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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 ¹³C labeled flagellates and diatoms during 3 weeks. Fatty acid (FA) and fatty alcohol compositions were determined by gas chromatography, ¹³C 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; ¹³C 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;

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.

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 m² 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 *Contricibra weissflogii*) and flagellates (*Rhodomonas salina* and *Dunaliella salina*) at concentrations $\gg 1000$ cells mL⁻¹ ($\gg 20$ µg Chl *a* L⁻¹). Algal cells were counted with a haemocytometer (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 ¹³C 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 $1.3 \pm 0.8\%$ day⁻¹. Mortality rates of *O. similis* were highest during the first 9 days ($3.1 \pm 0.7\%$ day⁻¹) and low thereafter until termination of the experiment ($0.9 \pm 0.5\%$ day⁻¹). Overall, the average mortality of *O. similis* was $3.0 \pm 0.8\%$ 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 H₂SO₄ 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),

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 ¹³C 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 (CO₂). The isotopic composition of different amounts of reference gas (CO₂, 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):

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

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

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):

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

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

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

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

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

Since the dietary FAs did not only contain ¹³C but also ¹²C, 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):

$$\text{PA} = \frac{\text{ATE}}{L} \quad (4)$$

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 ¹³C/¹²C ratio (De Troch et al., 2012):

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

Statistics

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

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 $\mu\text{g C lipid L}^{-1}$ 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 $2.8 \pm 0.6 \mu\text{g C ind}^{-1}$.

Carbon assimilation in total lipid occurred throughout the experiment, and the amount of assimilated carbon reached $1.4 \pm 0.1 \mu\text{g C}_{\text{assi}} \text{ind}^{-1}$, which represented $54.6 \pm 0.1\%$ of the total lipid carbon, when the experiment was terminated. Thus, the assimilation rate of labeled carbon into copepod total lipid carbon was $2.6\% \text{ day}^{-1}$.

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 \pm standard deviation from single algae cultures (n = 8)

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

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

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

Table III: *P. minutus* and *O. similis*. Carbon assimilation into main copepod FAs and alcohols (mean \pm standard deviation expressed as $10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$) at the end of the feeding experiment.

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

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

carbon assimilation was determined in 18:1(n-9) with $242.8 \times 10^{-3} \pm 84.3 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$ after 21 days ($P < 0.001$). 16:1(n-7) and 20:5(n-3) had the second highest carbon assimilation, with $202.4 \times 10^{-3} \pm 50.7 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$ and $180.1 \times 10^{-3} \pm 38.5 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$, respectively ($P < 0.001$). In fatty alcohols, maximum carbon assimilation was determined in 16:0, with $248.9 \times 10^{-3} \pm 129.4 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$ ($P < 0.01$), followed by 14:0 with $190.4 \times 10^{-3} \pm 75.8 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$ ($P < 0.01$). 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 $75.6 \pm 6.9\%$ of carbon was renewed in 20:5(n-3), and this exchange reached $45.7 \pm 4.7\%$ in 16:0 and $46.9 \pm 6.9\%$ in 22:6(n-3) ($P < 0.001$). In 18:0, $4.2 \pm 1.2\%$ 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 $54.3 \pm 14.9\%$ were assimilated in 18:1(n-9), and in the C_{18} PUFA assimilation ranged between $9.5 \pm 4.5\%$ and $22.9 \pm 3.4\%$. In the fatty alcohols (Fig. 1), a linear carbon exchange occurred, reaching between $62.6 \pm 11.7\%$ and $76.7 \pm 9.6\%$ after 21 days ($P < 0.001$).

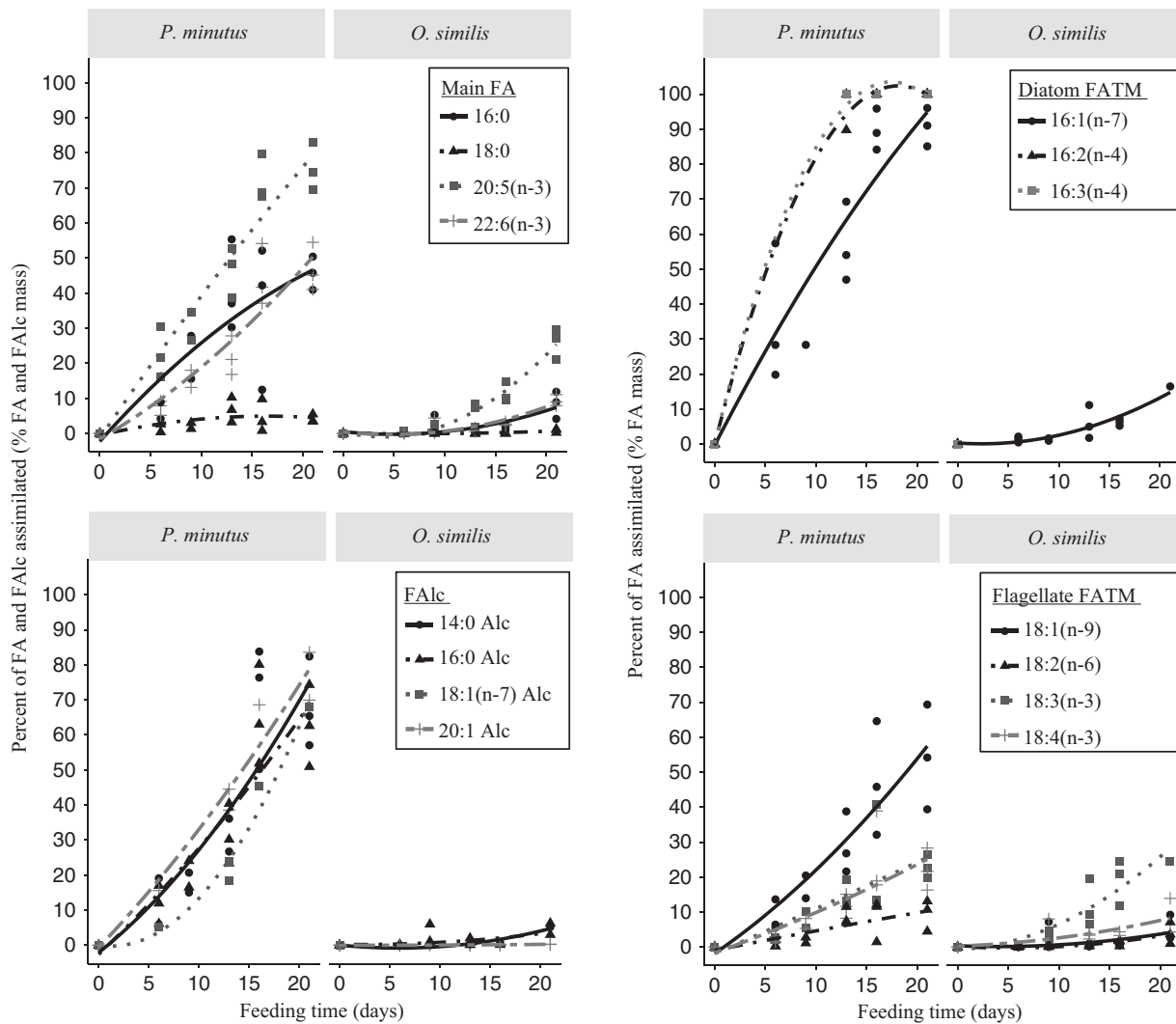


Fig. 1. *P. minutus* and *O. similis*. Proportion of carbon assimilated into main FAs and alcohols (expressed as % of FA and alcohol carbon mass) during the experiment (21 days of feeding).

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 $2.2 \times 10^{-1} \pm 0.1 \times 10^{-1} \mu\text{g C ind}^{-1}$ ($P < 0.01$). By Day 21, the amount of assimilated total lipid carbon, calculated from the labeling results, reached $0.2 \times 10^{-1} \pm 0.1 \times 10^{-1} \mu\text{g C ind}^{-1}$ ($P < 0.01$), which corresponded to $9.4 \pm 0.2\%$ of the total lipid mass. Overall, the daily carbon turnover rate of total lipid was $0.9 \times 10^{-3} \mu\text{g C day}^{-1}$, 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 II), 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 $3.2 \times 10^{-3} \pm 2.3 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$ ($P < 0.01$). It was followed by 18:1(n-9) ($1.6 \times 10^{-3} \pm 1.5 \times 10^{-3}$) and 22:6(n-3) ($1.6 \times 10^{-3} \pm 1.2 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ ind}^{-1}$). Fatty alcohol assimilation was in the same range compared to that of the FAs and the increase of assimilated carbon

was significant ($P < 0.05$). At the end of the experiment, $1.1 \times 10^{-3} \pm 0.7 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ind}^{-1}$ were assimilated in 16:0 and $0.3 \times 10^{-3} \mu\text{g C}_{\text{ass}} \text{ind}^{-1}$ ($n = 1$) in 14:0. Initial assimilation in FAs was not yet detected at Day 6 (Fig. 1). By Day 21, around $25.9 \pm 4.5\%$ of carbon was assimilated in 20:5(n-3). The portion of assimilated carbon reached $8.3 \pm 3.9\%$ in 16:0 and $9.3 \pm 1.6\%$ in 22:6(n-3) ($P < 0.01$). In 18:0, only negligible amounts of carbon were assimilated ($0.8 \pm 0.5\%$, $P > 0.05$). 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 $9.1 \pm 6.8\%$ in 18:4(n-3). In 18:1(n-9), only $5.0 \pm 3.7\%$ were assimilated, and $3.9 \pm 3.1\%$ 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 $4.7 \pm 2.3\%$ 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

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 ($2.6\% \text{ day}^{-1}$) 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 $2.7\% \text{ day}^{-1}$ 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* ($0.5\% \text{ day}^{-1}$) 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 $0.5\% \text{ day}^{-1}$ 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 C₁₆ 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; Svendsen 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

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 (Prahel *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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