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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 (FA) turnover in Arctic pteropods. Thecosome pteropods, i.e. *Limacina helicina* (juveniles and adults) and *L. retroversa* (adults), were collected in summer/autumn in Kongsfjorden and Isfjorden (Svalbard, 78° N) and, for the first time, successfully fed with ^{13}C-labeled algae for 6 d. The gymnosome pteropod *Clione limacina* was sampled in summer in northern Svalbard and fed with ^{13}C-labeled *L. retroversa* for 23 d. FA compositions were determined by gas chromatography, and ^{13}C enrichment of FAs was analyzed by compound-specific isotope analysis. Among the thecosomes, maximum lipid turnover occurred in *L. retroversa* adults (1.3% d^{-1}). *L. helicina* adults and juveniles showed lower lipid turnover rates (0.1 and 0.2% d^{-1}, respectively). The thecosomes exhibited the ability to assimilate omega-3 FAs (up to 8.0% d^{-1}). The lipid turnover rate of *C. limacina* averaged at only 0.07% d^{-1}. However, *C. limacina* clearly showed the unusual capacity of de novo synthesis of odd-chain FAs (up to 1.2% d^{-1}). Lipid turnover rates of pteropods were lower than those reported for Arctic copepods. However, pteropods may play a substantial role in the transfer of lipids to higher trophic levels, especially in autumn, when copepods have descended from the upper layers of the water column. The pteropods also showed the capacity to channel particular compounds such as omega-3 and odd-chain FAs, and therefore could be important for the functional diversity of the Arctic zooplankton community.

KEY WORDS: Fatty acid assimilation · Trophic transfer · Omega-3 fatty acids · ^{13}C labeling · Compound-specific isotope analysis · CSIA · Arctic

1. INTRODUCTION

The Arctic environment is characterized by extreme seasonality in light conditions, with continuous sunlight in summer and complete darkness during the polar winter. Strong pulses of primary production are followed by extended periods of food scarcity, especially for herbivorous species. Abundant zooplankton species such as copepods and euphausiids have adapted to the seasonal food availability by storing large amounts of energy-rich lipids, usually as triacylglycerols (TAGs) and/or wax esters (Sargent & Falk-Petersen 1988, Falk-Petersen et al. 2000, Lee et al. 2006). This strategy allows them to survive during long periods of food paucity, while reducing their metabolism (Hagen & Auel 2001, Freese et al. 2015, 2016, Huenerlage et al. 2016). Pteropods also have the capacity to store lipids as diacylglycerol ethers (DAGEs) and/or TAGs to cope with the strong seasonality in food supply (Lalli & Gilmer 1989, Gilmer &

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Harbison 1991, Böer et al. 2006). However, pteropods are 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).

Limacina helicina is the main representative of thecosome pteropods in Arctic waters and reaches a maximum size of 13 mm (Conover & Lalli 1972, Gilmer & Harbison 1991). In contrast, *L. retroversa* is advected into the Arctic with Atlantic water masses and does not grow larger than 3 mm (Kattner et al. 1998, Lischka & Riebesell 2012). Both species can reach high abundances and are preyed upon by other carnivorous zooplankton, such as ctenophores (Larson & Harbison 1989) and gymnosome pteropods (Conover & Lalli 1972), as well as various species of fish, seabirds and baleen whales (Gilmer & Harbison 1991, Karnovsky et al. 2008). *L. helicina* and *L. retroversa* are omnivorous and usually feed by excreting a mucous web, in which food particles become entangled (Gilmer 1972, Harbison & Gilmer 1992). They accumulate moderate lipid reserves (<20% of dry mass, %DM), which are mainly deposited as TAGs (Kattner et al. 1998, Falk-Petersen et al. 2001, Gannefors et al. 2005). Major fatty acids (FAs) are the saturated 16:0 and 18:0, and the polyunsaturated FAs (PUFAs) 20:5(n-3) (eicosapentaenoic acid, EPA) and 22:6(n-3) (docosahexaenoic acid, DHA), typical components of membrane phospholipids. Essential FAs (EFAs) can be used as trophic markers (FATMs) and thus reveal preferences and changes in feeding behavior (Dalsgaard et al. 2003 and references therein). In *Limacina* spp., the portion of 16:1(n-7) lipids 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).

L. helicina has a life cycle of 1–2 yr and spawns in late summer/autumn (Kobayashi 1974, Gannefors et al. 2005, Bednaršek et al. 2012). *L. helicina* is a protandrous hermaphrodite; individuals develop to males during early summer and to females in July to August. Females die after spawning, and individuals overwinter as veligers and juveniles. (Lalli & Wells 1978, Gannefors et al. 2005). Veligers accumulate lipids from the phytoplankton diet and probably use them during the dark period, in addition to relying on omnivorous feeding (Gannefors et al. 2005). However, it remains under debate whether their metabolism is reduced during overwintering to save energy (Hunt et al. 2008, Bednaršek et al. 2012). The life cycle of *L. retroversa* is less known than that of *L. helicina*. Studies conducted in subpolar environments suggested a 1 yr life cycle with constant reproductive activities throughout the year, including 2 intense spawning events in spring and in autumn (Dadon & de Cidre 1992).

Many recent studies on *Limacina* spp. have focused on the impact of climate change, due to the high sensitivity of the species' aragonite shell to acidification (e.g. Comeau et al. 2009, Lischka & Riebesell 2012, Bednaršek et al. 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 web (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 their 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 FA biosynthesis and energy transfer to higher trophic levels.

Clione limacina is the only gymnosome pteropod species 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 (Lalli 1970). It seems to feed exclusively on *Limacina* spp. (Lalli 1970, Conover & Lalli 1972). Such a monophagous feeding strategy, with a strong dependency on 1 species with a patchy distribution, implies a high feeding efficiency and starvation tolerance, hence the need for energy reserves to survive long periods of food paucity (Böer et al. 2005). *C. limacina* is able to assimilate carbon from its prey with an efficiency of more than 90% (Conover & Lalli 1972, 1974). The species is characterized by high lipid contents of up to 50% DM (Ikeda 1972, Lee 1974, Böer et al. 2005), mainly deposited as TAGs and DAGEs (Kattner et al. 1998, Falk-Petersen et al. 2001). DAGEs are used as long-term energy reserves, whereas TAGs serve as short- and medium-term energy stores (Kattner et al. 1998). Both lipid classes are used to fuel growth and reproduction processes, as well as metabolism during starvation, in combination with very low metabolic activity and body shrinkage (Phleger et al. 1997, Böer et al. 2005, 2007).

Major FAs 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 FAs, 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 a de novo synthesis of odd-chain FAs by *C. limacina*. This presumably starts with propionate (3 carbon atoms), a molecule that may originate from dimethylsulfoniopropionate (DMSP), which *Limacina* spp. accumulate from their phytoplankton diet (Kattner et al. 1998, Falk-Petersen et al. 2001, Böer et al. 2005). Propionate would subsequently be elongated and desaturated to form the odd-chain FAs (Kattner et al. 1998). The efficiency of gymnosomes to synthesize lipid components from their diet has not yet been analyzed in detail. Most studies have focused on their capacity to synthesize odd-chain FAs (Kattner et al. 1998, Böer et al. 2005), but the metabolic turnover of specific FAs remains poorly understood.

Our study aimed at elucidating how the life strategies of pteropod species and their developmental stages are reflected by the turnover of their total lipids and single FAs, in order to better understand the role of pteropods in the transfer of lipid carbon through the Arctic marine food web.

Two major working hypotheses were put forward:

(1) Boissonnot et al. (2016) demonstrated that herbivorous copepod species show higher lipid turnover rates than omnivorous species. We hypothesized that the omnivorous *Limacina* spp. exhibit lower lipid turnover rates than herbivorous zooplankton species, but higher rates than the carnivorous *C. limacina*, reflecting the different feeding strategies of these pteropods.

(2) Kattner et al. (1998) demonstrated that *C. limacina* has the exceptional ability to synthesize odd-chain FAs. We hypothesized that all Arctic pteropod species exhibit specific FA assimilation and synthesis pathways. Thus, they are an important source of complex FAs for higher trophic levels, although total lipid turnover rates may be rather low.

To address these questions, feeding experiments were conducted with *L. helicina* juveniles and adults, *L. retroversa* adults and *C. limacina* adults. To monitor the assimilation of specific dietary FAs as well as the turnover rates of lipid carbon, *Limacina* spp. were fed with ^{13}C-labeled algae. Subsequently, labeled *L. retroversa* adults were offered as food to *C. limacina*. We analyzed the ^{13}C enrichment of FAs by compound-specific isotope analysis (CSIA) following the method described by 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*.

2. MATERIALS AND METHODS

2.1. Sampling and experimental work

Limacina retroversa adults were sampled for feeding experiments by vertical hauls from 100 to 0 m with a WP3 net (1000 µm mesh size, 1.0 m^2 net opening) in Kongsfjorden, near the west coast of Spitsbergen (Norway), on board RV 'Helmer Hanssen' on 23 September 2014. Following the same procedure, *L. retroversa* adults that were used as food for *Clione limacina* were sampled in Isfjorden, a fjord south of Kongsfjorden, every 3 to 4 d from 20 September to 10 October 2014. *L. helicina* adults were sampled in Isfjorden on 28 July 2015. Considering the time of sampling and the shell diameter of the individuals (>5 mm), they were all considered to be females in this study (Gannefors et al. 2005). *L. helicina* specimens 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 (L. Boissonnot pers. obs.). *L. helicina* juveniles were sampled by vertical net hauls from 50 to 0 m with a WP2 net (200 µm mesh size, 0.25 m^2 net opening) in Isfjorden on 29 September 2015 on board a small boat ('Polarcirkel') from the University Centre in Svalbard (UNIS). All thecosomes were transferred to containers filled with 0.7 µm filtered seawater at 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. *C. limacina* specimens were sampled in the Arctic Ocean north of Svalbard in July 2014 onboard RV 'Lance' by vertical hauls from bottom to surface with a Method Isaac Kidd (MIK) net (opening: 3.14 m^2, mesh size: 1.5 mm, net bag: 7 m long). Before being transported to the UNIS laboratories, the organisms were maintained onboard for 2 wk in 20 l buckets filled with seawater, which was exchanged twice a week.

2.2. 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 the use of antibiotics to prevent bacterial infections (reviewed by Howes et al. 2014), but in our study even small concentrations of antibiotics induced high mortalities of the thecosomes. Hence, this approach was abandoned for both *Limacina* spp. and *C. limacina*.

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

Immediately after arrival at UNIS, clearly active *Limacina* spp. specimens were sorted using glass pipettes. Due to their larger 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 2 species was based on shape and size, as described by Lalli & Wells (1978). *L. retroversa* dominated the thecosome community in Kongsfjorden and Isfjorden in 2014. They were easily recognizable by their pointy spiral shell, and adults measured more than 1.0 mm in diameter, as described by Hsiao (1939). *L. helicina* prevailed in 2015. They were distinguished from *L. retroversa* by their flat-coiled shell. The shell diameter of the adults ranged between 5.0 and 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 (Day 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 a surface reference sensor in air; LI-190, LI-COR). Two-thirds of the water in all bottles was changed every second day to feed the thecosomes and maintain high oxygen concentrations. In parallel, 3 bottles were emptied and the animals were deep-frozen (–80°C) in triplicates for lipid analysis (Table 1). Mortality was low during the first 4 d, with <26% of all individuals dead by Day 4. It increased to 70% of all individuals by Days 5–6 and >95% after Day 6. Therefore, the experiments were stopped after 6 d.

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 a 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 natural concentrations to avoid an overload of the isotope-ratio mass spectrometry (IRMS) detector (Faraday cups). Therefore, the standard method has gradually decreased the quantity of ^{13}C for the algae labeling since 2005 (Graeve et al. 2005), and the atom% reached in the algae FAs was 3–4 times lower in 2015 than in 2014 (see 'Results').

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, and *C. weissflogii* and *D. salina* were maintained at 15°C, since they grew faster than at 4°C. All cultures were grown at a 12 h light:12 h dark cycle. Before feeding the thecosomes, algal cells were counted with a hemocytometer (Schoen 1988), and chl *a* concentrations were measured by fluorometry using methanol as the extracting solvent (Holm-Hansen & Riemann 1978). On average, 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 × 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 that was sieved over 60 µm mesh to exclude larger zooplankton. The bottles contained 50, 100 and 200 individuals (2 replicates for each density). In addition, 3 bottles without pteropods served as controls. The bottles were attached to a slowly rotating plankton wheel and incubated for 24 h at an ambient temperature of ca. 5°C and in the dark to avoid algal growth. At the end of the experiment, the juveniles were retrieved from the bottles, and mortality was found to be low (on average <19% of all individuals). Chl *a* concentrations were determined at the end of the experiment according to the protocol described above, using 3 subsamples of 200 ml per bottle. Ingestion rates were calculated as chl *a* removal rates, using linear least square regression according to Frost (1972) with slight modifications:

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

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

$$[\text{Chl } a] = -3.4 \times 10^{-4} \times n_{Limacina} + 0.27 \quad (p < 0.01) \quad (1)$$

where [chl *a*] is the concentration of chl *a* (µg l^{-1}) at the end of the experiment, $n_{Limacina}$ is the density of *L. helicina* juveniles (ind. l^{-1}), and 0.27 is the *y*-intercept of the model. The absolute value of the slope was equated with the ingestion rate, resulting in a rate of 3.4×10^{-4} µg chl *a* ind.$^{-1}$ d^{-1} for *L. helicina* juveniles. These ingestion rates expressed as µg chl *a* ind.$^{-1}$ d^{-1} were converted to µg lipid C ind.$^{-1}$ d^{-1} using the measured content of chl *a* per algal cell and the measured lipid content per cell.

2.4. Feeding of *C. limacina*

C. limacina, which were sampled in July 2014, were kept without food for 10 wk 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*, since they did not appear in the fjord before September. Half of the water in the aquaria was renewed twice a week to maintain sufficiently high 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 3 d. 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 Days 0 and 8) and thereafter 3 (on Days 14, 17 and 20) active *L. retroversa* were transferred to the bottles. Feeding rates were determined by counting the number of emptied shells after 24 and 48 h. Non-swimming *L. retroversa* specimens were removed after 48 h, since *C. limacina* apparently feeds only on active *Limacina* spp. (Lalli 1970). The feeding experiment lasted for 23 d. Individuals were frozen 3 d after their last feeding episode (Table 2) and analyzed separately (total number of samples: 22). Ingestion rates were calculated from the number of *L. retroversa* specimens and converted to lipid carbon, considering that *L. retroversa* contained on average 3.0 µg lipid C ind.$^{-1}$ (see 'Results').

2.5. FA analysis

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

Table 2. Feeding experiments with *Clione limacina*, showing the day of feeding and the number of adult *Limacina retroversa* successfully ingested (number in parentheses indicates the number of *C. limacina* specimens which ingested the respective number of *L. retroversa* specimens. e.g. Day 11: 3 *C. limacina* ingested 9 *L. retroversa*, 2 *C. limacina* ingested 10 *L. retroversa*)

Day	No. of *L. retroversa* ingested (no. of *C. limacina*)
0	0 (3)
11	9 (3), 10 (2)
17	5 (1), 9 (1), 10 (1)
20	8 (2), 9 (1)
23	7 (1), 9 (1), 10 (3), 17 (1), 18 (2)

Total FAs 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) (see Boissonnot et al. 2016). The chromatograms were evaluated using ChemStation software (Agilent Technologies). Total lipid mass per individual was calculated by summing up single FA masses.

2.6. Carbon isotopic ratios

The ^{13}C isotopic enrichment in FAs was measured using a Thermo gas chromatography-combustion-isotope-ratio mass spectrometry (GC-c-IRMS) system (Thermo Scientific) (see Boissonnot et al. 2016). For each analytical run, 2 reference gas pulses were used for data calibration at the start and at the end, together with the internal standard 23:0 FA methyl ester (FAME) (δ –32.50 vs. Pee Dee belemnite [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 are normally expressed in δ notation according to Eq. (2):

$$\delta^{13}C\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (2)$$

where *R* is the ratio $^{13}C/^{12}C$, and the commonly used standard is Vienna PDB: $R_{standard} = 0.0112372$.

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. δ-values were converted according to the following equation:

$$AT = [R_{sample}/(R_{sample} + 1)] \times 100 \quad (3)$$

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

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

$$ATE(\%) = AT_{t=i} - AT_{t=0} \quad (4)$$

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

The dietary FAs do not only contain ^{13}C but also ^{12}C. To calculate the percentage of carbon assimilated in the pteropod FAs (PA), the ATE was therefore divided by the total average labeling in the FAs of the food source. For thecosome pteropods, average FA AT in the algae mixture was used in the calculation ($AT_{algae,\ 2014} = 13.7\%$, $AT_{algae,\ 2015} = 3.7\%$). The average AT of *L. retroversa* FAs on Day 3 was used to estimate the portion of carbon assimilated by *C. limacina* ($AT_{L.\ retroversa,\ Day3} = 2.2\%$):

$$PA = ATE/AT_{food} \quad (5)$$

To estimate the assimilation of carbon (C_{assi}) as mass (µg C_{assi} ind.$^{-1}$) (see Eq. 6 below), the PA was multiplied by the mass of each FA (B) expressed as carbon mass (in µg C ind.$^{-1}$). The carbon mass was calculated on a mole base for each FA. The molecular mass of each labeled FA was calibrated by its carbon AT to incorporate the carbon mass variation according to the $^{13}C/^{12}C$ ratio (De Troch et al. 2012):

$$\begin{aligned} C_{assi}\ (\mu g\ C\ ind.^{-1}) &= B \times PA \\ &= B \times ATE/AT_{food} \end{aligned} \quad (6)$$

Finally, the relative assimilation of FAs was calculated as Eq. (7), with TL_{assi} being the total assimilated lipid mass:

$$Relative_{assi} = (C_{assi}/TL_{assi}) \times 100 \quad (7)$$

2.7. Statistical analyses

Statistical analyses were performed using the free software R 3.2.1 (R Development Core Team 2010). Normal distribution of the total lipid and FA turnover rates was tested with a Shapiro-Wilk test. One-way ANOVAs followed by Tukey HSD tests were performed on linear regressions. Portions were arcsine-transformed prior to the tests.

Principal component analyses (PCAs) were performed to explore the thecosomes' FA compositions and to determine their similarity. Variables were arcsine-square root-transformed for better separation of observations when analyzing portions. FAs comprising <1% were removed and the rest recalculated to 100%. Data are summarized as means, and measures of variability are reported as standard deviations (SD).

3. RESULTS

3.1. FA composition and ^{13}C uptake of algal cultures

In all experiments (2014 and 2015), major FAs 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 FA masses at a ratio of about 60% diatoms to 40% flagellates in 2014 and 50% to 50% in 2015 (Table 3). The monounsaturated FA (MUFA) 16:1(n-7) and the PUFAs 16:2(n-4) and 16:3(n-4) were mostly provided by diatoms (>89%). The PUFAs 18:3(n-3) and 18:4(n-3) were largely synthesized by flagellates (>88%). The FA 18:2(n-6) was exclusively detected 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 ($p > 0.05$). In 2014, maximum enrichment occurred in 16:3(n-4) with 18.9 atom%. 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%).

3.2. Total lipid and FA composition of pteropods

During the feeding experiments, neither the total lipid mass nor the FA masses changed significantly in juvenile and adult *Limacina* spp. and adult *Clione limacina* specimens ($p > 0.05$) (Table 4).

The total lipid mass of *L. helicina* juveniles averaged 0.04 ± 0.01 µg C ind.$^{-1}$ (Table 4). Major FAs 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 MUFAs 22:1(n-9) and 22:1(n-7) (>3.7%). The total lipid mass of *L. helicina* adults averaged at

Table 3. Absolute and relative fatty acid (FA) compositions of the algal food from the 2014 and 2015 experiments (mean ± SD) and contributions of diatoms (*Contricribra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*) to the FA masses in the mixture. The ^{13}C enrichment is expressed as mean ± SD atom% (AT%). The presented values are calculated from single-algae cultures and averaged for the mixture, for the entire culture period (n = 6 on each date, 30 d of culturing). SD could not be calculated when the number of replicates was <3. TL: total lipid

FA	Mass (µg C l^{-1})	Relative mass (%TL)	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
20:3(n-3)	37 ± 8	0.6 ± 4.3	29.8	70.2	11.6 ± 5.1
20:3(n-6)	100 ± 65	0.7 ± 6.8	0.0	100.0	12.0 ± 5.8
20:5(n-3)	1272 ± 483	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 ± 89	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)	332 ± 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
20:3(n-3)	55 ± 25	0.5 ± 4.3	27.4	72.6	1.8 ± 0.3
20:3(n-6)	87 ± 85	0.8 ± 6.5	0.0	100.0	2.4 ± 0.8
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

203 ± 72 µg C ind.$^{-1}$. The major FAs 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 FA 14:0 and the MUFAs 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 FAs 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 to 1235 µg C ind.$^{-1}$, with an average of 148 ± 255 µg C ind.$^{-1}$. The major FAs 16:0, 18:0, 20:5(n-3) and 22:6(n-3) accounted for 63.5% of the total lipid mass. The FA 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 FAs 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*.

To visualize the differences in FA composition related to species and developmental stages, a PCA was performed on *L. helicina* (adults, juveniles) and *L. retroversa* (adults) (Fig. 1). Two principal components together 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 the PCA. Adults of *L. helicina* and *L. retroversa* had similar FA compositions, mostly driven by the PUFAs 20:5(n-3) and 22:6(n-3). *L. helicina* juveniles formed a well-differentiated group, with high concentrations of the saturated FAs 16:0, 18:0 and 20:0, as well as C_{16} PUFA and the long-chain MUFA 22:1 (both isomers: n-11 and n-9).

3.3. Carbon assimilation

3.3.1. Total lipids

The ingestion rate of *L. helicina* juveniles averaged at 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 6 d (Fig. 2). The daily total lipid turnover rate was of 1.2 × 10^{-4} µg C ind.$^{-1}$ d^{-1}, which corresponds to 0.2 ± 0.05% of total lipid C d^{-1}. *L. helicina* adults had a lower carbon turnover rate than the juveniles, with only 0.3 ± 0.01% exchanged by Day 6 (significant increase: $p < 0.01$). The daily rate of total lipid turnover was 0.04 µg C ind.$^{-1}$ d^{-1}, equaling 0.1 ± 0.01% of total lipid C d^{-1}. In *L. retroversa* adults, 7.7 ± 0.5% of the total lipid carbon was replaced by Day 6. The portion

Table 4. Fatty acid (FA) compositions of *Limacina helicina* juveniles and adults, *L. retroversa* adults and *Clione limacina* adults (mean % total lipid mass ± SD) during the entire experiment (number of replicates in parentheses). Concentrations below detection limit are indicated as (–). FATM: FA trophic marker, MUFA: monounsaturated FA

Fatty acid	*L. helicina* juveniles (11)	*L. helicina* adults (10)	*L. retroversa* (10)	*C. limacina* (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	–
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
Structural FA	57.4 ± 8.2	68.2 ± 9.9	71.5 ± 8.8	63.5 ± 14.6
Diatom FATM	6.0 ± 1.7	3.5 ± 1.3	2.9 ± 0.8	3.5 ± 1.9
Flagell. FATM	6.3 ± 1.5	5.9 ± 0.8	2.6 ± 0.6	4.1 ± 2.4
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

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

3.3.2. Fatty acids

In *L. helicina* juveniles, the highest ^{13}C assimilation was detected in the structural FAs 16:0, 18:0, 20:5(n3) and 22:6(n-3), together contributing to 51.0 ± 2.9% of the total lipid carbon assimilated (Table 5). The turnover of these FAs (i.e. the percentage of each FA exchanged in a certain interval) ranged between 0.4 ± 0.1% and 2.2 ± 0.4% of the total mass of the respective FAs by Day 6 ($p < 0.01$) (Fig. 3). In diatom FATMs, ^{13}C was assimilated at considerable rates, especially in 16:1(n-7), with a mean of 6.2 ± 1.3% exchanged by Day 6 ($p < 0.001$). In flagellate FATMs, ^{13}C was exchanged at a lower rate with 0.4 ± 0.2% to 1.1 ± 0.6% exchanged by Day 6 ($p < 0.001$). The long-chain MUFAs 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 ^{13}C of 20:1(n-9) were exchanged ($p > 0.05$). In the odd-chain FA 15:0, 1.6 ± 0.2% were exchanged by Day 6 ($p < 0.01$), while only traces of the EFA 20:3(n-6) were exchanged by Day 6 ($p > 0.05$).

In *L. helicina* adults, carbon assimilation was also the highest in structural FAs with 44.7 ± 11.4% of total lipid assimilated by Day 6. High assimilation was also detected in diatom FATMs, with 21.8 ± 6.0% of total lipid assimilated (Table 5). In relation to the total mass of specific FAs, the exchange rates were low, although they were mostly significant. Of the total diatom FA masses, 0.4 ± 0.1% ($p < 0.05$) to 1.2 ± 0.4% were exchanged by Day 6 (Fig. 4). Similarly low values were detected for flagellate markers, with 0.8 ± 0.4% exchanged in 18:3(n-3) and 0.5 ± 0.2% in 18:4(n-3) exchanged by Day 6 ($p < 0.05$). In 18:2(n-6), no significant changes were observed ($p > 0.05$). The odd-chain 15:0 was exchanged at 1.5% by Day 6 ($p < 0.01$, n = 1 at Day 6). In the EFAs 20:3(n-3) and 20:3(n-6), 0.5 ± 0.1% ($p < 0.01$) and 0.2 ± 0.4% ($p < 0.05$) of their total mass were exchanged by Day 6.

In *L. retroversa* adults, maximum carbon assimilation occurred in the flagellate FATMs 18.2(n-6), 18:3(n-3) and 18:4(n-3) (51.0 ± 3.0% of total lipid

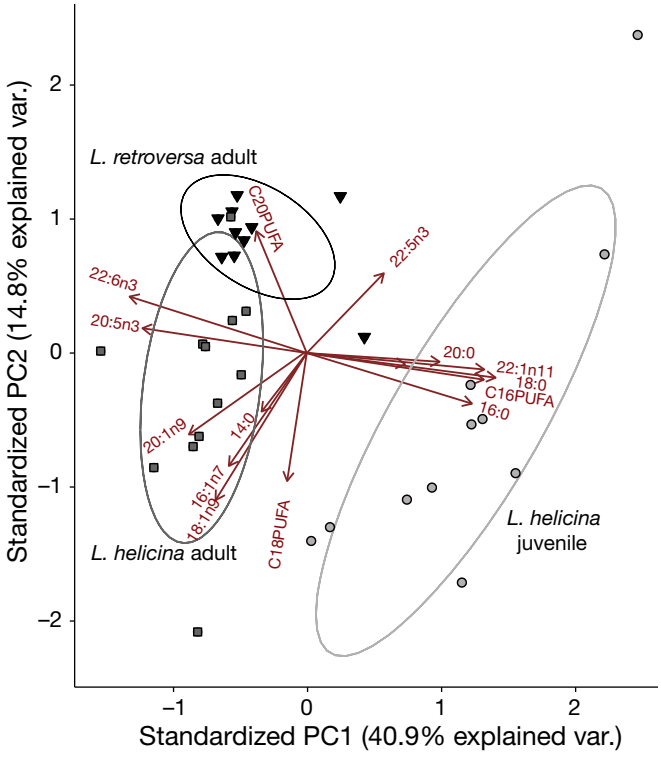

Fig. 1. Biplot of principal component analysis (PCA) of the fatty acid compositions of *Limacina helicina* juveniles (circles) and adults (squares) and *L. retroversa* adults (triangles). Variables are arcsin (sqrt) transformed. The ellipses indicate confidence intervals (68%). PUFA: polyunsaturated fatty acid

assimilated) (Table 5). In terms of exchange rates of specific FAs, flagellate markers also showed the highest rates, with 13.9 ± 0.9% ($p < 0.001$) to 59.9 ± 0.3% ($p < 0.001$) of the FA mass exchanged by Day 6 (Fig. 5). In the structural FAs 16:0, 18:0, 20:5(n-3) and 22:6(n-3), 1.2 ± 0.1 to 5.9 ± 0.7% ($p < 0.001$) of their FA masses were renewed by Day 6. Between 0.8 ± 0.7 and 1.6 ± 0.6% of the diatom FATM masses were exchanged by Day 6 ($p < 0.05$). In the long-chain MUFAs 20:1(n-7), 20:1(n-9) and 22:1(n-9), 0.4 ± 0.01% to 4.6 ± 2.6% ($p < 0.001$) of their total masses were exchanged by Day 6. In the EFA 20:3(n-3), 4 ± 0.4% ($p < 0.01$) of the total mass were exchanged, and in 20:3(n-6), 0.8 ± 0.1% ($p < 0.01$) were exchanged by Day 6.

In *C. limacina* maximum carbon assimilation again occurred in the structural FAs 16:0, 18:0, 20:5(n-3) and 22:6(n-3) (50.7 ± 8.1% of total lipid assimilated) (Table 5). Carbon exchange rates were low, even though the experiment lasted longer (23 d). Nevertheless, a ^{13}C increase was detectable in most FAs (Fig. 6). The exchanged carbon in structural FAs reached between 0.1 ± 0.01% ($p < 0.05$) and 1.3 ± 0.9% ($p < 0.001$) of their total mass by Day 23. In the diatom FATM 16:1(n-7), 1.8 ± 1.4% ($p < 0.01$) were exchanged, while 1.1 ± 0.9% ($p < 0.01$) were exchanged in 16:3(n-4) by Day 23. 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% by Day 23, $p < 0.01$). In long-chain MUFAs, only 20:1(n-7) and 20:1(n-9) were exchanged in detectable amounts (2.2 ± 1.4 and 0.7 ± 0.3% [$p < 0.001$] of their total mass by Day 23). In odd-chain FAs, 0.9 ± 0.4 to 1.2 ± 0.7% were exchanged by Day 23 ($p < 0.01$).

4. DISCUSSION

4.1. Experimental conditions and feeding strategies of pteropods

CSIA was first developed in the early 1990s to evaluate natural isotope abundances of single compounds (Meier-Augenstein 1999, Boschker & Middelburg 2002). It can also be applied in feeding experiments based on a ^{13}C-labeled diet to detect processes of carbon assimilation into specific compounds such as FAs, even when the concentrations of the respective compounds remain unchanged (Graeve et al. 2005). The transfer of lipid carbon from a producer to one or several trophic levels of consumers can thus be accurately followed and its turn-

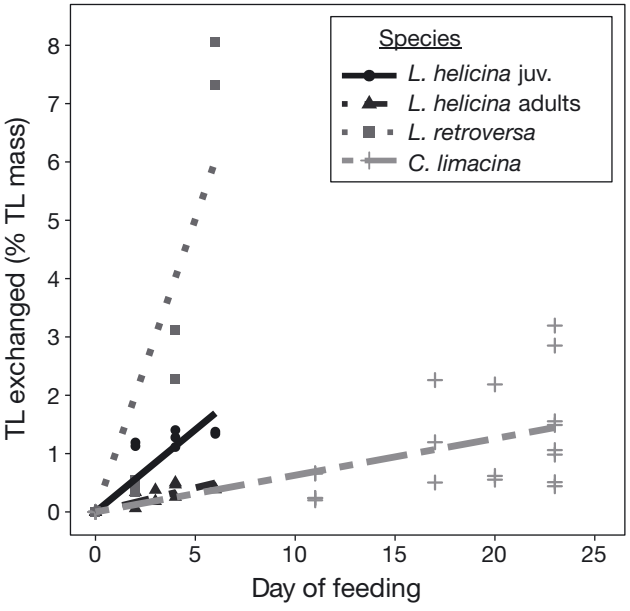

Fig. 2. Percent of total lipid (TL) exchanged, expressed as % of TL mass, in *Limacina helicina* juveniles and adults, *L. retroversa* adults and *Clione limacina* adults during the feeding experiment (6 d for *Limacina* spp., 23 d for *C. limacina*). Data of all replicates are presented

Table 5. Relative composition of fatty acids (FAs) assimilated by *Limacina helicina* juveniles and adults, *L. retroversa* adults and *Clione limacina* adults (mean % total FAs assimilated ± SD). Only FAs that are shown in the figures of assimilation are presented in this table. Averages over Day 4 and Day 6 were calculated for *Limacina* spp., since both days reflected the same assimilation pattern. Day 23 was used for *C. limacina* (number of replicates in brackets). Concentrations below detection limit are indicated as (–). FATM: FA trophic marker, MUFA: monounsaturated FA

Fatty acid	*L. helicina* juveniles (4)	*L. helicina* adults (4)	*L. retroversa* (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	–
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	–	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
Structural FA	51.0 ± 2.9	44.7 ± 11.4	41.9 ± 3.0	50.7 ± 8.1
Diatom FATM	13.8 ± 7.0	21.8 ± 6.0	1.8 ± 0.3	5.9 ± 3.7
Flagellate FATM	4.0 ± 2.7	23.3 ± 9.1	51.0 ± 3.0	24.5 ± 5.5
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

over rates quantified. During our experiment, the labeling of algal FAs was successful and allowed us to trace the transfer of specific dietary FAs to the lipids of the pteropods. Only the FA 18:0 showed very low concentrations of ^{13}C label (2.4 atom% in 2014 and 1.2 atom% in 2015). Low labeling of 18:0 has previously been reported (Boissonnot et al. 2016) and might be due to the structure and physical behavior of the 18:0 FA, e.g. due to less efficient absorption of stearic acid in the gut, but also its central role as substrate for further desaturation processes to oleic acid 18:1(n-9) (Sampath & Ntambi 2005, Li et al. 2014).

Limacina species are notoriously difficult to keep in the laboratory over an extended period of time, even in large water volumes (e.g. Lischka et al. 2010, Niehoff et al. 2013, Howes et al. 2014). During our experiment, the mortality rates of *Limacina* spp. increased after 6 d for unknown reasons. During the first 5 d, however, mortality rates were low, *Limacina* specimens exhibited normal swimming behavior, and the animals appeared to be in good condition. The major challenge concerns the appropriate uptake of food by these fragile and sensitive organisms in culture. Early studies suggested that thecosome pteropods ingest material that is transported by cilia through the mantle cavity (Morton 1954 and references therein). During SCUBA dives, Gilmer & Harbison (1986) observed the production of mucous nets by *Limacina retroversa*, and for almost 3 decades, ingesting particles captured by these nets was considered the only feeding mechanism in thecosomes (Howes et al. 2014). However, this feeding behavior has not yet been observed in the laboratory in any species of Thecosomata (Hunt et al. 2008). Recently, Howes et al. (2014) suggested that under high food concentrations, thecosome pteropods feed by cilia instead of collecting food items by mucous nets. In our experiment, the thecosomes were fed with a mixture of flagellates and diatoms at a concentration of more than 800 µg C l^{-1}, which matches ad libitum food concentrations (Howes et al. 2014). Although our experimental set-up may not have been adequate for the pteropods to produce mucous nets (see Table II in Howes et al. 2014), the analyses showed that all investigated *Limacina* species and stages assimilated ^{13}C-labeled dietary FAs. This clearly indicates that the animals ingested the labeled algae we provided and suggests ciliary feeding by *Limacina* (Howes et al. 2014).

For the first time, we were able to experimentally determine grazing rates of juvenile *L. helicina* (mean of 9.0 × 10^{-4} µg lipid C ind.$^{-1}$ d^{-1}). The lipid assimilation rate averaged 1.2 × 10^{-4} µg lipid C ind.$^{-1}$ d^{-1}, which corresponded to a total lipid turnover of 0.2% d^{-1}. For adult *L. retroversa* and *L. helicina*, ingestion rates as determined by feeding experiments have not been published, and the only data available have been derived from gut fluorescence analyses (Pakhomov et al. 1997, Pakhomov & Froneman 2004, Hunt et al. 2008). These *in situ* rates cannot be compared to experimentally determined lipid ingestion rates, as they are often much higher than for animals fed under laboratory conditions (Paffenhöfer 1988). Therefore, lipid assimilation rates can only be based on the incorporation of labeled ^{13}C. Adult *L. helicina* exhibited a total lipid exchange of only 0.1% d^{-1}, and adult

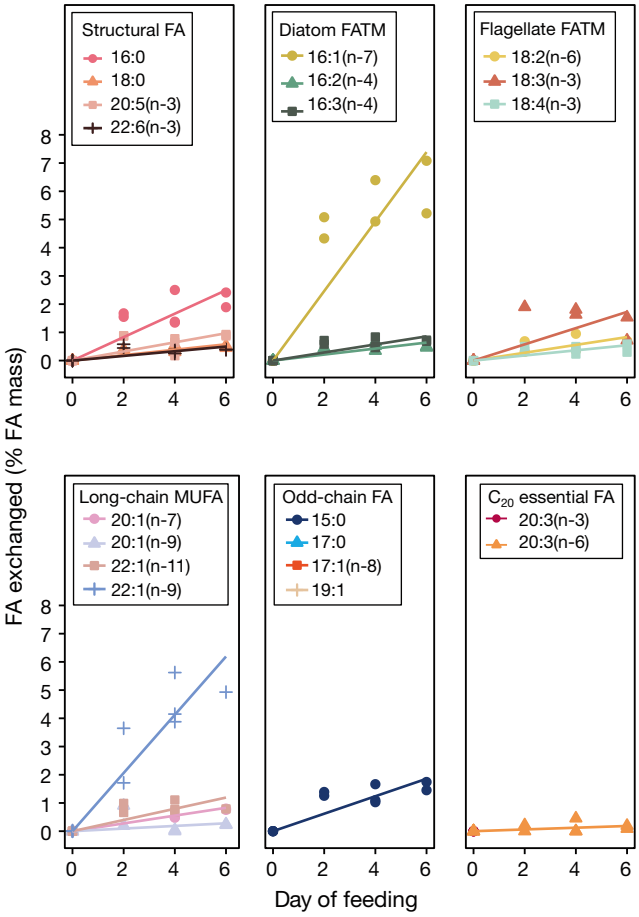

Fig. 3. Percent of fatty acids (FAs) exchanged by *Limacina helicina* juveniles (expressed as % of FA mass) during the feeding experiment (6 d). Data of all replicates are presented. FATM: FA trophic marker, MUFA: monounsaturated FA

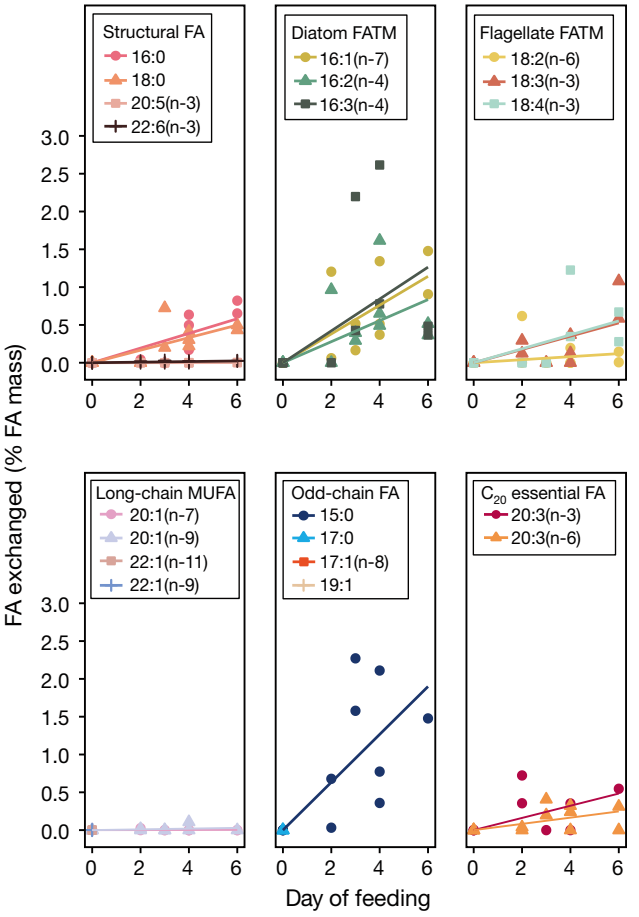

Fig. 4. Percent of fatty acids (FAs) exchanged by *Limacina helicina* adults (expressed as % of FA mass) during the feeding experiment (6 d). Data of all replicates are presented. FATM: FA trophic marker, MUFA: monounsaturated FA

L. retroversa specimens exchanged 1.3 % d^{-1}. In the absence of mucous net production, these assimilation rates of the thecosomes are probably at the lower end of their capacities.

In contrast to the *Limacina* species, the gymnosome *Clione limacina* survived more than 12 wk in our laboratory and (after a longer starvation period) they preyed on labeled *L. retroversa* adults. However, total lipid assimilation was very low (1.4 % of the ingested lipids at the end of the experiment). In their experiments with *C. limacina*, Böer et al. (2006) found that the ash-free dry mass increased by 80 % within 1 wk, suggesting much higher lipid carbon assimilation rates than during our experiment. This difference may be partially explained by the amount of carbon offered during the experiments: Böer et al. (2006) fed *C. limacina* with *L. helicina*, and the total dietary lipid provided was 1.2 mg ind.$^{-1}$, corresponding to approx. 0.9 mg C ind.$^{-1}$. We fed *C. limacina* with 3–4 individuals of the much smaller *L. retroversa*, and total dietary lipid provided corresponded to 0.01 mg C ind.$^{-1}$. Thus, total lipid mass available for *C. limacina* was 2 orders of magnitude (about 100 times) lower in our experiments, with lipid assimilation rates about 70 times lower than those reported by Böer et al. (2006). Another reason for the low assimilation rates may be the timing of sampling. In our study, *C. limacina* specimens were collected during their period of high reproductive activity (Mileikovsky 1970) and subsequently they starved for 10 wk, because no *Limacina* specimens were available. Body shrinkage associated with metabolic reduction can be expected during this period (Böer et al. 2005). The exchange rate of lipids increased throughout our feeding experiment, suggesting that *C. limacina* first invested the diet-derived energy into metabolic activity and somatic growth, and only after this 'recovery phase' did they begin to deposit

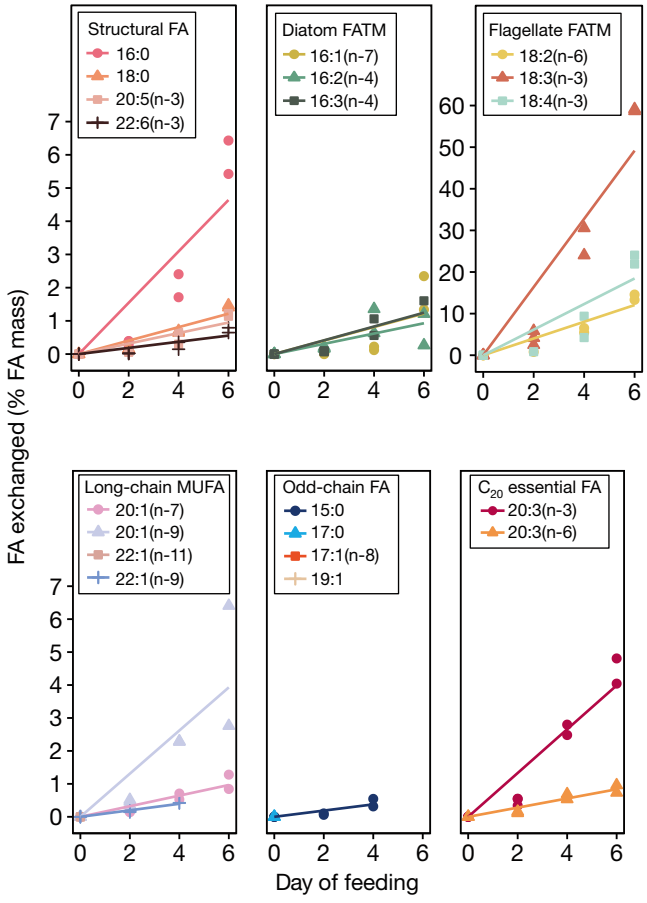

Fig. 5. Percent of fatty acids (FAs) exchanged by *Limacina retroversa* adults (expressed as % of FA mass) during the feeding experiment (6 d). Note the different scale used for flagellate FA trophic markers (FATMs). Data of all replicates are presented. MUFA: monounsaturated FA

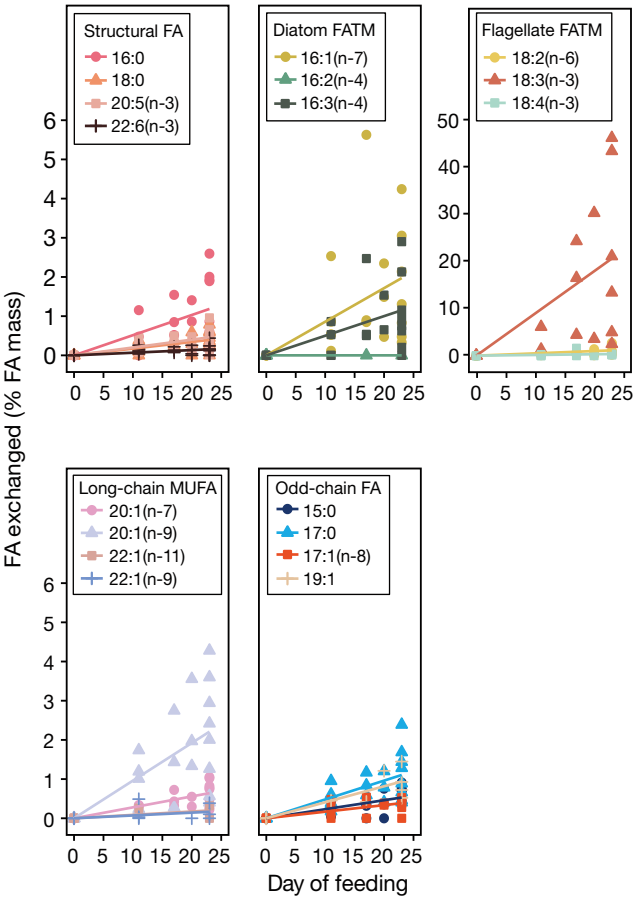

Fig. 6. Percent of fatty acids (FA) exchanged by *Clione limacina* adults (expressed as % of FA mass) during the feeding experiment (23 d). Note the different scale used for flagellate FA trophic markers (FATMs). Data of all replicates are presented. MUFA: monounsaturated FA

lipids. Somatic growth is also reflected by maximum turnover rates of the structural FAs 16:0, 20:5(n-3) and 22:6(n-3), which are major components of biomembranes and essential for zooplankton growth (Müller-Navarra et al. 2000, Wacker & von Elert 2001). Methodological differences may also have cotributed to the low lipid assimilation rates in *C. limacina*, as compared to those determined by Böer et al. (2006). In contrast to our study, Böer et al. (2006) did not remove the guts of *C. limacina*; hence, their lipid signals possibly included undigested food.

4.2. Biosynthetic pathways and transfer of FAs through the food chain

Phytoplankton synthesize all FAs de novo starting with 2 carbon units, step by step adding 2 more carbon units (elongation). PUFAs with first double bonds in the positions (n-3) and (n-6) are typical biosynthetic products of diatoms and flagellates (Graeve et al. 1994, Scott et al. 1999). They are called EFAs, as they are not synthesized by heterotrophic organisms but are crucial for their development (Pohl & Zurheide 1979, Gurr & Harwood 1991). For instance, EPA and DHA are considered the most important long-chain PUFAs for zooplankton organisms, as they are essential components of cell membranes and have various beneficial effects related to health (Brett et al. 2009, Jónasdóttir et al. 2009). Among other dietary FAs, these PUFAs (also called omega-3 FAs) are transferred unchanged through the Arctic marine food web from lower to higher trophic levels.

At capture, the adults of *L. helicina* had significantly higher concentrations of the 2 omega-3 FAs EPA and DHA than the juveniles. The concentrations of these EFAs were the highest yet observed in *L. helicina* in this season (Gannefors et al. 2005). EPA

and DHA are key components of biomembranes and are essential for gonad maturation processes and hatching success in many zooplankton species (Müller-Navarra et al. 2000, Wacker & von Elert 2001, Jónasdóttir et al. 2009), and they probably have similar functions in pteropods. When adults of *L. helicina* were sampled in late July, their gonads were probably mature, as *L. helicina* spawns in late summer/autumn in that area (Gannefors et al. 2005). Also, veliger larvae were abundant in the water column from August onwards (L. Boissonnot pers. obs.), indicating that spawning had occurred shortly after collecting the adults. *L. retroversa* adults exhibited similarly high concentrations of EPA and DHA as *L. helicina* adults; *L. retroversa*, like *L. helicina*, most likely use these PUFAs for reproductive processes (see Jónasdóttir et al. 2009 for copepods).

Adult and juvenile *L. helicina* differed with respect to the carbon turnover of the omega-3 FA 18:4(n-3). In adults, it reflected nearly 15% of the assimilated carbon, compared to only 2% in the juveniles. Gannefors et al. (2005) detected high amounts of 18:4 (n-3) in egg ribbons. Hence, this flagellate FATM could also be essential for reproductive processes of *L. helicina*.

MUFAs do not occur in an algal diet and are synthesized de novo by zooplankton species. For example, calanoid copepods are known to biosynthesize large amounts of long-chain MUFAs (20:1 and 22:1 isomers) and the respective alcohols from non-lipid dietary precursors (Sargent 1978, Sargent & Henderson 1986, Kattner & Krause 1989). They serve as long-term energy reserves, usually stored as wax esters, and may fuel metabolism during winter and reproductive processes in late winter/spring (Sargent & Falk-Petersen 1988, Kattner et al. 2007). Long-chain MUFAs have also been found in *L. helicina* (<5% of total lipid mass) from the Arctic and the Southern Ocean and have so far been associated with feeding on copepods (Kattner et al. 1998, Falk-Petersen et al. 2001, Gannefors et al. 2005). We also detected long-chain MUFAs in *L. helicina* and *L. retroversa*, and our CSIA results show that carbon was assimilated in these compounds. The assimilation was statistically significant, despite a high standard deviation of the FA masses. However, in our experiments, copepods or copepod debris were not offered to the thecosomes and can thus be excluded as a source for these FAs. Hence, *L. helicina* and *L. retroversa* may also have the potential to synthesize these long-chain MUFAs. Such a capacity has been demonstrated for benthic gastropods (Ackman & Hooper 1973), and possible pathways may include the desaturation and elongation of saturated short-chain FAs. Further work is needed to address this question and clarify the origin of long-chain MUFAs in *Limacina* species.

It has been emphasized that *C. limacina* has a high capacity for de novo FA biosynthesis. In particular, the unusual production of odd-chain FAs is remarkable and requires further explanation (Kattner et al. 1998, Böer et al. 2005). The biosynthetic pathway of the odd-chain FAs in *C. limacina* is still unclear, although the utilization of DMSP for FA biosynthesis has been suggested (Kattner et al. 1998). DMSP is most likely provided by *Limacina* specimens, which strongly accumulate this component, due to feeding on detritus and phytoplankton (Gilmer & Harbison 1991, Levasseur et al. 1994). After ingestion of *Limacina* by *C. limacina* and cleavage of DMSP to DMS and acrylic acid, the propionate moiety is formed, which could be the starter molecule (3 carbon atoms instead of the typical starter units with 2 carbon atoms, see above) for the biosynthesis of odd-chain FAs (Kattner et al. 1998). Odd-chain FAs are mainly incorporated by *C. limacina* into DAGEs, which are long-term storage lipids (Kattner et al. 1998, Böer et al. 2005), but are also found in significant amounts in triacylglycerols and phospholipids (Phleger et al. 1997, Kattner et al. 1998). Our results confirm that *C. limacina* produces these FAs de novo, since individuals assimilated substantial amounts of the odd-chain FAs 17:0, 17:1(n-8) and 19:1, but none of these were detectable in *L. retroversa,* the only prey ingested by *C. limacina*. In *C. limacina*, assimilation rates were similar for odd-chain and for even-chain FAs, directly derived from the diet. The reason for the formation of odd-chain FAs is not yet clear, and the complete pathways of biosynthesis and incorporation of these unusual FAs into the storage lipid classes TAG and DAGE still need to be resolved.

4.3. Ecological implications

Thecosome and gymnosome pteropods can occur in high numbers in polar oceans (e.g. Walkusz et al. 2003, 2009, Tsurumi et al. 2005) and are preyed upon by numerous species, including zooplankton, fish, sea birds and whales (Lalli & Gilmer 1989, Bathmann et al. 1991, Bednaršek et al. 2012). Hence, they are important components of the Arctic food web (Lalli & Gilmer 1989, Manno et al. 2010). This is the first study to assess lipid assimilation rates in pteropods by means of labeled feeding experiments and CSIA. It revealed relatively low lipid turnover rates by the

pteropods compared to herbivorous copepods. In similar experiments in the Arctic, Graeve et al. (2005) showed that the large herbivorous species *Calanus hyperboreus, C. glacialis* and *C. finmarchicus* exchanged approximately 3% of their total body lipids per day. Also, the much smaller herbivorous copepod *Pseudocalanus minutus* showed a relatively high total lipid carbon turnover rate of 2.6% d^{-1} (Boissonnot et al. 2016). In contrast, the copepod species *Oithona similis* exchanged only 0.5% total lipid C d^{-1}, possibly related to its omnivorous feeding mode (Boissonnot et al. 2016). In our experiments, only the rates of adult *L. retroversa* (1.6% total lipid C d^{-1}) fell in the range of those of the copepods, whereas the rates of juvenile and adult *L. helicina* (0.2 and 0.1% total lipid C d^{-1}, respectively) and those of *C. limacina* (0.07% total lipid C d^{-1}) were considerably lower.

The transfer of lipids from primary producers, i.e. phytoplankton, to higher trophic levels is, of course, not only dependent on lipid turnover rates of the consumers, but also on the total amount of lipid assimilated and on the abundance of the organisms. Usually, the large *Calanus* species prevail in surface waters of the Arctic only for a short period in spring/early summer (Sargent & Falk-Petersen 1988, Hagen & Auel 2001, Søreide et al. 2010). In mid- to late summer, they descend to great depths for overwintering and subsequently, small copepods together with other taxa dominate the zooplankton community near the surface (Lischka & Hagen 2005, Narcy et al. 2009, Bednaršek et al. 2012). In late summer/early autumn, juvenile *L. helicina*, for example, can reach abundances between 700 and 8200 ind. m^{-3} in Svalbard waters, while adults show lower abundances of 90 to 250 ind. m^{-3} (Walkusz et al. 2003, 2009, Gannefors et al. 2005, Blachowiak-Samolyk et al. 2008). When we simply average these abundance data of *L. helicina*, the calculated amount of lipids provided by the species adds up to 2 µg C m^{-3} d^{-1}. This is far lower than that of the most abundant lipid-storing copepods in Svalbard waters in July. *C. glacialis* copepodite stage IV (CIV) contribute 191 µg C m^{-3} d^{-1} and *P. minutus* copepodite stage V (CV) 53 µg C m^{-3} d^{-1}, according to the same calculation based on lipid carbon uptake and abundance (Boissonnot 2017). However, especially in autumn/winter, when adult *Calanus* species are absent in surface waters, pteropods may be an important lipid source for zooplanktivores. They could buffer fluctuations in food supply, thus stabilizing the zooplankton community (Paffenhöfer 1993, Narcy et al. 2009).

Among thecosome pteropods, the larger *L. helicina* adults may represent the most important vector of total lipid transfer, providing 7 µg C m^{-3} d^{-1}, while *L. retroversa* adults contribute only 0.5 µg C m^{-3} d^{-1} according to our estimations (see Boissonnot 2017).

Besides the total amount of lipids, the quality of the lipids that a prey provides is of major importance. Many of the EFAs such as 18:2(n-6), 18:3(n-3), 20:5 (n-3) and 22:6(n-3) are assimilated from their diet by both copepods and pteropods (Böer et al. 2005, Gannefors et al. 2005, Graeve et al. 2005). Comparing our CSIA data on copepods with those from the present experiment suggests that the long-chain EFAs 20:3(n-3) and 20:3(n-6) were assimilated by *Limacina* spp., especially by *L. retroversa* adults, but not by copepods. These FAs have been shown to serve as anti-inflammatory compounds in immune system processes in other organisms (Fan & Chapkin 1998). *L. helicina* adults and juveniles had low exchange rates of these FAs, but *L. retroversa* exchanged up to 4% of them until Day 6. Thus, while thecosome pteropods transfer less lipid from primary producers to higher trophic levels than the dominating copepod species, they may provide specific essential FAs absent in copepods.

With the rapid climate change occurring in the Arctic, the phytoplankton composition may shift from a diatom-dominated system to a community dominated by small flagellates (e.g. Hegseth & Tverberg 2013). In our study, adult *L. helicina* assimilated FATMs from both flagellates and diatoms in similar proportions, confirming previous findings that they do not prefer either algal group (Gilmer 1972, Falk-Petersen et al. 2001, Gannefors et al. 2005). In contrast, the juveniles assimilated higher proportions of diatom markers than those of flagellates (14 vs. 4% of assimilated FAs), even though they were sampled in September, when flagellates prevail in the water column (Leu et al. 2006, Søreide et al. 2010, Hegseth & Tverberg 2013). This highlights the potential importance of diatom-derived FAs for the development of young thecosomes. In the Arctic, growth of juvenile *Limacina* specimens ceases in winter, and growth resumes at the onset of the phytoplankton bloom (Lischka & Riebesell 2012). If, as some studies suggest, diatom spring blooms decline due to climate change (Li et al. 2009, Gao et al. 2012, Hegseth & Tverberg 2013), fewer diatom-derived FAs will be available for *Limacina* juveniles. Whether and how this will impact the development of the new generations needs to be evaluated by future studies. In contrast, *L. retroversa* exhibited a much higher assimilation rate of FAs derived from flagellates than from diatoms (51% vs. 2%). Therefore, this species could thrive in a regime where the phytoplankton community composition shifts to a dominance of flagellates.

The increasing amounts of warmer, more saline Atlantic water masses entering the Arctic may also result in an increase in *L. retroversa* abundances (Bauerfeind et al. 2014). In our study, *L. retroversa* had the highest lipid turnover rates of all investigated pteropods. This could potentially channel more lipids from primary producers to higher trophic levels than *L. helicina* does, resulting in a higher total carbon and FA transfer. However, ocean acidification may lead to a drastic decrease of shelled pteropods in the future Arctic Ocean (Comeau et al. 2012). Such a diversity loss could result in a loss of functionality. First, the buffering function of pteropods as a food source for zooplanktivores in surface waters in winter would weaken. Second, the quality of lipids transferred up the food web would also be altered. Some long-chain omega-3 and omega-6 PUFAs as well as certain de novo synthesized odd-chain FAs are mainly provided by pteropods. A loss of these essential compounds might hamper the growth of zooplanktivores and thus result in a lower productivity at higher trophic levels (e.g. fish, seabirds, whales). We therefore expect that climate change and ocean acidification can have negative implications for the overall lipid transfer in Arctic marine food webs.

4.4. Conclusions

Our study indicates that thecosome and gymnosome pteropods are less efficient than copepods in assimilating lipids. However, they still contribute to an important extent to the lipid transfer in the Arctic marine food web. After the large, lipid-rich *Calanus* species have descended to greater depths in late summer and autumn, pteropods are still abundant in surface waters and provide essential FAs, which are not synthesized by copepods. In the future, ocean acidification may lead to severe decreases in the abundance of the shelled *Limacina* species, and hence of their monospecific predator *C. limacina* (Comeau et al. 2012, Lischka & Riebesell 2012). As a result, specific long-chain EFAs of the (n-3) and (n-6) series and the odd-chain FAs 17:0, 17:1(n-8) and 19:1, which, to our knowledge, are transferred only by pteropods, may become scarce for higher trophic levels, with yet unknown implications for the Arctic marine food web.

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LITERATURE CITED

Ackman RG, Hooper SN (1973) Non-methylene-interrupted fatty acids in lipids of shallow-water marine invertebrates: a comparison of two molluscs (*Littorina littorea* and *Lunatia triseriata*) with the sand shrimp (*Crangon septemspinosus*). Comp Biochem Physiol B Biochem Mol Biol 46:153–165

Bathmann UV, Noji TT, von Bodungen B (1991) Sedimentation of pteropods in the Norwegian Sea in autumn. Deep Sea Res I 38:1341–1360

Bauerfeind E, Nöthig EM, Pauls B, Kraft A, Beszczynska-Möller A (2014) Variability in pteropod sedimentation and corresponding aragonite flux at the Arctic deep-sea long-term observatory HAUSGARTEN in the eastern Fram Strait from 2000 to 2009. J Mar Syst 132:95–105

Bednaršek N, Tarling GA, Bakker DCE, Fielding S and others (2012) Extensive dissolution of live pteropods in the Southern Ocean. Nat Geosci 5:881–885

Bednaršek N, Tarling GA, Bakker DC, Fielding S, Feely RA (2014) Dissolution dominating calcification process in polar pteropods close to the point of aragonite undersaturation. PLoS One 9:e109183

Blachowiak-Samolyk K, Søreide JE, Kwasniewski S, Sundfjord A, Hop H, Falk-Petersen S, Hegseth EN (2008) Hydrodynamic control of mesozooplankton abundance and biomass in northern Svalbard waters (79–81°N). Deep Sea Res II 55:2210–2224

Böer M, Gannefors C, Kattner G, Graeve M, Hop H, Falk-Petersen S (2005) The Arctic pteropod *Clione limacina*: seasonal lipid dynamics and life-strategy. Mar Biol 147: 707–717

Böer M, Graeve M, Kattner G (2006) Impact of feeding and starvation on the lipid metabolism of the Arctic pteropod *Clione limacina*. J Exp Mar Biol Ecol 328:98–112

Böer M, Graeve M, Kattner G (2007) Exceptional long-term starvation ability and sites of lipid storage of the Arctic pteropod *Clione limacina*. Polar Biol 30:571–580

Boissonnot L (2017) Turnover of trophic markers and lipid carbon in Arctic marine food webs: the contribution of key zooplankton species. PhD thesis, University of Bremen

Boissonnot L, Niehoff B, Hagen W, Søreide JE, Graeve M (2016) Lipid turnover reflects life-cycle strategies of small-sized Arctic copepods. J Plankton Res 38: 1420–1432

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol Ecol 40:85–95

Brenna JT, Corso TN, Tobias HJ, Caimi RJ (1997) High precision continuous flow isotope ratio mass spectrometry.

Mass Spectrom Rev 16:227–258

Brett MT, Müller-Navarra DC, Persson J (2009) Crustacean zooplankton fatty acid composition. In: Arts M, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer, New York, NY, p 115–146

Comeau S, Gorsky G, Jeffree R, Teyssié JL, Gattuso JP (2009) Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). Biogeosciences 6: 1877–1882

Comeau S, Alliouane S, Gattuso JP (2012) Effects of ocean acidification on overwintering juvenile Arctic pteropods *Limacina helicina*. Mar Ecol Prog Ser 456:279–284

Conover RJ, Lalli CM (1972) Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusc. J Exp Mar Biol Ecol 9:279–302

Conover RJ, Lalli CM (1974) Feeding and growth in *Clione limacina* (Phipps), a pteropod mollusc. II. Assimilation, metabolism, and growth efficiency. J Exp Mar Biol Ecol 16:131–154

Dadon JR, de Cidre LL (1992) The reproductive cycle of the thecosomatous pteropod *Limacina retroversa* in the western South Atlantic. Mar Biol 114:439–442

Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

De Troch M, Boeckx P, Cnudde C, Van Gansbeke D, Vanreusel A, Vincx M, Caramujo MJ (2012) Bioconversion of fatty acids at the basis of marine food webs: insights from a compound-specific stable isotope analysis. Mar Ecol Prog Ser 465:53–67

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57: 178–191

Falk-Petersen S, Sargent JR, Kwasniewski S, Gulliksen B, Millar RM (2001) Lipids and fatty acids in *Clione limacina* and *Limacina helicina* in Svalbard waters and the Arctic Ocean: trophic implications. Polar Biol 24:163–170

Fan YY, Chapkin RS (1998) Importance of dietary γ-linolenic acid in human health and nutrition. J Nutr 128:1411–1414

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Freese D, Niehoff B, Søreide JE, Sartoris FJ (2015) Seasonal patterns in extracellular ion concentrations and pH of the Arctic copepod *Calanus glacialis*. Limnol Oceanogr 60: 2121–2129

Freese D, Søreide JE, Niehoff B (2016) A year-round study on digestive enzymes in the Arctic copepod *Calanus glacialis*: implications for its capability to adjust to changing environmental conditions. Polar Biol 39:2241–2252

Frost BW (1972) Effects on size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus finmarchicus*. Limnol Oceanogr 17:805–815

Gannefors C, Böer M, Kattner G, Graeve M and others (2005) The Arctic sea butterfly *Limacina helicina*: lipids and life strategy. Mar Biol 147:169–177

Gao K, Xu J, Gao G, Li Y and others (2012) Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. Nat Clim Chang 2:519–523

Gilmer RW (1972) Free-floating mucus webs: a novel feeding adaptation for the open ocean. Science 176:1239–1240

Gilmer RW, Harbison GR (1986) Morphology and field behavior of pteropod molluscs: feeding methods in the families Cavoliniidae, Limacinidae and Peraclididae (Gastropoda: Thecosomata). Mar Biol 91:47–57

Gilmer RW, Harbison GR (1991) Diet of *Limacina helicina* (Gastropoda: Thecosomata) in Arctic waters in midsummer. Mar Ecol Prog Ser 77:125–134

Graeve M, Hagen W, Kattner G (1994) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep Sea Res I 41:915–924

Graeve M, Albers C, Kattner G (2005) Assimilation and biosynthesis of lipids in Arctic *Calanus* species based on feeding experiments with a ^{13}C labelled diatom. J Exp Mar Biol Ecol 317:109–125

Gurr MI, Harwood JL (1991) Lipid biochemistry, an introduction. Chapman & Hall, London

Hagen W, Auel H (2001) Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology 104:313–326

Harbison GR, Gilmer RW (1992) Swimming, buoyancy and feeding in shelled pteropods: a comparison of field and laboratory observations. J Molluscan Stud 58:337–339

Hegseth EN, Tverberg V (2013) Effect of Atlantic water inflow on timing of the phytoplankton spring bloom in a high Arctic fjord (Kongsfjorden, Svalbard). J Mar Syst 113-114:94–105

Holm-Hansen O, Riemann B (1978) Chlorophyll *a* determination: improvements in methodology. Oikos 30:438–447

Howes EL, Bednaršek N, Büdenbender J, Comeau S and others (2014) Sink and swim: a status review of thecosome pteropod culture techniques. J Plankton Res 36: 299–315

Hsiao SC (1939) The reproductive system and spermatogenesis of *Limacina* (*Spiratella*) *retroversa* (Flem.). Biol Bull (Woods Hole) 76:7–25

Huenerlage K, Graeve M, Buchholz F (2016) Lipid composition and trophic relationships of krill species in a high Arctic fjord. Polar Biol 39:1803–1817

Hunt BPV, Pakhomov EA, Hosie GW, Siegel V, Ward P, Bernard K (2008) Pteropods in Southern Ocean ecosystems. Prog Oceanogr 78:193–221

Ikeda T (1972) Chemical composition and nutrition of zooplankton in the Bering Sea. In: Takenouti YA (ed) Biological oceanography of the northern North Pacific Ocean. Idemitsu Shoten, Tokyo, p 433–442

Jónasdóttir SH, Visser AW, Jespersen C (2009) Assessing the role of food quality in the production and hatching of *Temora longicornis* eggs. Mar Ecol Prog Ser 382:139–150

Karnovsky NJ, Hobson KA, Iverson S, Hunt GL Jr (2008) Seasonal changes in diets of seabirds in the North Water Polynya: a multiple-indicator approach. Mar Ecol Prog Ser 357:291–299

Kattner G, Krause M (1989) Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. Mar Chem 26:261–275

Kattner G, Hagen W, Graeve M, Albers C (1998) Exceptional lipids and fatty acids in the pteropod *Clione limacina* (Gastropoda) from both polar oceans. Mar Chem 61: 219–228

Kattner G, Hagen W, Lee RF, Campbell R and others (2007) Perspectives on marine zooplankton lipids. Can J Fish Aquat Sci 64:1628–1639

Kobayashi HA (1974) Growth cycle and related vertical distribution of the thecosomatous pteropod *Spiratella* (‘*Limacina*’) *helicina* in the central Arctic Ocean. Mar Biol 26:295–301

Lalande C, Bauerfeind E, Nöthig EM, Beszczynska-Möller A

(2013) Impact of a warm anomaly on export fluxes of biogenic matter in the eastern Fram Strait. Prog Oceanogr 109:70–77

Lalli CM (1970) Structure and function of the buccal apparatus of *Clione limacina* (Phipps) with a review of feeding in gymnosomatous pteropods. J Exp Mar Biol Ecol 4: 101–118

Lalli CM, Gilmer RW (1989) Pelagic snails: the biology of holoplanktonic gastropod mollusks. Stanford University Press, Stanford, CA

Lalli CM, Wells FE (1978) Reproduction in the genus *Limacina* (Opisthobranchia: Thecosomata). J Zool 186: 95–108

Larson RJ, Harbison GR (1989) Source and fate of lipids in polar gelatinous zooplankton. Arctic 42:339–346

Lee RF (1974) Lipids of zooplankton from Bute inlet, British Columbia. J Fish Res Board Can 31:1577–1582

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306

Leu E, Falk-Petersen S, Kwasniewski S, Wulff A, Edvardsen K, Hessen DO (2006) Fatty acid dynamics during the spring bloom in a High Arctic fjord: importance of abiotic factors versus community changes. Can J Fish Aquat Sci 63:2760–2779

Levasseur M, Gosselin M, Michaud S (1994) A new source of dimethylsulfide (DMS) for the arctic atmosphere: ice diatoms. Mar Biol 121:381–387

Li WK, McLaughlin FA, Lovejoy C, Carmack EC (2009) Smallest algae thrive as the Arctic Ocean freshens. Science 326:539

Li HY, Lu Y, Zheng JW, Yang WD, Liu JS (2014) Biochemical and genetic engineering of diatoms for polyunsaturated fatty acid biosynthesis. Mar Drugs 12:153–166

Lischka S, Hagen W (2005) Life histories of the copepods *Pseudocalanus minutus, P. acuspes* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard). Polar Biol 28:910–921

Lischka S, Riebesell U (2012) Synergistic effects of ocean acidification and warming on overwintering pteropods in the Arctic. Glob Change Biol 18:3517–3528

Lischka S, Büdenbender J, Boxhammer T, Riebesell U (2010) Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth. Biogeosci Discuss 7:8177–8214

Manno C, Tirelli V, Accornero A, Fonda Umani S (2010) Importance of the contribution of *Limacina helicina* faecal pellets to the carbon pump in Terra Nova Bay (Antarctica). J Plankton Res 32:145–152

Meier-Augenstein W (1999) Applied gas chromatography coupled to isotope ratio mass spectrometry. J Chromatogr A 842:351–371

Mileikovsky SA (1970) Breeding and larval distribution of the pteropod *Clione limacina* in the North Atlantic, Subarctic and North Pacific Oceans. Mar Biol 6:317–334

Morton JE (1954) The biology of *Limacina retroversa.* J Mar Biol Assoc UK 33:297–312

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77

Narcy F, Gasparini S, Falk-Petersen S, Mayzaud P (2009) Seasonal and individual variability of lipid reserves in *Oithona similis* (Cyclopoida) in an Arctic fjord. Polar Biol 32:233–242

Niehoff B, Knüppel N, Daase M, Czerny J, Boxhammer T (2013) Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in an Arctic fjord. Biogeosciences 10:1391–1406

Paffenhöfer GA (1988) Feeding rates and behavior of zooplankton. Bull Mar Sci 43:430–445

Paffenhöfer GA (1993) On the ecology of marine cyclopoid copepods (Crustacea, Copepoda). J Plankton Res 15: 37–55

Pakhomov EA, Froneman PW (2004) Zooplankton dynamics in the eastern Atlantic sector of the Southern Ocean during the austral summer 1997/1998—Part 2: Grazing impact. Deep Sea Res II 51:2617–2631

Pakhomov EA, Verheye HM, Atkinson A, Laubscher RK, Taunton-Clark J (1997) Structure and grazing impact of the mesozooplankton community during late summer 1994 near South Georgia, Antarctica. Polar Biol 18:180–192

Phleger CF, Nichols PD, Virtue P (1997) Lipids and buoyancy in Southern Ocean pteropods. Lipids 32:1093–1100

Pohl P, Zurheide F (1979) Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. In: Hoppe HA, Levring T, Tanaka Y (eds) Marine algae in pharmaceutical science, Vol 1. Walter de Gruyter, Berlin, p 473–523

R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Sampath H, Ntambi JM (2005) The fate and intermediary metabolism of stearic acid. Lipids 40:1187–1191

Sargent JR (1978) Marine wax esters. Sci Prog 65:437–458

Sargent JR, Falk-Petersen S (1988) The lipid biochemistry of calanoid copepods. Hydrobiologia 167-168:101–114

Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) The biological chemistry of marine copepods. Clarendon Press, Oxford, p 59–108

Schoen S (1988) Cell counting. In: Lobban CS, Chapman DJ, Kremer BP (eds) Experimental phycology. A laboratory manual. Cambridge University Press, Cambridge, p 16–22

Scott CL, Falk-Petersen S, Sargent JR, Hop H, Lønne OJ, Poltermann M (1999) Lipids and trophic interactions of ice fauna and pelagic zooplankton in the marginal ice zone of the Barents Sea. Polar Biol 21:65–70

Søreide JE, Leu E, Berge J, Graeve M, Falk-Petersen S (2010) Timing of blooms, algal food quality and *Calanus glacialis* reproduction and growth in a changing Arctic. Glob Change Biol 16:3154–3163

Tsurumi M, Mackas DL, Whitney FA, DiBacco C, Galbraith MD, Wong CS (2005) Pteropods, eddies, carbon flux, and climate variability in the Alaska Gyre. Deep Sea Res II 52:1037–1053

Wacker A, von Elert E (2001) Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata.* Ecology 82:2507–2520

Walkusz W, Storemark K, Skau T, Gannefors C, Lundberg M (2003) Zooplankton community structure; a comparison of fjords, open water and ice stations in the Svalbard area. Pol Polar Res 24:149–165

Walkusz W, Kwasniewski S, Falk-Petersen S, Hop H, Tverberg V, Wieczorek P, Weslawski JM (2009) Seasonal and spatial changes in the zooplankton community of Kongsfjorden, Svalbard. Polar Res 28:254–281

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