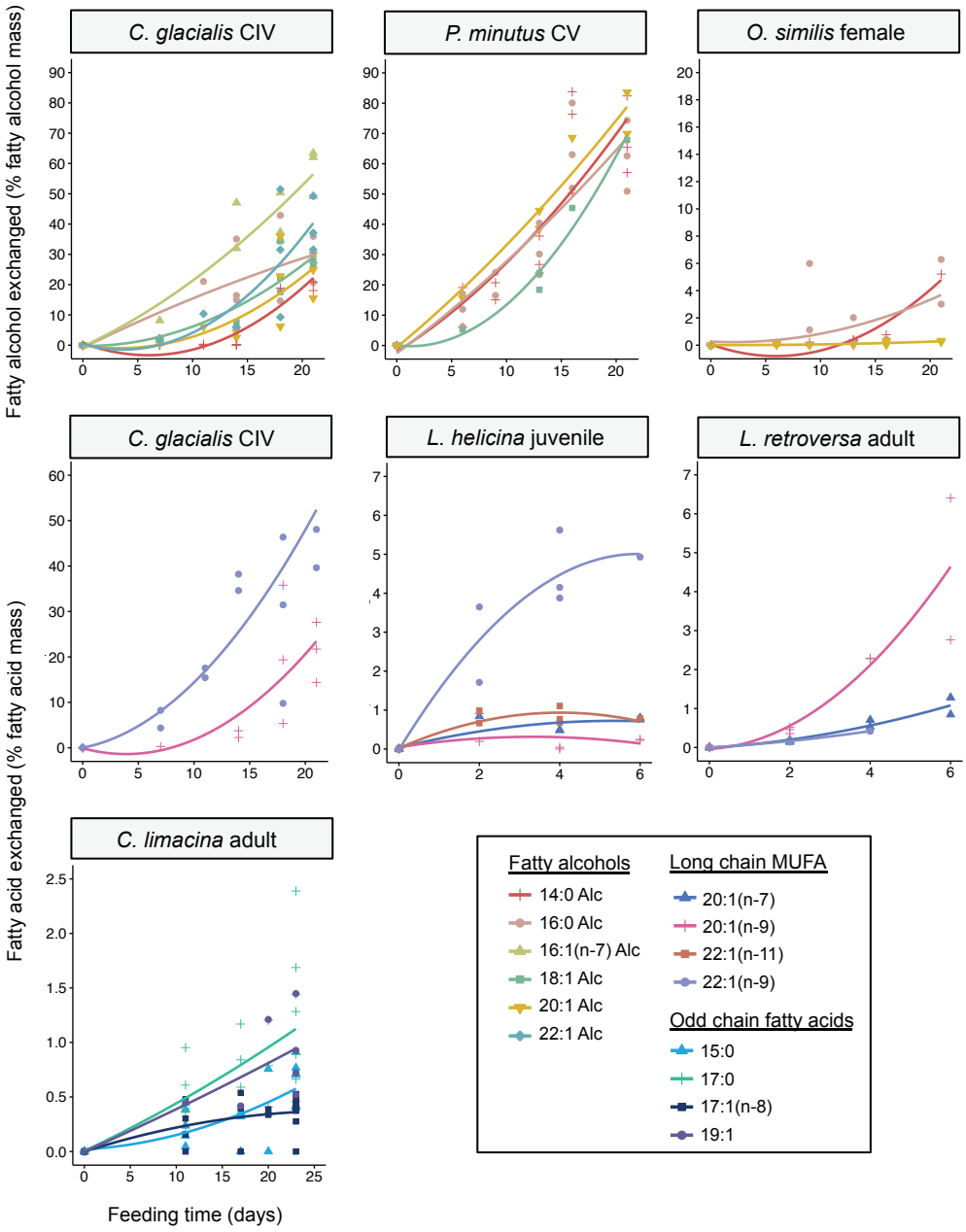

Figure 14: Turnover of *de novo* synthesized compounds (% total fatty acid/alcohol mass) of zooplankton species during the feeding experiments.

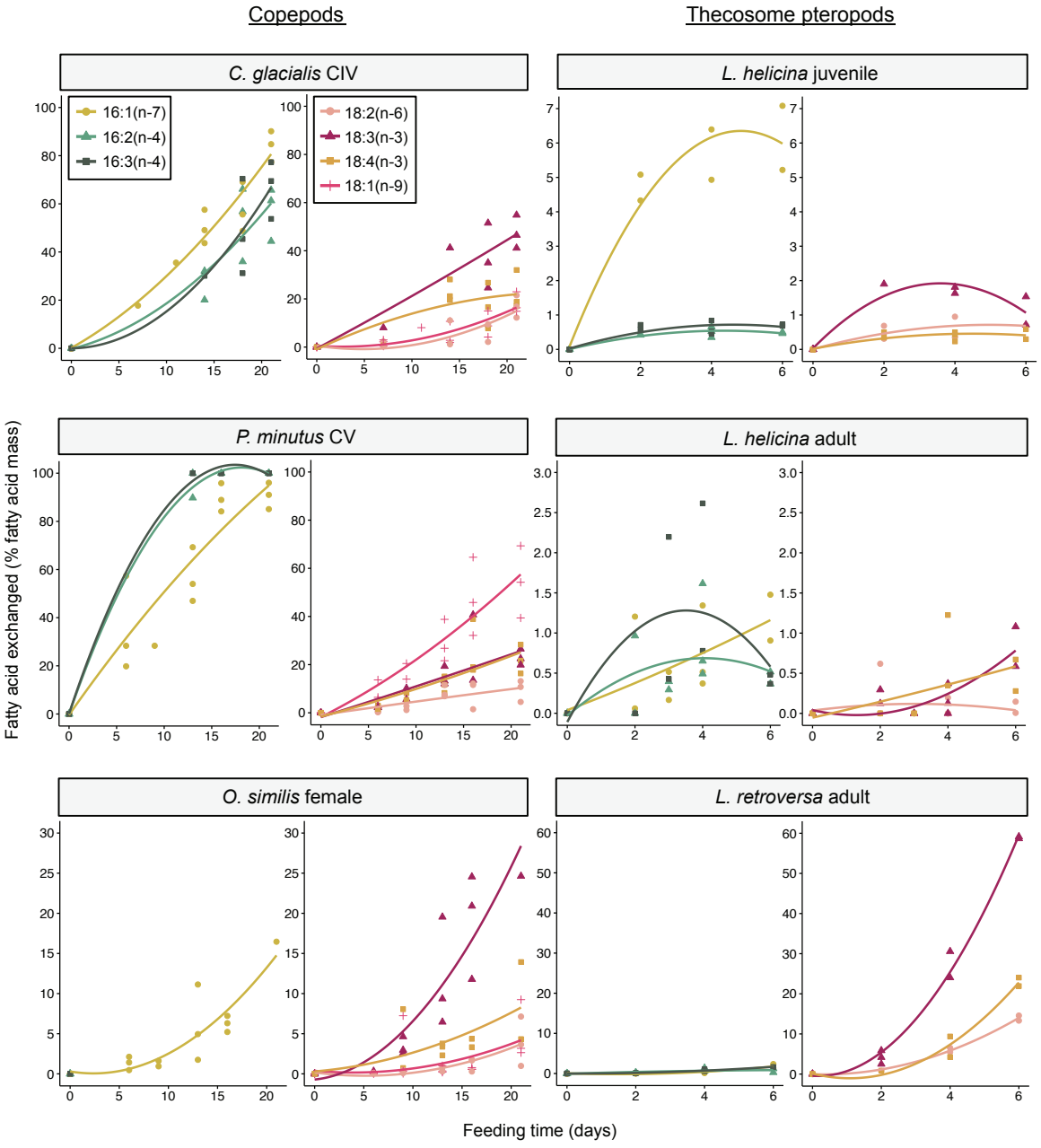

Figure 15: Turnover of FATM (% total lipid mass) of Arctic copepods (left) and thecosome pteropods (right) during the feeding experiments.

al. 2001, Gannefors et al. 2005). Hence, both species do not need to exchange their total lipids at a high rate, as food is continuously available for them. Extensive lipid accumulation does not seem essential in their life-cycle strategy (manuscript I, III). *L. retroversa* showed a high lipid turnover efficiency, reaching the same rate as that of *C. glacialis* (1.3% d^{-1}) (Table 7). This is unexpected given the fact that this species is an omnivorous feeder that is not known to rely on lipid reserves to a large extent (Falk-Petersen et al. 2001, Gannefors et al. 2005) (manuscript III).

Despite their omnivorous strategy, *O. similis* and *Limacina* spp. exhibited capacities of *de novo* synthesis, which are usually related to a strategy of long term energy storage . *O. similis* exchanged fatty alcohols and respectively wax esters at a rate of 0.2% d^{-1} (Fig.14). This confirmed that wax esters are important in *O. similis* life cycle (manuscript I). They are used by this species as an additional energy to buffer the poor food supply in winter and to fuel reproductive processes (Lischka and Hagen 2007). The results strongly suggested that *L. helicina* and *L. retroversa de novo* synthesized long chain MUFA (0.3 and 0.5% d^{-1} respectively) (Fig.14) (see manuscript III for more details about possible pathways). This result is in contrast with previous studies that did not consider thecosome pteropods to being able to *de novo* synthesize long chain MUFA. Even though these compounds have been found in significant amounts in *L. helicina* from the Arctic and Southern Ocean, they have always been associated with feeding on copepods (Kattner et al. 1998, Falk-Petersen et al. 2001, Gannefors et al. 2005).

The omnivorous *O. similis* and *L. helicina* appeared to be equally efficient in ingesting and/or assimilating fatty acids from diatoms and flagellates (0.7 vs. 0.5% d^{-1} for *O. similis* and 0.1 vs. 0.1% d^{-1} for *L. helicina* adult) (Fig.15). *O. similis* has been characterized as preferring motile prey (Drits and Semenova 1984, Svensen and Kiørboe 2000) but some studies suggest that it can feed on diatoms (Kattner et al. 2003, Lischka and Hagen 2007). Our results confirm that *O. similis* feeds on diatoms and that it pursues an opportunistic feeding strategy (manuscript I). Likewise, *L. helicina* is known to feed opportunistically (Gilmer 1972, Harbison and Gilmer 1992, Falk-Petersen et al. 2001, Gannefors et al. 2005), explaining its capacity to assimilate fatty acids from both algae taxa at the same rate (manuscript III). *L. retroversa* was the only studied species that ingested/assimilated preferentially flagellate FATM. At Day 6, the portion of exchanged flagellate FATM was 23 times higher than that of diatom FATM (Fig. 15), reflecting a daily rate of 5.3 v.s 0.2% d^{-1}. This confirms previous studies that indicated that *L. retroversa* feeds primarily on flagellates and, at a lower level, on diatoms of ingestible size

and shape (Morton 1954, Perissonotto 1992, manuscript III). Given the fact that *L. retroversa* and *L. helicina* are closely related species with a similar ecology, the difference in their FATM assimilation rates is however surprising and should be further investigated.

4.2.3 Carnivorous feeding strategy

The only carnivorous species investigated in this study, *C. limacina*, exchanged its total lipids at the slowest rate of all investigated species (0.07% d^{-1}) (Table 7). This is very low as compared to previous studies, which report a carbon turnover of nearly 100% within 1-2 weeks (Conover and Lalli 1974, Böer et al. 2006). These markedly different results may be due to large differences in methodology and in the physiological state of *C. limacina* among the studies (manuscript III). Firstly, Conover and Lalli (1974) used a completely different method, in which they determined assimilated carbon by subtracting egested to ingested material. Böer et al. (2006) used a similar method as in the present study, but did not remove the stomachs of *C. limacina*. Therefore, the lipid signal may have been masked by undigested food and did not truly reflect lipid accumulation. Secondly, in the present study, *C. limacina* specimens were collected during the period of high reproductive activity (Mileikovsky 1970) and then starved for ten weeks during which they probably decreased their metabolism (Böer et al. 2005). It is hence likely that the individuals first needed to reactivate their digestive enzymes after the long starvation period, as previously observed in copepods (Freese et al. 2016). A further explanation may be that the individuals used the diet-derived energy in priority to sustain their metabolism. Thereafter, they had enough subsidiary energy to invest into lipid deposits (see manuscript III for more details). Thirdly, the amount of food offered may have been a limiting factor for the metabolic uptake. In the present study, *C. limacina* were fed with 3-4 *L. retroversa* every five days, which corresponds to a total lipid mass of 11 μg C. In contrast, *L. helicina*, the main prey of *C. limacina* in Arctic waters (Meisenheimer 1905, Lalli 1970, Conover and Lalli 1972, Hopkins 1985, 1987), contains 203 μg C lipid, i.e. 20 times more. It is therefore likely that *C. limacina* needs high amounts of ingested lipids to trigger energy storage processes. Unfortunately, it was not possible to compare this result with feeding on *L. helicina*, because the respective experiment conducted in 2015 was not successful. Surprisingly, the very few *C. limacina* specimens that achieved feeding on *L. helicina* died few days later. Most *C. limacina* showed a predator behavior up to more than 24 h, swimming in circles around *L. helicina* and evaginating their tentacles trying to catch it. This operation lasted for a very long time compared to previous observations that reported <6 h (Conover and Lalli 1972, Böer et

al. 2006, pers. obs.). Hence, *C. limacina* might have used too much energy to catch their prey and subsequently died of exhaustion. This experiment would need to be repeated to better understand these observations.

The occurrence of odd chain fatty acids as minor components is not unusual in marine organisms. However, exceptional amounts have been found in *C. limacina*, in which they contribute up to 34% to the fatty acid composition (Kattner et al. 1998, Böer et al. 2005, manuscript III). Since odd chain fatty acids are in negligible amounts in *C. limacina*'s unique prey *Limacina* spp., it has been suggested that they are *de novo* synthesized, probably from dimethylsulphoniopropionate (DMSP) using the propionate moiety as a starter molecule (Kattner et al. 1998, Falk-Petersen et al. 2001, Böer et al. 2005). In this study, despite a possible feeding limitation (see section 4.2.3), *C. limacina* assimilated substantial amounts of the odd chain fatty acids 17:0, 17:1(n-8) and 19:1 (ca. 0.03% d^{-1}) while none of these were detectable in *L. retroversa* (Fig.14), confirming the previous assumptions. Assimilation rates in *C. limacina* were as high for odd chain fatty acids as for even chain fatty acids that were directly derived from the diet. This suggests that these compounds play an important role for the physiology and ecology of *C. limacina* and are very efficiently synthesized (see manuscript III for more details). Odd chain fatty acids are especially enriched in DAGE, which are considered as a long term energy storage that is used for reproduction and during periods of food scarcity (Phleger et al. 1997, Falk-Petersen et al. 2001, Böer et al. 2005).

Key-messages of section 4.2

• Arctic herbivorous species (here *C. glacialis* and *P. minutus*) exhibit a highly efficient total lipid turnover, which, combined with the rapid *de novo* synthesis of wax esters, reflects the importance of quickly storing energy reserves to successfully fulfill their life cycle. These species preferentially ingest/assimilate diatom FATM, confirming that diatoms play an essential role for growth and reproduction.

• Omnivorous species show lower total lipid turnover rates than herbivorous, reflecting a life strategy that is less dependent on lipid reserves. Nonetheless they *de novo* synthesize significant amounts of long term energy storage such as fatty alcohols (*O. similis*) and long chain MUFA (*Limacina* spp.). *O. similis* and *L. helicina* have similar ingestion/assimilation rates of flagellate and diatom FATM, mirroring their opportunistic feeding strategy. In contrast, *L. retroversa* seems to prefer flagellates over diatoms.

• Carnivorous species (*C. limacina*) exhibit a very slow lipid turnover, which in this study may be related to the direct investment of energy into metabolism after a long period of starvation. *C. limacina de novo* synthesize odd chain fatty acids that are used as long term energy reserves to overcome the variability of presence of its unique food source *Limacina* spp.

4.3 Temporal variations of lipid and fatty acid turnover

Most species of the Arctic pelagic food web match their life cycle to the large seasonal changes (Clarke 1983), which may result in seasonally changing lipid turnover rates. Lipid turnover may also change over the course of life cycle, because different lipid amounts and different specific compounds are needed depending on the developmental stage (Brett et al. 2009).

4.3.1 Season-specific requirements

Herbivorous copepods such as *C. glacialis* and *P. minutus* are able to turnover large amounts of storage lipids during the productive period (Lischka and Hagen 2007, Søreide et al. 2008, manuscript I). In winter during dormancy, the lipid turnover is probably low since metabolism

is reduced to a minimum (Norrbin et al. 1990, Norrbin 1994, Falk-Petersen et al. 2009, Vader et al. 2015). The lower lipid turnover rate of *C. glacialis* as compared to *P. minutus* in this study (see section 4.2.1) may support this hypothesis. *C. glacialis* builds up large lipid reserves mainly during the short phytoplankton spring bloom, and starts to descend to deep layers in late July/early August for diapause (Sargent and Falk-Petersen 1988, Hagen 1999, Hagen and Auel 2001, Lee et al. 2006, Søreide et al. 2010, Daase et al. 2013) (Fig.16). In contrast, *P. minutus* feeds on phytoplankton in surface waters for a longer time, since it does not descend to deep layers before October/November (Lischka and Hagen 2005). When the feeding experiments were conducted in late July, it is hence likely that *C. glacialis* was already starting to decrease its metabolism for overwintering and thus reduced feeding and lipid assimilation while *P. minutus* was still in a phase of regular feeding and lipid assimilation. This would indicate that lipid assimilation efficiency is mainly driven by internal factors and not by external triggers such as food availability.

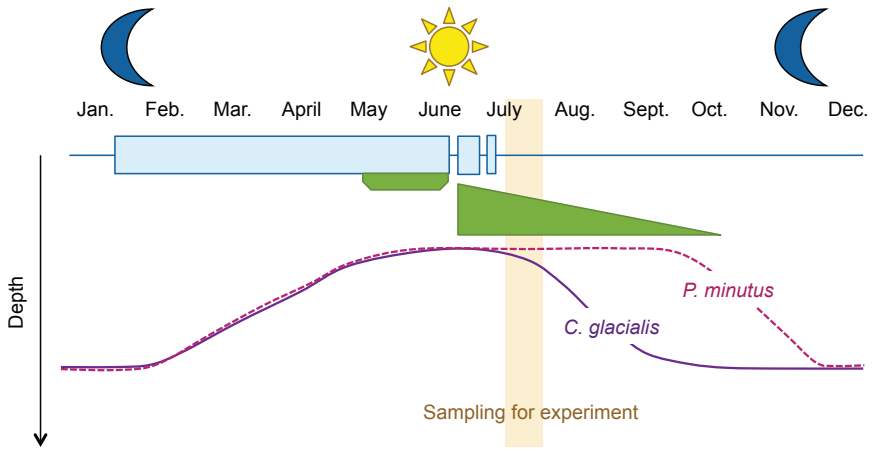

Figure 16: Schematic representation of the light and primary production regime in high Arctic ecosystems, as well as the seasonal vertical distribution of the calanoid copepods *Calanus glacialis* and *Pseudocalanus minutus*.

Omnivorous species are expected to turnover their total lipids throughout the year, feeding on phytoplankton in summer and on detritus in winter with no major reduction of metabolism (Conover and Huntley 1991, Lischka et al. 2007). However phytoplankton offers a high quality of food, which is enriched for example in EFA such as the PUFA 20:5(n-3) and 22:6(n-3) (Ackman 1989, Lee et al. 2006, Leu et al. 2006). It is therefore possible that even omnivorous species have a slightly higher turnover rate during the productive period. This may be reflected in this study by the differences in lipid assimilation between *L. helicina* and *L. retroversa* (see section 4.2.2). High latitude species, e.g. *L. helicina*, are particularly efficient to accumulate

lipids during a short primary production cycle, whereas sub-polar species, e.g. *L. retroversa* are adapted to feed during a longer productive period (Madsen et al. 2001, Ringuette et al. 2002, Søreide et al. 2008, Falk-Petersen et al. 2009). Therefore, *L. helicina* may be more efficient to build up lipid reserves in spring than in late summer, when the experiment was conducted. On the contrary, *L. retroversa* may be efficient to accumulate lipids during a longer period than *L. helicina*, which would explain its high lipid turnover rate in this study (manuscript III).

Several studies report a changing fatty acid signature of zooplankton species over the seasons (Lischka and Hagen 2007, Søreide et al. 2008, Falk-Petersen et al. 2009). These changes are the result of shifts in the diet, between a diatom-based diet in spring and a flagellate and/or detritus-based diet in autumn and winter (Drits and Semenova 1984, Gilmer and Harbison 1991, Gannefors et al. 2005). It is likely that not only the composition of fatty acids but also their turnover changed depending on seasons. The results of this study are based on a diatom-flagellate mixed diet that reflects *in situ* summer conditions; it is hence not possible to conclude about seasonality of specific fatty acid turnover.

4.3.2 Stage-specific requirements

Turnover of total lipids and fatty acids in zooplankton may vary among developmental stages, depending on requirements for stage-specific processes such as growth, gonad maturation and egg production (Brett et al. 2009, Jónasdóttir et al. 2009). Here *L. helicina* juveniles and adults were investigated and can illustrate these stage-related differences.

L. helicina adults exchanged their total lipids at a lower rate as compared to the juveniles. They may have been at the end of their life cycle since the experiment was conducted in late July/early August and previous studies suggested that they die in August/September, shortly after reproduction (Gannefors et al. 2005, Hunt et al. 2008). Before they disappear from the water column, they may reduce feeding to only fuel basic metabolism, and therefore exhibit reduced lipid content (Gannefors et al. 2005) as well as low turnover rate (manuscript III). Adults had significantly higher concentrations of 20:5(n-3) and 22:6(n-3) than juveniles but they exhibited a a negligible turnover rate of these compounds. These fatty acids are essential for gonad maturation processes and hatching success of copepods (Jónasdóttir et al. 2009) and it is very possible that they have a similar function in pteropods. This results suggest that females were already mature when sampled for the experiment and that the turnover of these

compounds reflects processes of gonad maturation (manuscript III). The high lipid turnover rate of juveniles combined with high turnover rate of the long chain MUFA suggests an investment into growth as well as a storage of energy (Paranjape 1968, Lalli and Gilmer 1989, manuscript III). Also juveniles assimilated higher portions of diatom markers as compared to flagellate markers (0.4 vs. 0.1% d^{-1}) while adults assimilated diatom and flagellate FATM in the same portions (see section 4.2.2). This highlights the potential importance of diatom-derived fatty acids for the development of young thecosomes.

Stage-related differences of specific compound turnover rates that were observed in this study for *L. helicina* are likely to concern other zooplankton species. For example copepods of stages CIV and CV invest in storage lipids (Falk-Petersen et al. 2009) and may hence turnover their long chain MUFA and fatty alcohols at a high rate. Females that invest mainly in reproduction processes (Søreide et al. 2008) may show a higher turnover rate of the fatty acids 20:5(n-3) and 22:6(n-3).

The differences of lipid and fatty acid turnover among seasons and development stages of zooplankton species are still not quantified. This study is the first to compare different species but it presents only a snapshot of the actual zooplankton physiological capacities related to lipid biosynthesis. It is crucial to conduct further experiments with different development stages and during different seasons to better comprehend the yearly turnover rates.

Key-messages of section 4.3

• The lipid assimilation efficiency of herbivorous zooplankton species may show a pronounced seasonality, with highest rates during the productive season in spring and low rates during overwintering. In contrast, omnivorous species may have a more constant capacity to assimilate lipids throughout the year.

• Energetic needs may vary quantitatively and qualitatively depending on life-stages because processes such as growth, reproduction, and metabolism during food scarcity involve and require different amounts of lipids as well as specific compounds.

4.4 Role of zooplankton in the transfer of energy compounds through the Arctic pelagic food web

Fatty acids are among the most important molecules transferred across marine food webs from primary producers up to top predators (Brett et al. 2009). They occupy a crucial role in different processes of all organisms such as somatic growth, survival, development, reproduction and metabolism during food scarcity periods (Müller-Navarra 1995, Sargent et al. 1999). The efficiency of lipid transfer throughout the food web depends on how quickly key organisms respond to dietary inputs and assimilate fatty acids and alcohols (Brett et al. 2009). The amount of lipid carbon provided by zooplankton for higher trophic levels depends on different factors that include intrinsic capacities of organisms to assimilate lipids from their diet and their respective biomass and abundances. Since all these parameters are expected to highly vary throughout the year (see section 4.3), this section estimates the role of zooplankton in the lipid-driven Arctic pelagic food web, exclusively however during summer/autumn.

4.4.1 Total lipids

The daily transfer of total lipids from phytoplankton to zooplankton differed among the studied species (Fig.17) as a result of their individual capacities to assimilate lipids from diet (Table 7, manuscript I, III) and their respective abundances (Table 8).

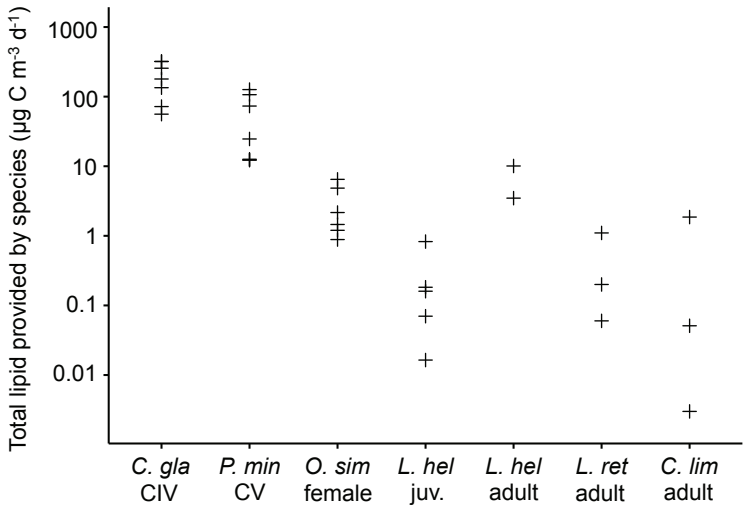

Figure 17: Total lipids (μg C m^{-3} d^{-1}) provided by main Arctic zooplankton species. The y-axis is represented in a $\log_{10}$ scale for easier visualization.

Table 8: Averaged summer/autumn abundances in the entire water column (< 300 m deep) of main zooplankton species and stages in Svalbard fjords.

Species	Month	Abundance (ind m^{-3})	Reference
C. glacialis CIV	July	90; 400; 168; 70; 400; 320; 224	Walkusz et al. 2003, Walkusz et al. 2004, Blachowiak-Samolyk et al. 2008, Walkusz et al. 2009, Freese 2015, Boissonnot et al. in prep., Stübner unpubl.
P. minutus CV	July	1046; 175; 180; 1524; 1804; 352; 176	Węsławski et al 1991, Walkusz et al. 2003, Walkusz et al. 2004, Blachowiak-Samolyk et al. 2008, Walkusz et al. 2009, Kwasniewski et al. 2010, Stübner unpubl.
O. similis female	July	884; 2161; 1200; 6475; 4846; 1456	Węsławski et al 1991, Walkusz et al. 2003, Walkusz et al. 2004, Blachowiak-Samolyk et al. 2008, Walkusz et al. 2009, Stübner unpubl.
L. helicina juv.	September	700; 8264; 1824; 164; 1600	Walkusz et al. 2003, Gannefors et al. 2005, Blachowiak-Samolyk et al. 2008, Ehrenfels 2016, Manuscript II
L. helicina adult	July	87; 252	Gannefors et al. 2005, Walkusz et al. 2009
L. retroversa adult	September	10; 3; 55	Wencky 2001, Weydmann et al. 2014, Manuscript II
C. limacina adult	October	62; 1.7; 0.1	Węsławski et al 1991, Walkusz et al. 2009, Stübner unpubl.

Due to their high individual biomass combined with rapid total lipid turnover rates as well as high abundances, copepods transferred the largest amount of lipids from phytoplankton, with 247 μg C m^{-3} d^{-1} (Fig.17). Among copepods, *C. glacialis* and *P. minutus* were the species that provided the highest amounts of lipid carbon for higher trophic levels (191 and 53 μg C m^{-3} d^{-1}, respectively). Such elevated daily transfer confirms the major role of calanoid copepods in the lipid-based Arctic pelagic food web, as suggested by previous studies, which were mainly conducted on large calanoids (Falk-Petersen et al. 1990, 2004, 2009, Dahl et al. 2003). In contrast, the small cyclopoid *O. similis* provided 3 μg C m^{-3} d^{-1} total lipids on average, which suggests that despite its very high abundance, the species does not occupy a major role in the transfer of lipids in the Arctic pelagic food web. However, *O. similis* may be more important during particular periods, i.e. autumn and winter when calanoid copepods undergo diapause (Lischka and Hagen 2005, Narcy et al. 2009, Svensen et al. 2011 section 4.3). The species may therefore act as stabilization factor of planktonic communities (Paffenhöfer 1993, Narcy et al. 2009).

Among pteropods, the large *L. helicina* adults represented the most important vector of total lipid transfer, with a daily supply of 7 μg C m^{-3} d^{-1} for higher trophic levels. This result

confirms the suggestions of previous studies that *L. helicina* is a key species in the Arctic pelagic food web (Gilmer and Harbison 1991, Falk-Petersen et al. 2001, Böer 2005, Lischka et al. 2012), however at a much lower level than calanoid copepods.

The low total lipid production of *L. retroversa* (0.5 μg C m^{-3} d^{-1}) despite its high individual lipid turnover rate (manuscript III) strongly supports that the combination of physiological capacities, biomass and abundance plays a role in the capacity of species to transfer lipids across the food web. Namely a species that exhibits a low lipid turnover rate but has a high biomass can have the same impact on the lipid transfer in the food web as a species that exhibit a high lipid turnover rate but has a low biomass. The similar range of total lipids provided by *L. helicina* adults and *O. similis* females despite their contrasting sizes and abundances is in line with this observation.

The zooplankton community of Arctic fjords in summer/autumn is mainly composed of the species and developmental stages investigated in this thesis (Walkusz et al. 2003, Blachowiak-Samolyk et al. 2008, Kwasniewski et al. 2010). Therefore I consider that the range of turnover rates calculated in this study is a good estimate of that of the zooplankton community. Based on my data (Table. 9), I suggest that the lipid turnover rate of the zooplankton community can be approximated to 0.6 % total lipids d^{-1} in summer/autumn. With such an average rate, the total lipids of the zooplankton community may be entirely exchanged in less than 5 months. This seems in agreement with Falk-Petersen et al. (1990) who suggested that fatty acids may be transported through the Arctic pelagic food web from algal blooms to top predators within 6-8 months.

Table 9: Average abundances calculated from Table 8 and lipid turnover of zooplankton species.

Species	**Average abundance (ind m^{-3})**	**Lipid turnover (% total lipids ind^{-1} d^{-1})**
C. glacialis CIV	239	1.3
P. minutus CV	751	2.6
O. similis female	2837	0.5
L. helicina juvenile	2510	0.2
L. helicina adult	170	0.1
L. retroversa adult	23	1.3
C. limacina adult	21	0.07

4.4.2 Essential fatty acids

A variety of PUFA that have their first double bond in the position (n-3) and (n-6) (see Box.2) are identified as EFA. Among all lipid compounds transferred across the food web, these compounds are considered as the most important and have been the focus of several studies (i.e. Klein Breteler et al. 1999, Sargent et al. 1999, Arts et al. 2001, Parrish et al. 2007). The first fatty acids described as essential were 18:2(n-6) and 18:3(n-3) because they could eliminate acute deficiency symptoms in rats (Burr and Burr 1930, Gurr and Harwood 1991). Currently, 23 PUFA are recognized as being essential (Cunnane 2000), among which 10 are recurrent in Arctic marine species (Parrish 2009, manuscript I, III). These compounds are involved in several processes such as neural development (Connor 2000, Ruxton et al. 2004), cell membranes function (Lee et al. 1971, Ruxton 2004), reproduction (Jónasdóttir et al. 2009) and diseases prevention/healing (Kris-Etherton et al. 2002, Simopoulos 2002, Shahidi and Miraliakbari 2004). In the biosynthesis of PUFA, the first double bond is usually inserted in the middle of the molecule, for example in the (n-9) position of 18:0. While primary producers introduce the subsequent double bonds between the first double bond and the methyl end, animals are only able to introduce double bonds between the first double bond and the carboxyl end (Parrish 2009). Consequently animals cannot synthesize (n-3) and (n-6) PUFA and need to assimilate them from their diet. In the Arctic pelagic food web, zooplankton ensures the transfer of EFA from primary producers to higher trophic levels. According to the results of this study, zooplankton species assimilate and turnover their EFA at different rates (Table 10).

As for the total lipids (section 4.4.1), calanoid copepods were the most efficient in supplying EFA to higher trophic levels, since *C. glacialis* and *P. minutus* could transfer 7 and 10 μg C m^{-3} d^{-1}, respectively (Fig. 18). Thus, these organisms do not only play a major role in the transfer of lipid carbon but also in transferring high quality fatty acids to higher trophic levels (Falk-Petersen et al. 1990, 2004, 2009, Dahl et al. 2003). The pteropod community exhibited a daily transfer of EFA of only 3 μg C m^{-3} d^{-1} (Fig. 18). Among this community, *L. helicina* adults had the highest capacity to provide EFA to higher trophic levels (2 μg C m^{-3} d^{-1}). Their large biomass seems to counterbalance their low individual EFA turnover rate (Gannefors et al. 2005, section 4.4.1). This stresses the importance of the thecosome community in the transfer of EFA, which to my knowledge, has never been demonstrated.

Table 10: Daily EFA assimilation (10^{-5} μg C ind^{-1} d^{-1}) and EFA turnover of zooplankton species (% total EFA ind^{-1} d^{-1}).

	EFA assimilation (10^{-5} μg C ind^{-1} d^{-1})	EFA turnover (% total EFA ind^{-1} d^{-1})	EFA assimilation (10^{-5} μg C ind^{-1} d^{-1})	EFA turnover (% total EFA ind^{-1} d^{-1})	EFA assimilation (10^{-5} μg C ind^{-1} d^{-1})	EFA turnover (% total EFA ind^{-1} d^{-1})
	C. glacialis CIV		*P. minutus* CV		*O. similis* female	
18:2(n-6)	27.15	0.80	24.92	0.45	0.69	0.19
18:3(n-3)	132.25	2.26	67.65	1.09	1.83	1.17
18:3(n-6)	23.38	0.27	–	–	–	–
18:4(n-3)	150.64	0.92	61.23	1.05	0.78	0.43
20:3(n-3)	–	–	–	–	–	–
20:3(n-6)	–	–	–	–	–	–
20:4(n-6)	28.83	0.46	NA	NA	NA	NA
20:5(n-3)	1468.31	1.57	NA	NA	NA	NA
22:5(n-3)	41.84	1.43	857.54	3.60	15.15	1.24
22:6(n-3)	965.75	1.60	334.94	2.23	7.74	0.45
	L. helicina juv.		*L. helicina* adult		*L. retroversa* adult	
18:2(n-6)	0.08	0.12	56.92	0.01	98.02	2.32
18:3(n-3)	0.14	0.19	289.15	0.14	789.08	9.82
18:3(n-6)	–	–	–	–	–	–
18:4(n-3)	0.29	0.07	372.52	0.08	169.71	3.83
20:3(n-3)	–	–	221.72	0.09	108.85	0.74
20:3(n-6)	0.03	0.02	98.28	0.03	17.06	0.14
20:4(n-6)	0.06	0.12	0.00	0.00	17.48	0.59
20:5(n-3)	0.77	0.15	13.33	0.00	167.11	0.20
22:5(n-3)	0.74	0.94	56.78	0.03	3.49	0.11
22:6(n-3)	0.32	0.06	280.28	0.00	138.64	0.12
	C. limacina adult					
18:2(n-6)	101.51	0.06				
18:3(n-3)	423.45	0.95				
18:3(n-6)	–	–				
18:4(n-3)	16.81	0.01				
20:3(n-3)	–	–				
20:3(n-6)	–	–				
20:4(n-6)	57.19	0.02				
20:5(n-3)	247.27	0.02				
22:5(n-3)	15.79	0.01				
22:6(n-3)	142.52	0.01				

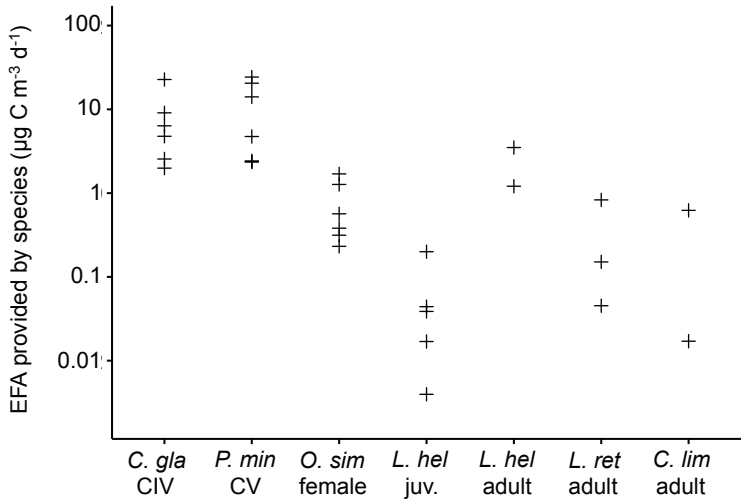

Figure 18: Total EFA (μg C m^{-3} d^{-1}) provided by main Arctic zooplankton species. The y-axis is represented in a $\log_{10}$ scale for easier visualization.

The EFA 20:5(n-6) and 22:6(n-3) are considered the most important long chain PUFA for zooplankton organisms (Brett et al. 2009, Jónasdóttir et al. 2009) but also for higher levels, e.g. fish (Sargent et al. 1999, Montero et al. 2003, 2004) and mammals (Simopoulos 2002, Ruxton et al. 2004, Shahidi and Miraliakbari 2004, Wijendran and Hayes 2004). They are essential components of cell membranes and have several beneficial effects related to health (Parrish 2009). It is therefore not surprising that these two compounds were the most assimilated EFA by the zooplankton species in this study, representing 51% and 27% of the total EFA provided by zooplankton, respectively (Fig.19). Among zooplankton, calanoid copepods seemed particularly efficient to provide 20:5(n-6) and 22:6(n-3) (Fig.20).

The C_{18} EFA, and especially 18:2(n-6) and 18:3(n-3) are crucial in the neural development of several higher trophic species (Burr and Burr 1930, Gurr and Harwood 1991). In the present study, they represented 2% and 8% of total EFA assimilated by all zooplankton species (Fig.19). *L. helicina* adults showed the same efficiency as calanoid copepods to provide C_{18} EFA, which may be explained by their respective feeding strategies, since *L. helicina* assimilates flagellate fatty acids in larger amounts than calanoids do (see section 4.2.2, manuscript III).

The long chain EFA 20:3(n-3) and 20:3(n-6) are particularly involved in the immune system processes as anti-inflammatory compounds (Fan and Chapkin 1998, Yang-Yi and Chapkin 2000). In the present study, they represented together 3% of the total EFA transferred by the zooplankton community (Fig.19). The results revealed that among the zooplankton community,

only thecosome pteropods showed the ability of providing these compounds for higher trophic levels (Fig.20), evidencing here again their crucial role among the zooplankton community.

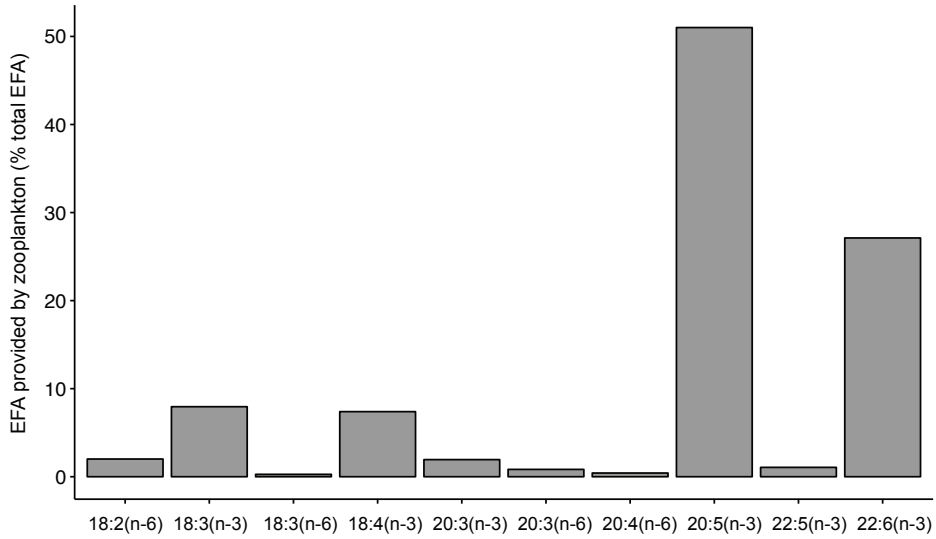

Figure 19: EFA (% total EFA) provided by the Arctic zooplankton community.

Assuming that the species and stages investigated in this study are representative for the zooplankton community, I suggest that the EFA turnover rate of the zooplankton community may average 0.6 % total EFA d^{-1}, which is similar to the total lipid turnover rate.

KEY-MESSAGES OF SECTION 4.4

• The transfer of lipids across the Arctic pelagic food web by zooplankton is mainly ensured by calanoid copepods as a result of their high lipid content, efficient lipid turnover and high abundances. *L. helicina* adults also supply significant amounts of lipids to higher trophic levels, despite their low turnover rates.

• EFA are largely assimilated by zooplankton species from their diet and therefore available for higher trophic levels. Copepods and pteropods supply different compounds to their predators. This complementarity suggests that both taxa are crucial for an efficient transfer of EFA across the food web.

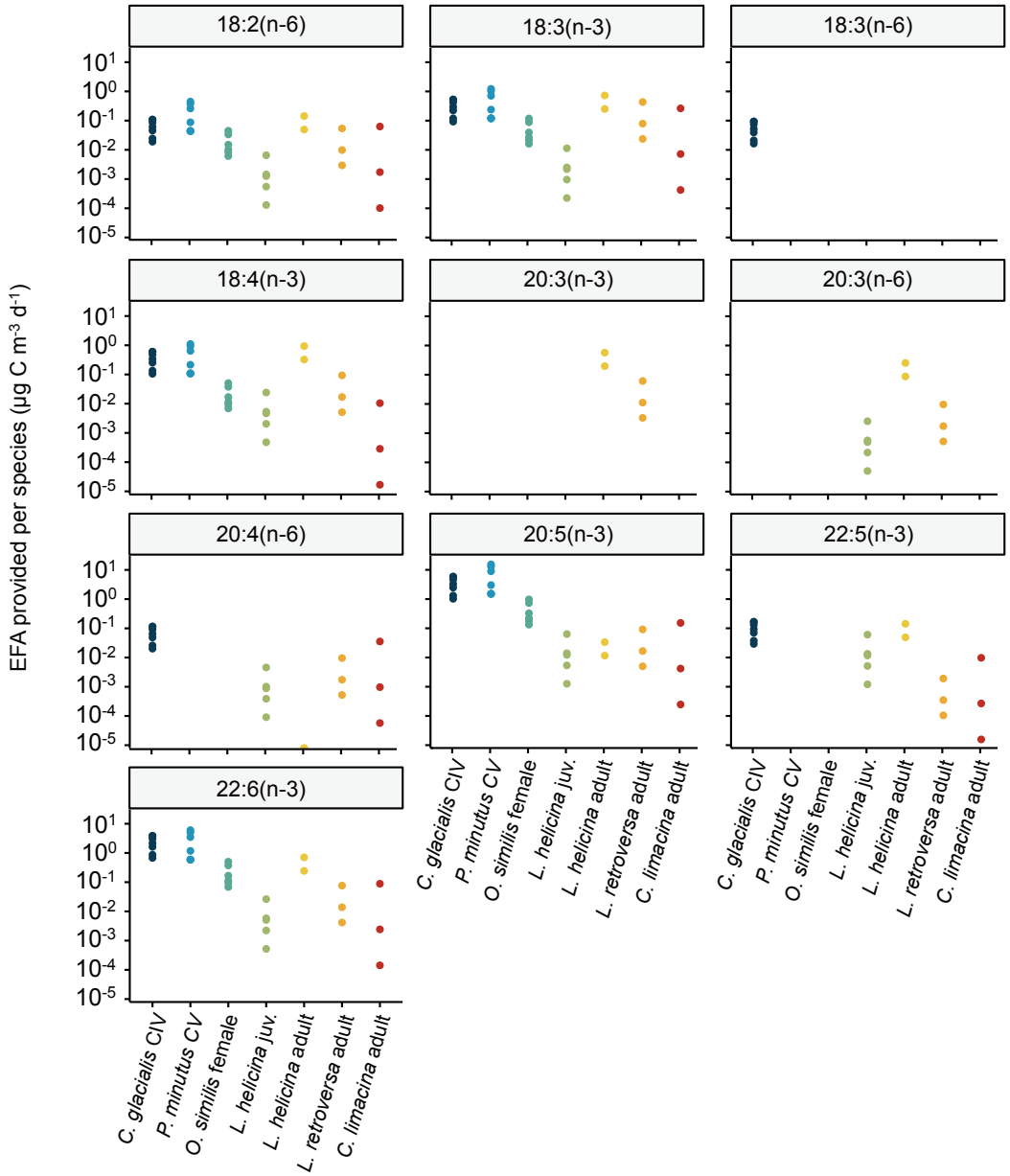

Figure 20: EFA (μg C m^{-3} d^{-1}) provided by main Arctic zooplankton species. The y-axis is represented in a $\log_{10}$ scale for easier visualization.

4.5 Impacts of climate warming on lipid transfer in the Arctic pelagic food web

Recent estimates of the effects of climate change on the Arctic environment suggest higher Atlantic inflow, surface water warming, sea ice melting, increased rates of carbon uptake and enhanced stratification (Dickson et al. 2000, Hansen et al. 2004, Orr et al. 2005, Nghiem et al. 2007, Serreze et al. 2007, Holliday et al. 2008, 2009, McNeil and Matear 2008, Timmermans and Proshutinsky 2015, 2016). Such changes may cause serious alterations in the pelagic food web structure (Hansen et al. 2003). Primary producers and zooplankton species will be directly and indirectly affected by these environmental shifts, which will have repercussions on the transfer of lipids across the food web. The determination of lipid turnover rates in zooplankton offers the possibility to evaluate changes in the Arctic pelagic food web with a functional approach. This approach is novel and complementary to other studies that base predictions on static observations of lipid biomass (e.g. Falk-Petersen et al. 2007, Søreide et al. 2010, Berge et al. 2012), considering zooplankton as an "energy reservoir" without taking fluxes into account. This section aims at developing some major impacts of climate change on the lipid turnover of the Arctic pelagic food web.

4.5.1 Changes in the fatty acid primary production

In the future decades, the warming and stratification of Arctic seas may result in a shift of the phytoplankton species composition, as flagellates are likely to thrive while diatom abundances may decrease (Li et al. 2009, Ardyna et al. 2011). Such change may have large consequences on the basal production of total lipids and fatty acids, in terms of quantity and quality. Firstly, small cells such as flagellates produce less lipid carbon than large cells such as diatoms (Li et al. 2009). In our experiments, flagellate cells contained 2 times less lipid carbon than diatom cells (manuscript I, III). In a situation where flagellates dominate the phytoplankton community, a high abundance of this taxa may compensate for its low lipid content. Yet zooplankton species may have to feed at higher rates to ensure a sufficient consumption of carbon lipid. Secondly, flagellates and diatoms do not have the same fatty acid signature, especially regarding EFA (Fig.21) (manuscript I, III). A dominance of flagellates would result in an increased production of the C_{18} PUFA (Graeve et al. 1994b). In contrast, the long chain 20:3(n-6) and 20:4(n-6) are apparently not synthesized by flagellates, and this will have negative implications for higher trophic levels that are unable to synthesize them (Parrish 2009).

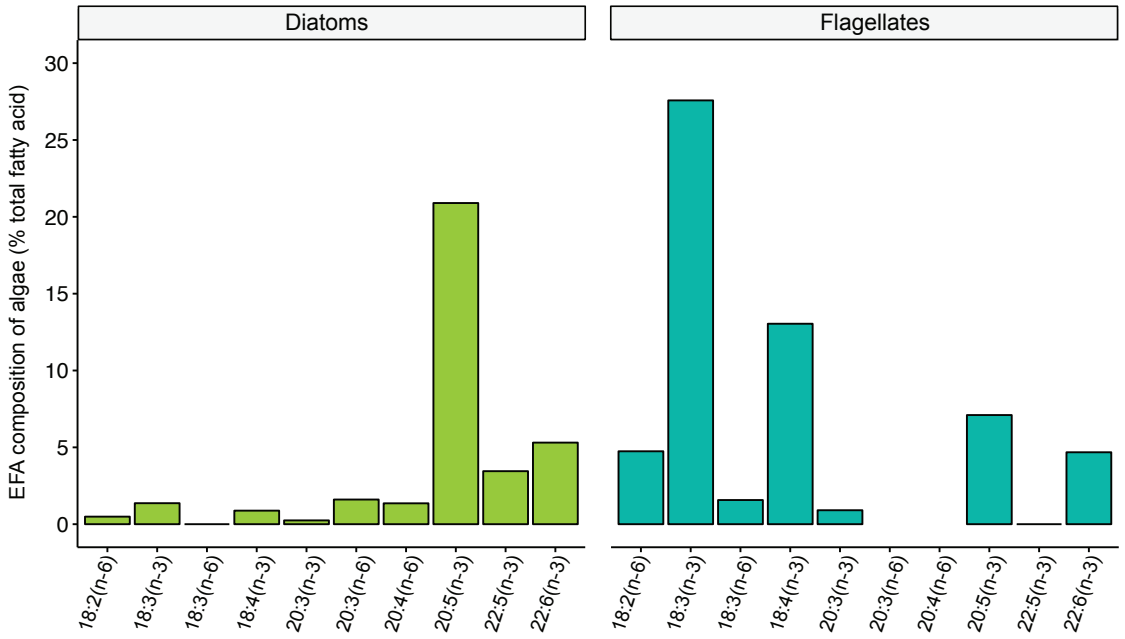

Figure 21: EFA composition (% total fatty acid mass) of diatoms and flagellates grown during the feeding experiments in the present study.

The phytoplankton community composition shift to a dominance of flagellates may directly affect zooplankton dynamics. Species that prefer diatoms will face a decrease of their main food source while species that prefer flagellates may have a higher success and thrive under this new regime. The present study suggests that herbivorous zooplankton, i.e. *C. glacialis* and *P. minutus*, assimilate FATM at a higher rate from diatoms than flagellates, when feeding on a mix-diet (see section 4.2.1, manuscript I). From my data, I cannot conclude whether these copepods may switch to feeding solely on flagellates when there is no other food source in the water column as some studies suggest (Levinsen et al. 2000, Lischka and Hagen 2007), and more investigations are needed to comprehend to which extend they can perform lipid biosynthesis only from this diet. As suggested by the present study's results, omnivorous species such as *O. similis*, *L. helicina* and *L. retroversa* (Gilmer 1972, Perissonotto 1992, Svensen and Kiørboe 2000) assimilate FATM at least as much from flagellates as from diatoms (see section 4.2.2). In a situation of flagellate dominance, these species may thrive. Since the omnivorous species *O. similis* and *Limacina* spp. generally provide less lipids and fatty acids than the large calanoid copepods (see sections 4.4.1 and 4.4.2), higher trophic levels of the food web may be affected, as they would get less energy form their diet and would hence need to feed at a higher rate.

Some studies suggest that the prymnesiophyte *Phaeocystis pouchetti* may dominate the phytoplankton community in the future decades, as a result of the warming of the water column and intensified inflow of Atlantic water masses (Hegseth et al. 2008, Lasternas and Augusti

2010, Hegseth and Tverberg 2013). Several studies report reduced zooplankton feeding and abundance during blooms of *Phaeocystis* (Estep et al. 1990, Turner et al. 2002, Nejstgaard et al. 2007). Microzooplankton may be particularly vulnerable to this shift of phytoplankton species composition, as it cannot feed on the large colonies of *Phaeocystis* (Weisse et al. 1994, Calbet et al. 2010). Labeling experiments using this algal species as a food source need to be conducted to understand the transfer of lipids through the future Arctic pelagic food web.

As sea ice becomes thinner, it will break up earlier in spring, resulting in an earlier onset of the pelagic primary production (Hegseth and Sundfjord 2008). The growth season for sea ice algae will become shorter as they will lose their substrate (Hegseth 1998, Tremblay et al. 2006, Arrigo et al. 2008, Pabi et al. 2008). As a result, the time between the ice-associated and pelagic blooms will shorten (Søreide et al. 2010). This will potentially have direct impacts on the herbivorous zooplankton species, which highly rely on the algae and phytoplankton blooms to accumulate lipid reserves. The life cycle of *C. glacialis* is synchronized to the primary production regime (Niehoff et al. 2002, Søreide et al. 2008, Falk-Petersen et al. 2009). Females use the high-quality ice algal bloom to fuel early maturation and reproduction and their resulting offspring can used the phytoplankton bloom two months later. An earlier sea ice break-up may lead to a mismatch between the two primary production peaks of high-quality food and the life cycle of *C. glacialis*, resulting in negative impacts on the reproduction, growth, and abundance of *C. glacialis* (Søreide et al. 2010). *P. minutus*, although less studied than *C. glacialis*, is also known to take advantage of the ice-algal production (Conover et al. 1986, Runge and Ingram 1991). A shortened ice-algae bloom may therefore also have negative consequences for its development. Since *C. glacialis* and *P. minutus* constitute key species in the transfer of total lipids and fatty acids in the Arctic pelagic food web (see sections 4.4.1 and 4.4.2), several important species of higher trophic levels may be negatively affected by the changes in their life cycles.

4.5.2 Shifts in the zooplankton community

Invasion of boreal species

Over the past decades, ArW warming in combination with an elevated inflow of atypical warm AW (Dickson et al. 2000, Hansen et al. 2004, Holliday et al. 2008, 2009, Schauer et al. 2008, Beszczynska-Möller et al. 2012, Bauerfeind et al. 2014) have led to a growing presence of sub-Arctic species. For example, in the early 20^{th} century, *L. retroversa* was considered as a

sub-polar species, occurring south of 65 °N (Lebour 1932, Redfield 1939), while it can currently be found at up to 79 °N (van der Spoel and Heyman 1983, Meinecke and Wefer 1990, Bathmann et al. 1991, Bauerfeind et al. 2009). A long term study looking at sedimentation rates in the Fram Strait suggested a shift of the thecosome community since 2005/2006, pointing towards a dominance of *L. retroversa* (Bauerfeind et al. 2014). An increase of *L. retroversa* abundance can not be confirmed by this study since *L. retroversa* were on average 4 times less abundant than *L. helicina* (see manuscript II). Further investigations are needed to clarify this possible shift of the community composition. If the predictions of Bauerfeind et al. (2014) come true, the Arctic pelagic food web may face large alterations. While *L. retroversa* seems to have a higher lipid turnover rate than *L. helicina* (see section 4.2.2), its low body mass makes it less efficient to provide lipids for higher trophic levels (see section 4.4.1). Higher trophic levels would therefore have to feed on a higher number of animals to fulfill their metabolic requirements. This is supported by the fact that *C. limacina* seems to turnover its total lipids at a much higher rate when feeding on one *L. helicina* than when feeding on 3.4 *L. retroversa* (Böer et al. 2006, manuscript III, section 4.2.3).

Effects of acidification

Ocean acidification is occurring at a high rate in Arctic seas (Orr et al. 2005, McNeil and Matear 2008). Recent studies predict that most thecosomes will not be able to precipitate $CaCO_3$ to build their shells by the end of this century (Lischka et al. 2011, Comeau et al., 2012). Lischka and Riebesell (2012) suggested that thecosomes already experience aragonite under-saturation nowadays, at least during winter. Net dissolution of the shells in combination with additional energy demands to construct them may lead to a lower survival success of the thecosome community, which in turn will result in decreasing abundances (Comeau et al. 2012, Lischka et al. 2012). In such an extreme scenario, the transfer of lipids across the food web may be highly perturbed, since less lipids would be available from the thecosome community. Also, the EFA 20:3(n-3) and 20:3(n-6) may not be transferred towards higher trophic levels since pteropods seem to be the only zooplankton taxa to ensure their transfer (see section 4.4.2). With severe decrease in thecosome pteropod abundances, *C. limacina* may disappear. Higher trophic levels that count *C. limacina* as a main food source, such as baleen whales (Lebour 1931, Lalli 1970), would then face a major dietary change.

Key-messages of section 4.5

• Global changes that affect the Arctic ecosystem will qualitatively and quantitatively alter the primary production of lipids and fatty acids since small cells will likely dominate the phytoplankton community.

• Particularly herbivorous zooplankton will be strongly affected by a change of their diet, which will considerably change the transfer of lipid carbon and specific EFA to higher trophic levels.

• The zooplankton community composition may change, with increasing success of sub-Arctic species and decreasing success of Arctic species. Especially a disappearance of thecosome pteropods due to ocean acidification may have dramatic repercussions on the transfer of certain EFA that are only provided by pteropods for higher trophic levels.

5

CONCLUDING REMARKS

This study has gathered (1) physiological data acquired from experimental work and (2) ecology knowledge documented from field observations and literature to better understand the role of zooplankton in the transfer of lipids across the Arctic pelagic food web in a rapidly changing context. It is among the few that combined CSIA and labeled feeding experiments to accurately determine lipid assimilation rates and assess lipid turnover capacities of main Arctic zooplankton species and their developmental stages, i.e. *C. glacialis*, *P. minutus*, *O. similis*, *L. helicina*, *L. retroversa* and *C. limacina*. This thesis has also increased our understanding of thecosome pteropods ecology, filling gaps of knowledge that are essential to better assess their place in the Arctic pelagic ecosystem.

The findings of this thesis have raised new questions, which may be particularly promising for further studies. This section presents different axes of research that would be essential to develop to improve our understanding of the lipid-driven Arctic pelagic food web.

A first aspect is that this thesis is based on experiments that were exclusively conducted during the late productive period (summer/early autumn). The findings suggested a high seasonality of zooplankton lipid turnover and therefore lipid transfer throughout the Arctic pelagic food web. However lipid turnover rates could not be quantified for different seasons. Feeding exper-

iments using ^{13}C diet conducted with the same zooplankton species as in this study and at a different time of the year would allow for solving this problematic. These experiments should include a diet composition that is representative of the *in situ* conditions during respective seasons. In addition, using *Phaeocystis* as the main food source would allow for investigating the lipid turnover capacities of zooplankton species in future conditions.

In this study, the potential transfer of lipids by zooplankton was calculated using zooplankton distribution data that was gathered from previous estimates. This allowed for an evaluation of the average daily supply of lipids by zooplankton, which offers a valuable basis for a better understanding of ecosystem dynamics. It would be of major interest to further develop this research by investigating zooplankton community patchiness in Arctic seas as well as the species/stage composition of the patches. This knowledge combined with specific lipid turnover rates would allow for the identification of hotspots of lipid transfer in the Arctic pelagic food web. Developing a model based on these elements may be useful to predict areas of major importance for the lipid-driven pelagic food web, particularly in a context of high vulnerability of the Arctic ecosystem.

This thesis exclusively focused on the zooplankton community to evidence its major role in the transfer of lipids in the Arctic pelagic food web. The next step would be to extend these investigations to other communities for which there is currently not information about lipid turnover rates. For example, fish would represent an essential component of the food web and outputs of such investigations may be used in ecological studies as well as in fisheries management.

6

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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 örtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremerhaven, den 03/04/2017

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Lauris Boissonnot

Contribution

Contribution of the PhD candidate in % of the total work load (100% for each of the following categories):

Manuscript I
Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods
Lauris Boissonnot, Barbara Niehoff, Wilhelm Hagen, Janne E. Søreide and Martin Graeve

Experimental concept and design: ca. 60%
I developed the concept together with Martin Graeve, Barbara Niehoff and Janne E. Søreide.
Acquisition of (experimental) data: ca. 95%
I carried out the fieldwork alone. I performed the experiments with the support of Martin Graeve and Barbara Niehoff. I performed all lipid extractions.
Data analysis and interpretation: ca. 65%
I analyzed the data with the support of Martin Graeve. I interpreted with scientific advice from Barbara Niehoff and Martin Graeve.
Preparation of Figures and Tables: ca. 90%
I prepared all figures and tables and the co-authors commented on them.
Drafting of the manuscript: ca. 65%
I wrote the manuscript and co-authors commented and helped for improvement.

The manuscript was published in Journal of Plankton Research in 2016.

Manuscript II
Year-round population dynamics of *Limacina helicina* and *L. retroversa* in a high-Arctic fjord
Lauris Boissonnot, Janne E. Søreide, Barbara Niehoff, Peter Kohnert, Eike Stübner, Michael Schrödl and Martin Graeve

Experimental concept and design: ca. 50%
Janne E. Søreide, Barbara Niehoff and myself developed the idea of this study.
Acquisition of (experimental) data: ca. 10%
This study was based on previous field observations performed by Eike Stübner and Janne E. Søreide. I prepared sampled for the molecular analyses. I retrieved the meteorological data from the Norwegian Meteorological Institute.
Data analysis and interpretation: ca. 65%
I analyzed the data of the thecosome pteropod distribution as well as the meteorological data, with the contribution of Barbara Niehoff and Janne Søreide for interpretation. Peter Kohnert performed the molecular analyses with the help of Michael Schrödl, and did the interpretation.
Preparation of Figures and Tables: ca. 95%
I prepared all figures and tables. All co-authors commented on them.
Drafting of the manuscript: ca. 65%
I wrote the manuscript with scientific advice from Barbara Niehoff and Janne E. Søreide. Peter Kohnert wrote the molecular section of the material and methods.

The manuscript is in preparation for Polar Biology.

Manuscript III

Lipid and fatty acid turnover of the pteropods *Limacina helicina*, *L. retroversa* and *Clione limacina* from Svalbard waters

Lauris Boissonnot, Benedikt Ehrenfels, Barbara Niehoff, Janne E. Søreide, Wilhelm Hagen and Martin Graeve

Experimental concept and design: ca. 75%
I designed this study with Martin Graeve. The experimental concept was developed together with Martin Graeve, Barbara Niehoff and Janne E. Søreide.
Acquisition of (experimental) data: ca. 65%
I carried out the fieldwork with Benedikt Ehrenfels. I performed the feeding experiments of all pteropods with labeled diet. Benedikt Ehrenfels performed the feeding rate experiment of *L. helicina* juveniles with the help of Janne E. Søreide. I performed all lipid extractions with Benedikt Ehrenfels.
Data analysis and interpretation: ca. 60%
I analyzed the data with Benedikt Ehrenfels and with the support of Martin Graeve. I interpreted together with Benedikt Ehrenfels, with scientific advice from Martin Graeve and Barbara Niehoff.
Preparation of Figures and Tables: ca. 95%
I prepared all figures and tables and co-authors commented on them.
Drafting of the manuscript: ca. 55%
I wrote the manuscript in close collaboration with all co-authors.

The manuscript is submitted to Marine Ecology Progress Series.

I hereby certify that all the above information is true and correct,
Bremerhaven, 04/04/2017

_____

Lauris Boissonnot