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Impact of feeding and starvation on the lipid metabolism of the Arctic pteropod *Clione limacina*

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Abstract

Feeding and starvation experiments were carried out with *Clione limacina* sampled in Kongsfjorden (Svalbard, Arctic) during summer 2002. Dry mass and lipid mass, lipid class and fatty acid compositions were analysed. Specimens of *C. limacina* used for the feeding study had a mean length of 25 mm, a dry mass (DM) of 13.7 mg, and a moderate lipid content of 12.1%DM. Animals were allowed to ingest only one individual of its exclusive prey, *Limacina helicina* which had 8.0 mm in diameter, 21.4 mg DM and 8.7% lipid of ash-free DM. Five days after feeding, the dry mass of *C. limacina* had increased from 13.7 to 25.3 mg which corresponds to an uptake of about 80% of the ash-free DM (14.3 mg) of *L. helicina*. Lipid mass increased from 1.5 to 3.9 mg which is almost two times more the ingested lipid from *L. helicina* (1.2 mg lipid). Thus, the major portion of lipids was synthesised de novo by *C. limacina* from non-lipid compounds. These lipids were triacylglycerols (TAG) and 1-*O*-alkyldiacylglycerol ethers (DAGE), increasing from low proportions of 6.1% and 5.7% to 42.3% and 25.8%, respectively. Considerable de novo synthesis was observed for the monounsaturated fatty acids 16:1($n-7$), 17:1($n-8$), 18:1($n-9$), and 18:1($n-7$) and the alkyl moiety 16:0. The increase in the polyunsaturated fatty acids 22:6($n-3$), 20:5($n-3$), and 18:4($n-3$) corresponded with the amount available by ingestion of *L. helicina*, supporting that *C. limacina* is not able to synthesise polyunsaturates. After 15 days of digestion, dry mass and lipids dropped almost back to the initial values.

During the 100-day starvation experiment, two groups of animals were separately considered as storage lipid-rich and lipid-poor animals because of their large differences in the amount and proportion of TAG and DAGE. Storage lipid-rich *C. limacina* were only found until day 50, whereas lipid-poor animals were present throughout the experiment. In the lipid-rich specimens, the levels of TAG were about twice that of DAGE. The proportions of TAG decreased considerably during the 50 days of starvation (from 48.3% to 25.1% of total lipid). DAGE, varying between 16.5% and 20.5%, showed only a small decrease. The lipid-poor animals survived 100 days of starvation, exhibiting low initial amounts and proportions of storage lipids which were nearly exhausted at the end. In all *C. limacina* specimens, the total lipid content remained almost constant showing that lipid and non-lipid components were simultaneously utilised. This implies that body shrinkage may be an important adaptation to long-term starvation. Based on these results, it is possible to estimate the potential survival period of lipid-rich *C. limacina*

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under food limitation. A model, which considers maturity and reproduction (egg production), reveals that lipid-rich specimens might be able to survive up to 260 days without food.

Keywords: *Clione limacina*; Fatty acids; Feeding and starvation; Lipid classes; Lipids; Shrinkage

1. Introduction

The genus *Clione* is an important member of the Arctic and Antarctic zooplankton community. In the North Atlantic, *Clione limacina* accounts for up to 80% of the total gymnosome population (Raymont, 1983). Feeding studies revealed that in polar waters *C. limacina* feeds exclusively on the thecosome *Limacina helicina* and in temperate oceans on *L. retroversa* (Meisenheimer, 1905; Lalli, 1970; Conover and Lalli, 1972; Hopkins, 1985, 1987). Conover and Lalli (1974) have shown that *C. limacina* is very effective in food utilisation and assimilation with an efficiency for carbon of more than 90% and for nitrogen close to 100%. The disadvantage of monophagy is that *C. limacina* could be exposed to long periods without food and consequently has to be adapted to starvation stress (Lalli and Gilmer, 1989). Starvation and feeding experiments revealed that *C. limacina* is able to survive periods of up to 3 month without food and that the metabolism of starved animals is 20 times lower than that of fed animals (Lee, 1974; Conover and Lalli, 1974).

During the past three decades, only some studies have been performed on lipids of *C. limacina*. Ikeda (1972) reported on the lipid content of *C. limacina* from North Pacific, and other authors published data of lipid and fatty acid compositions for animals from temperate latitudes (Lee, 1974) and Arctic and Antarctic regions (Lee, 1975; Kattner et al., 1998; Falk-Petersen et al., 2001; Phleger et al., 1997, 2001; Böer et al., 2005). These studies revealed that high levels of triacylglycerols (TAG) and 1-*O*-alkyldiacylglycerol ethers (DAGE) are characteristically for *C. limacina*.

Lipids are the primary storage compounds because of their high energy content (39 J mg^{-1}) which is much higher than that of proteins and carbohydrates (both about 17 J mg^{-1}). Whereas TAG is the most common form to store energy, being easily mobilised for metabolic processes, DAGE is assumed to serve as long-term energy deposit (Lee and Patton, 1989; Sargent, 1989; Kattner et al., 1998). The biosynthesis of DAGE in zooplankton is suggested to be most pronounced in ecosystems with a high seasonality in food supply (Sargent, 1989) comparable to the wax ester storage in copepods (Sargent and Henderson, 1986). Böer et al. (2005) found highest DAGE levels in specimens of *C. limacina* which are in preparation for or have survived the winter season. They assumed that DAGE is necessary during periods of food scarcity, but may also serve as an additional energy source during reproduction. Enhanced TAG accumulation might be a prerequisite for successful growth and development of *C. limacina*. In addition, Phleger et al. (1997, 2001) proposed that DAGE may be important for buoyancy in *C. limacina* based on studies from the Antarctic Peninsula region.

The lipid metabolism of *C. limacina* and the biological importance of the less common neutral lipid, DAGE, are still poorly understood. Changes in lipids during feeding and starvation are key processes for the understanding of the life strategy of this highly specialised zooplankton species. The purpose of this study was to elucidate the impact of feeding and starvation of *C. limacina* on the lipid metabolism of the major storage lipids, TAG and DAGE, as well as their unusual odd-chain fatty acids.

2. Material and methods

2.1. Sampling and experimental work

Laboratory experiments with *C. limacina* were carried out at Ny-Ålesund (Svalbard) from 24 June to 23 August 2002. The long-term starvation experiment was extended until 3 October 2002 in our home laboratories in Bremerhaven. Specimens of *C. limacina* and *L. helicina* were collected at different sampling sites in Kongsfjorden (79°N, 12°E). Large specimens of both species were caught with a sieve (Ø 20 cm) on a rod directly from a landing stage in the

harbour and were also sampled with a plastic bottle during snorkelling. Live specimens were transferred into 10 L plastic buckets filled with seawater at ambient temperature and transported to the laboratory. Salinity in the fjord was 33.0, temperature 4.5 °C and pH 7.8 at 1 m depth.

All specimens were immediately transferred into 110 L V-shaped aquaria (40 × 40 × 70 cm). In order to keep the animals swimming, water was constantly circulated (Lange and Kaiser, 1995). The aquaria were filled with pre-filtered seawater (approximately 1 μm pore diameter; Hyrtex, Germany). The seawater was aerated by an air pump and permanently filtered with an external filter system (1000 L h^{-1}; Eheim 2217, Germany). Water temperature was maintained with a cooling device at 7–8 °C (SK 2, Aquamedic, Germany). For a constant light regime, fluorescent tubes (True-Lite, Osram, Germany) with illumination of 70 μmol s^{-1} m^{-2} E were used. In all aquaria temperature, salinity and pH were measured daily with a Cond 330i conductivity meter and a pH 330i pH-meter (both WTW, Weilheim, Germany). Additionally abiotic parameters (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, CO_2) were monitored every 4 days with a testlab aquarium test kit (JBL, Germany). The body lengths of all specimens were measured from the base of the anterior tentacles to the posterior end of the trunk (according to Conover and Lalli, 1972) after relaxation for a few minutes in a petri dish filled with cold seawater.

For the feeding experiment, about 80 specimens of *C. limacina* were kept in a V-shaped aquarium and starved for 14 days. Specimens of *L. helicina* were sampled just before the beginning of the experiment and placed in an additional aquarium as food. Two individuals were pooled to one sample, immediately frozen in liquid nitrogen and stored at −80 °C for later analyses. For each feeding event, one individual of *L. helicina* was transferred into the aquarium where it was captured by its predator within seconds up to some minutes. The fed *C. limacina* was transferred with a beaker into an aerated 5 L seawater aquarium and kept there until the end of the feeding process to avoid disturbance by competitors. In total, 16 specimens of *C. limacina* were fed with *L. helicina*. The whole procedure of ingestion lasted up to 45 min. For further digestion, all fed individuals were transferred to a second V-shaped aquarium. Sub-samples of 3 to 4 animals were taken before feeding and then after 5, 7 and 15 days, immediately frozen in liquid nitrogen and stored at −80 °C.

For the starvation experiment, which lasted for 100 days, 41 specimens of *C. limacina* were randomly chosen and kept in an aquarium. In order to determine the initial dry mass and lipid values, 5 animals were immediately frozen in liquid nitrogen and stored at −80 °C. Sub-samples of 3 to 7 specimens were taken at days 14, 30, 40, 50 and 60 of starvation in Ny-Ålesund. The starvation experiment was continued until day 100, the last sampling day, in Bremerhaven. For transportation, the animals were transferred in special plastic bags, filled with cold seawater (4 to 8 °C), covered with pure oxygen, and transported in a cooling box to the home laboratory. There, the animals were kept under the same conditions as described above.

2.2. Determination of dry mass and lipid analyses

For the determination of dry mass (DM) and total lipid mass (TL), samples of *C. limacina* and *L. helicina* were transferred into pre-weighted vials, lyophilised for 48 h, and DM was determined gravimetrically. Total lipid was extracted from the freeze-dried samples using dichloromethane/methanol (2:1; by volume) and measured gravimetrically (essentially after Folch et al., 1957).

Lipid class and fatty acid compositions were determined as described by Böer et al. (2005). Briefly, lipid classes were analysed by high performance thin layer chromatography (HPTLC) densitometry (Olsen and Henderson, 1989). Sample extracts and standard solutions were spotted on HPTLC plates, and separation of lipid classes was performed with hexane/diethylether/acetic acid (80:20:2; by volume). Standards were phospholipids (PL), sterols (ST), free fatty acids (FFA), triacylglycerols (TAG), wax esters (WE) and 1-*O*-alkyldiacylglycerol ethers (DAGE). Lipid classes were visualised by submerging the plate in manganese (II) chloride 4-water, methanol and sulphuric acid reagent followed by combustion at 120 °C for 20 min. The quantification was done with a TLC Scanner (CAMAG) at a wavelength of 550 nm considering the different response factors of the lipid classes. Concentrations were calculated on the basis of the total lipid mass.

Fatty acid compositions were determined by gas–liquid chromatography (GC) according to Kattner and Fricke (1986). Fatty acids of the lipid extracts were converted to their methyl esters and analysed with a Hewlett-Packard 6890 Series gas chromatograph with a DB-FFAP fused silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) using temperature programming. Fatty acids were identified with standard mixtures and by gas chromatography-mass spectrometry (GC-MS).

The 1-*O*-alkylglycerol ethers moieties of the DAGE were analysed by GC-MS (Myher et al., 1974). The lipid extract was saponified with potassium hydroxide in methanol (5% wt./vol.) under nitrogen for 3 h at 80 °C. After addition of 2 mL of water, glycerol ether diols were extracted with hexane (3 × 2 mL) and converted with 50 μL *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide to *O*-TMSi (trimethylsilyl) ethers (60 °C for 1 h). *O*-TMSi ethers were analysed by GC-MS on a Hewlett-Packard 6890 Series GC combined with an MSD 5973 fitted with a HP-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) using temperature programming (from 60–200 °C at 40 °C min^{-1} and from 200 to 300 °C at 2 °C min^{-1}, final hold 5.5 min). For recording and integration, Chem Station software (Agilent, Germany) was used.

2.3. Statistical analyses

Analyses of variance were performed to detect significant differences between the means of the various parameters of the feeding experiment. One-way ANOVA was calculated, and the Games–Howell post hoc test was applied for multiple comparisons using the SPSS software package for Macintosh.

3. Results

3.1. Feeding experiment

For the feeding studies, *C. limacina* specimens were starved for 14 days, then fed one individual of *L. helicina*, and digestion was followed for 15 days. *L. helicina* had a length of 8.0 mm and a dry mass (DM) of 21.4 mg. Since *C. limacina* only ingests the soft body without shell, the ash-free DM of *L. helicina* was calculated according to Gannefors et al. (2005) and accounted for 14.3 mg. The total lipid amount of *L. helicina* was 1.2 mg resulting in a lipid content of 8.7% of ash-free DM (Table 1). From the 16 fed *C. limacina* specimens, 3 animals smaller than 20 mm were not considered for statistical analysis to exclude the very low concentrations. The body length of the *C. limacina* specimens ranged over the time period (0–15 days) on average from 23.5 to 26.5 mm being similar at all sampling days. Dry mass was on average 13.7 mg on day 0 (i.e., after 14 days of starvation and before feeding). After ingestion of the *L. helicina* specimen, the dry mass of *C. limacina* increased to 25.3 mg at day 5 and was 22.0 mg at day 7. This is close to the value calculated by adding the dry mass of *C. limacina* at day 0 and the ash-free DM of *L. helicina* which gives 28 mg. Towards the end of the experiment, the dry mass decreased to 12.7 mg. In accordance with dry mass, lipid mass also increased significantly from 1.5 mg (day 0), to 3.9 mg (day 5 and 7) and decreased to 1.4 mg at the end (Fig. 1). The amount of lipid, which could be transferred by the ingestion of one *L. helicina* individual, was 1.2 mg lipid in maximum, neglecting any metabolic loss. This means that at least two thirds of the lipid increase in *C. limacina* was due to de novo biosynthesis from non-lipid compounds. The change in lipid content was similar starting from 12.1%DM, reaching a maximum value of 17.5%DM (day 7) and finally decreasing to 10.7%DM (Table 1, Fig. 1).

C. limacina contained high proportions of phospholipids (PL), triacylglycerols (TAG) and 1-*O*-alkyldiacylglycerol ethers (DAGE), moderate proportions of sterols (ST) as well as low proportions of free fatty acids (FFA) and wax esters (WE). These lipid classes were also found in *L. helicina* except for DAGE and WE which were absent (Table 1). The neutral lipids of *C. limacina*, TAG and DAGE, increased significantly during feeding (Fig. 2). The increase in TAG from 0.1 mg to 1.5 and 1.6 mg after 5 and 7 days of digestion, respectively, did not result from the assimilation of TAG of *L. helicina*, because it only contained 0.3 mg of TAG (23.8% of total lipid). DAGE, which is absent in *L. helicina*, increased from 0.1 to 1.0 mg after 7 days of digestion and decreased to 0.2 mg at day 15. TAG and DAGE exhibited significantly ($p < 0.05$) higher proportions after 7 days of digestion (TAG 42.3%, DAGE 25.8%) than at the beginning (TAG

Table 1
C. limacina and L. helicina: body length and chemical composition during the feeding experiment

Species	*L. helicina*	*C. limacina*			
Time of metabolism (days)		0	5	7	15[a]
		Mean ± S.D. (3)	Mean ± S.D. (3)	Mean ± S.D. (3)	Mean ± S.D. (4)
BL (mm)	8.0[b]	25.0 ± 4.4	26.5 ± 2.1	24.7 ± 1.5	23.5 ± 1.9
DM (mg ind^{-1})	21.4	13.7 ± 2.6	25.3 ± 6.3	22.0 ± 3.0	12.7 ± 1.4
AFDM (mg ind^{-1})	14.3[c]	–	–	–	–
TL (mg ind^{-1})	1.2	1.5 ± 0.1	3.9 ± 1.1	3.9 ± 1.1	1.4 ± 0.2
TL (%DM)	8.7[d]	12.1 ± 2.4	15.5 ± 0.3	17.5 ± 3.4	10.7 ± 1.3
PL (%)	46.7	73.4 ± 11.3	29.5 ± 4.6	26.7 ± 6.8	64.4 ± 20.4
ST (%)	12.0	6.1 ± 1.6	5.9 ± 0.8	3.6 ± 0.2	5.7 ± 1.4
FFA (%)	17.5	6.8 ± 5.2	0.7 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
TAG (%)	23.8	6.1 ± 5.5	37.9 ± 5.1	42.3 ± 6.9	14.5 ± 11.3
DAGE (%)	0.0	5.7 ± 4.3	24.4 ± 2.3	25.8 ± 1.6	14.8 ± 9.9
WE (%)	0.0	1.9 ± 2.4	1.5 ± 0.8	1.4 ± 0.7	0.5 ± 0.3

Body length (BL), dry mass (DM), ash-free DM (AFDM), total lipid (TL), total lipid content (TL (%DM)) and lipid class composition. Phospholipids (PL), sterols (ST), free fatty acids (FFA), triacylglycerols (TAG), 1-*O*-alkyldiacylglycerol ethers (DAGE) and wax esters (WE). Number of samples (*n*).

[a] Storage lipid-rich and -poor specimens combined.
[b] Diameter.
[c] Calculated with the formula of Gannefors et al. (2005).
[d] Lipid content of ash-free dry mass.

6.1%, DAGE 5.7%). At the end of the experiment, 2 specimens were again low in storage lipids similar to the initial values, whereas the other 2 specimens still had high proportions of TAG and DAGE. Both specimens showed no decrease in the proportions of DAGE (23.3%), but levels of TAG were reduced to about half of the maximum values to 23.9% still being high compared to the storage lipid-poor animals. PL showed an inverse development with lowest proportions of 26.7% after 7 days. Highest levels occurred at day 0 (73.4%) and at the end (64.4%) (Table 1). Until day 7, the amount of phospholipids was almost constant ranging from 1.0 to 1.2 mg and dropped to 0.9 mg at the end of the feeding experiment. The amount of PL in *L. helicina* was 0.6 mg and the proportion 46.7%. Enhanced proportions of FFA occurred in *C. limacina* at the beginning of the experiment (6.8%), and also lipids of *L. helicina* were rich in FFA (17.5%). However, levels decreased to less than 1% already at day 5. Throughout the experiment, the amount and proportion of sterols and wax esters were low showing only small variations (Table 1).

The composition and development of the fatty acids and alkyl moieties of *C. limacina* are presented in Table 2 and Fig. 2 supplemented with data on *L. helicina*. The amount of the major fatty acids

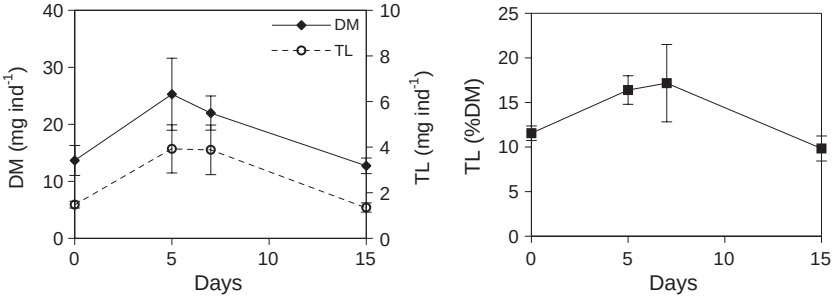

Fig. 1. *C. limacina*. Changes in dry mass (DM), total lipid (TL) and total lipid content (TL (%DM)) during the feeding experiment. Differences are significant ($p \leq 0.05$) for TL (mg) and TL (%DM), except from days 5 to 7.

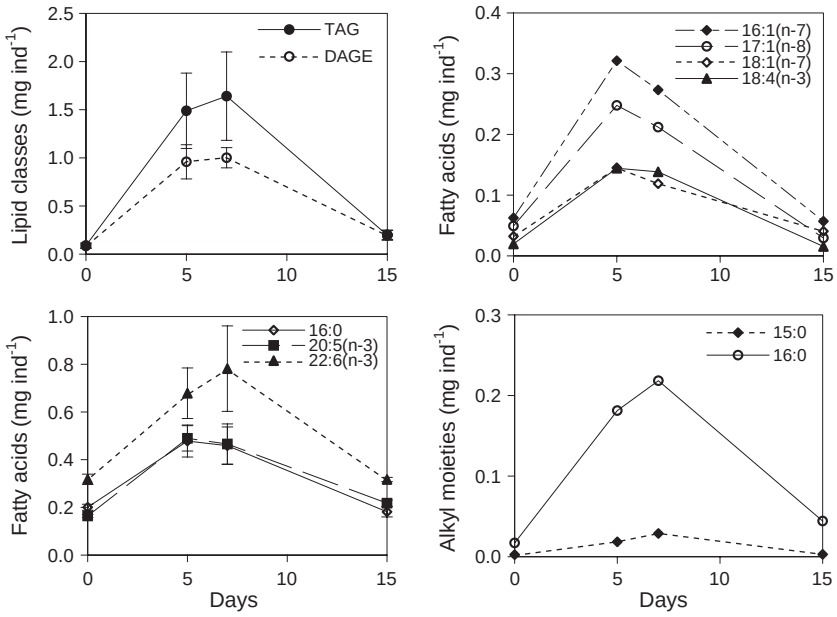

Fig. 2. *C. limacina*. Changes of the storage lipids, TAG and DAGE, and the fatty acids and alkyl moieties during the feeding experiment. Differences are significant ($p \leq 0.05$) for all lipid components, except from days 5 to 7.

16:0 and 20:5($n-3$) in *C. limacina* increased from about 0.2 to 0.5 mg after 5 and 7 days of digestion, whereas the proportions exhibited only small variations during the experiment; 16:0 ranged from 11.8% to 13.6% and 20:5($n-3$) from 11.1% to 16.1% of total fatty acids. The amount of the other major fatty acid 22:6($n-3$) rose from 0.3 (day 0) to 0.7 and 0.8 mg (day 5 and 7), however, proportions were lowest after 5 and 7 days varying between 17.3% and 20.1%. The same three fatty acids were also dominant in the prey, *L. helicina. L. helicina* had proportions of 16:0 and 22:6($n-3$) similar to those in *C. limacina*, but the proportion of 20:5($n-3$) was about twice as high. The increase of these polyunsaturated fatty acids in *C. limacina* was roughly equal to the amount ingested by feeding the one *L. helicina* specimen, only the increase in 16:0 was slightly larger. The same holds true for the minor fatty acid 18:4($n-3$) which increased by 0.14 mg in *C. limacina* being similar to the amount of 0.12 mg in *L. helicina*. 18:4($n-3$) was one of the more abundant fatty acids in *L. helicina* (9.3%) and increased in *C. limacina* from 1.3% to 3.7% until day 5. In contrast to these polyunsaturated fatty acids, where the increases can be explained by the amount of the same fatty acids incorporated through feeding the *L. helicina* specimen, the increase in 16:1($n-7$), 17:1($n-8$), 18:1($n-9$) and 18:1($n-7$) was not due to dietary uptake. 16:1($n-7$) increased from 0.06 to about 0.3 mg after 5 and 7 days of digestion, but the food contributed only 0.02 mg. The amount of 18:1($n-9$) and 18:1($n-7$) rose about fourfold in the same time but levels in *L. helicina* were also very low. The major odd-chain fatty acid 17:1($n-8$), which is absent in *L. helicina*, increased from 0.05 (day 0) to 0.25 mg after 5 days. The other odd-chain fatty acid 15:0 doubled its mass, and 17:0 rose slightly. The proportions of these fatty acids also strongly increased after 5 days of digestizon. 16:1($n-7$) accounted for 8.2%, whereas its proportion in *L. limacina* was only low (1.7%), and 17:1($n-8$) increased from 3.3% to 6.3%. At the end of the experiment after 15 days, most fatty acids showed similar amounts and proportions as at the beginning. The trace amounts of branched fatty acids might be of bacterial origin, however, its source is unknown (Table 2).

The proportions of the 1-*O*-alkyl moiety of the DAGE of *C. limacina* were relatively constant throughout the feeding experiment. The dominant

Table 2
C. limacina and *L. helicina*: fatty acid and 1-*O*-alkylglycerol composition (mass %) during the feeding experiment

Species	*L. helicina*	*C. limacina*			
Time of metabolism (days)		0	5	7	15[a]
		Mean ± S.D. (3)	Mean ± S.D. (3)	Mean ± S.D. (3)	Mean ± S.D. (4)
Fatty acids					
14:0	5.1	2.8 ± 1.6	2.8 ± 0.5	2.0 ± 0.4	1.4 ± 0.9
i-15:0	0.1	0.9 ± 0.4	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
a-15:0	0.1	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
15:0	0.4	4.7 ± 4.4	3.2 ± 0.3	2.3 ± 0.5	1.4 ± 0.5
i-16:0	0.2	0.9 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.0
a-16:0	–	0.4 ± 0.6	0.3 ± 0.3	0.1 ± 0.2	0.4 ± 0.2
16:0	13.3	13.6 ± 2.7	12.2 ± 1.0	11.8 ± 2.4	13.4 ± 2.0
16:1($n-9$)	0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.2
16:1($n-7$)	1.7	4.2 ± 2.2	8.2 ± 0.8	7.0 ± 1.9	4.2 ± 1.7
i-17:0	0.6	1.1 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	0.6 ± 0.0
a-17:0	1.1	2.0 ± 0.4	1.6 ± 0.2	1.7 ± 1.0	2.5 ± 0.5
16:2($n-4$)	0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
17:0	0.3	3.2 ± 0.6	2.1 ± 0.3	2.3 ± 0.3	2.0 ± 0.4
17:1($n-8$)	–	3.3 ± 2.2	6.3 ± 1.2	5.5 ± 0.8	2.2 ± 0.6
18:0	1.6	3.6 ± 1.1	2.1 ± 0.2	2.1 ± 0.9	4.8 ± 1.8
18:1($n-9$)	3.7	3.3 ± 1.2	3.7 ± 1.0	3.7 ± 0.7	2.7 ± 0.3
18:1($n-7$)	0.6	2.2 ± 0.0	3.7 ± 0.5	3.1 ± 1.0	3.0 ± 0.4
18:2($n-6$)	1.0	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
18:3($n-3$)	3.5	0.6 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	0.5 ± 0.2
18:4($n-3$)	9.3	1.3 ± 0.8	3.7 ± 0.9	3.6 ± 1.3	1.1 ± 0.9
20:0	–	0.6 ± 0.2	0.3 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
20:1($n-9$)	3.4	3.4 ± 1.1	2.3 ± 0.2	2.9 ± 1.2	2.7 ± 0.6
20:1($n-7$)	2.1	2.7 ± 0.2	1.9 ± 0.2	1.7 ± 0.7	3.1 ± 0.4
20:2($n-6$)	1.4	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.0	0.7 ± 0.7
20:4($n-6$)	0.4	1.8 ± 0.6	0.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.0
20:4($n-3$)	2.0	0.4 ± 0.1	0.9 ± 0.2	1.1 ± 0.4	0.7 ± 0.1
20:5($n-3$)	21.9	11.1 ± 1.4	12.5 ± 0.8	12.0 ± 2.6	16.1 ± 1.2
22:1($n-11$)	0.2	1.4 ± 1.8	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
22:1($n-9$)	–	0.4 ± 0.2	0.2 ± 0.3	0.4 ± 0.3	0.1 ± 0.1
22:5($n-3$)	1.1	0.7 ± 0.1	1.7 ± 0.8	3.8 ± 4.6	2.2 ± 1.6
22:6($n-3$)	23.5	21.5 ± 4.6	17.3 ± 1.5	20.1 ± 5.4	23.4 ± 0.8
Alkyl moieties					
15:0	–	12.0	8.2	10.3	6.0
i-16:0	–	0.3	0.3	0.4	–
16:1	–	0.7	1.1	1.0	0.7
16:0	–	79.6	80.1	77.7	90.3
i-17:0	–	1.0	1.4	2.0	–
17:1	–	2.5	2.9	3.3	1.4
17:0	–	3.2	5.3	4.9	1.6
18:1	–	–	–	–	–
18:0	–	0.4	0.7	0.6	–

Number of samples (n).
For indices, see Table 1.

alkyl moiety 16:0 varied between 77.7% and 90.3% of the total followed by 15:0 with 6% to 12%. The amount of the 16:0 alkyl moiety increased from 0.02 (day 0) to about 0.2 mg (days 5 and 7) (Fig. 2). Other minor compounds were 17:0, 17:1 and i-17:1 (Table 2).

3.2. Starvation experiment

During the starvation experiment, specimens of *C. limacina* exhibited a high variability in lipid composition which was partially due to variations in body length and different developmental phases. Individuals taken after 14 days showed large differences in size and body composition. Due to the highly variable proportions of storage lipids, animals were grouped into storage lipid-rich and lipid-poor specimens which was defined according to the proportions of TAG (more than 12% TAG of total lipid is termed lipid-rich and less lipid-poor). TAG proportions are mostly in accordance with high and low proportions of DAGE.

Dry mass was independent of the amount of storage lipids, decreasing slightly with starvation time, following an exponential trend. The same tendency was found for the total lipid mass, decreasing more in the lipid-rich animals. However, in both groups these changes in total lipid were not significant. The lipid-rich specimens varied between 13.4% and 24%DM and the lipid-poor between 13.6% and 17.1%DM, except at day 60 where lipids accounted for 25.6%DM (Fig. 3).

The most pronounced differences occurred in the amounts and proportions of the lipid classes of the lipid-rich *C. limacina* individuals during the first 50 days of starvation. Storage lipid-rich *C. limacina* were found until day 50, thereafter only lipid-poor animals were present. Changes were illustrated by exponential trend lines through the mean values (Fig. 4) which is a reasonable fit for the reduction of body components during starvation. The high amounts of TAG of 1.2 to 1.5 mg during the first 2 weeks dropped to 0.3 mg on day 50 of starvation. During the same period, the proportions of TAG decreased from 48.3% to 25.1% of total lipid. The initial amounts of DAGE of the lipid-rich animals (0.55 mg) were about half that of TAG and decreased slower than TAG. The proportions of DAGE (18.1% to 22.5%) remained relatively constant with a slightly decreasing tendency. The lipid-poor animals exhibited low proportions of TAG and DAGE from the beginning until the end of the experiment. TAG and DAGE varied between 3.1% and 7.7% until day 50 and were nearly depleted at the end of the 100-day starvation period. PL was the dominant lipid class with highest proportions in the lipid-poor animals. The PL contents of the lipid-rich

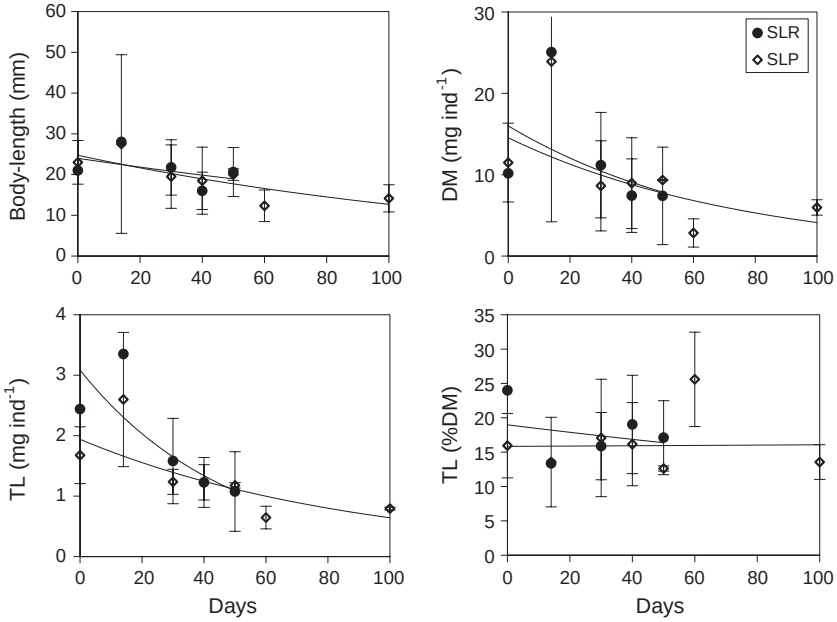

Fig. 3. *C. limacina*. Body-length, dry mass (DM), total lipid (TL) and total lipid content (TL (%DM)) of storage lipid-rich (SLR) and storage lipid-poor (SLP) animals during starvation.

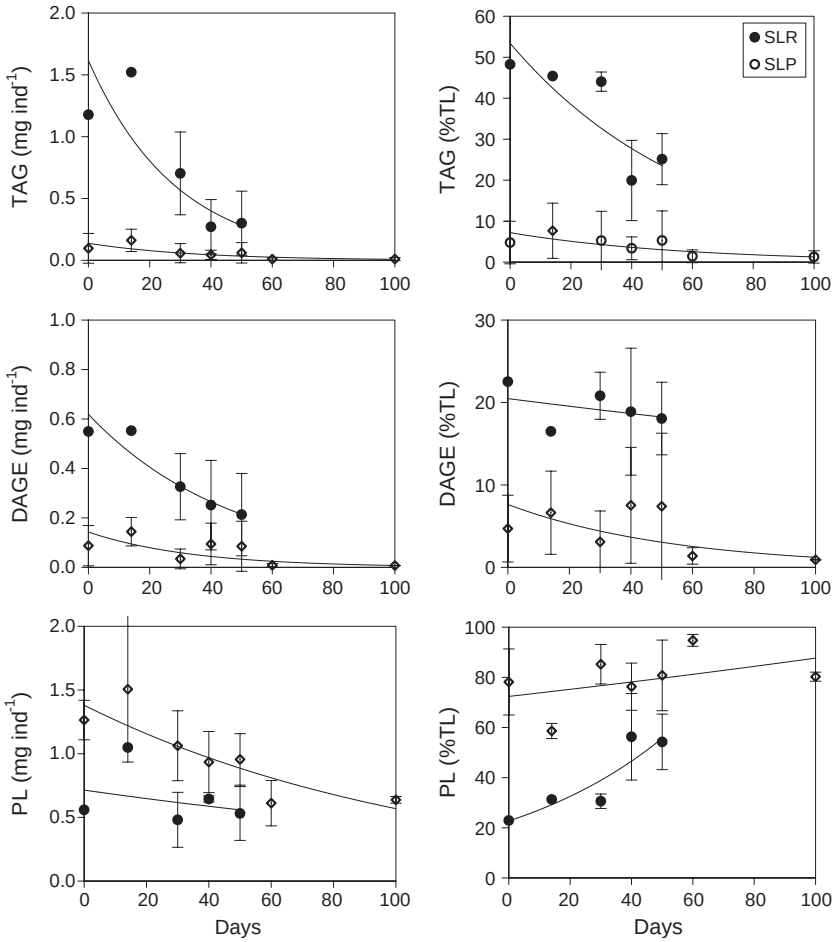

Fig. 4. *C. limacina.* Amounts and proportions of the storage lipids TAG and DAGE during starvation. For abbreviations, see Fig. 3.

specimens were almost constant until day 50, whereas the proportions increased due to lower storage lipid levels. In the lipid-poor animals, the PL contents decreased from 1.3 to 0.6 mg, whereas the proportions increased from 80% to 95% towards the end of starvation.

The dominant fatty acid 22:6($n-3$) increased in the lipid-poor animals (from 21.8% to 26.4% until day 100) and in the lipid-rich ones (from 16.3% to 18.5% until day 50) which is in accordance with the occurrence of PL. The fatty acids 20:5($n-3$) and 16:0 had almost the same proportions and were nearly constant, ranging from 11.3% to 14.3% in all animals. The fatty acids 16:1($n-7$), 18:1($n-7$) and 18:1($n-9$) exhibited only small variations in the lipid-rich animals, but were clearly lower in the lipid-poor ones. The proportions of 17:1($n-8$) and 18:4($n-3$) decreased in the lipid-rich specimens and were low in the lipid-poor individuals (Table 3). The concentrations of all fatty acids strongly decreased in the lipid-rich specimens, whereas in the lipid-poor specimens the decrease was lower which is in accordance with the relatively small variations in the percentage distribution. An exception was 18:4($n-3$) which remained constant in the lipid-poor group.

4. Discussion

Previous field studies on lipid changes within the life cycle of the Arctic pteropod *C. limacina* have shown that this species has at least a perennial life

Table 3
C. limacina: fatty acid composition (mass %) of storage lipid-rich and lipid-poor specimens during the starvation experiment

Time of starvation (days)	Storage lipid-rich		Storage lipid-poor	
	0	50	0	100
		Mean ± S.D. (4)	Mean ± S.D. (4)	Mean ± S.D. (3)
Fatty acids				
14:0	3.8	4.0 ± 1.5	2.6 ± 1.3	2.2 ± 1.5
i-15:0	0.5	0.8 ± 0.3	0.9 ± 0.4	0.7 ± 0.3
a-15:0	0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.1
15:0	3.2	2.4 ± 0.5	4.0 ± 3.8	1.9 ± 1.2
i-16:0	0.3	0.5 ± 0.1	0.9 ± 0.1	0.5 ± 0.1
a-16:0	0.2	0.4 ± 0.2	0.3 ± 0.5	0.9 ± 0.7
16:0	13.8	14.0 ± 1.3	14.3 ± 2.7	12.3 ± 1.5
16:1($n-9$)	0.4	0.6 ± 0.3	0.2 ± 0.2	0.4 ± 0.2
16:1($n-7$)	7.6	6.7 ± 1.6	3.9 ± 1.9	3.6 ± 1.3
i-17:0	0.6	0.7 ± 0.1	1.2 ± 0.2	0.6 ± 0.1
a-17:0	1.7	2.0 ± 0.2	2.1 ± 0.3	2.9 ± 0.7
16:2($n-4$)	0.4	0.5 ± 0.2	0.8 ± 0.2	0.4 ± 0.2
17:0	2.4	2.0 ± 0.2	3.3 ± 0.5	1.9 ± 0.1
17:1($n-8$)	5.4	3.3 ± 1.5	3.1 ± 1.9	1.7 ± 1.2
18:0	2.3	2.8 ± 0.3	3.8 ± 1.0	4.3 ± 1.0
18:1($n-9$)	3.7	3.2 ± 1.1	3.2 ± 1.0	2.5 ± 0.3
18:1($n-7$)	2.9	3.0 ± 1.2	2.2 ± 0.0	1.8 ± 0.6
18:2($n-6$)	1.6	1.1 ± 0.4	0.8 ± 0.1	0.7 ± 0.2
18:3($n-3$)	1.7	1.4 ± 0.4	0.6 ± 0.2	0.8 ± 0.4
18:4($n-3$)	6.1	4.7 ± 2.2	1.1 ± 0.8	1.4 ± 1.1
20:0	0.2	0.5 ± 0.1	0.7 ± 0.2	1.5 ± 0.7
20:1($n-9$)	2.6	2.6 ± 0.3	3.4 ± 0.9	3.2 ± 0.4
20:1($n-7$)	1.9	2.4 ± 0.4	2.8 ± 0.2	2.3 ± 0.4
20:2($n-6$)	0.6	0.9 ± 0.1	1.1 ± 0.3	1.7 ± 0.3
20:4($n-6$)	0.5	0.8 ± 0.2	2.0 ± 0.6	1.4 ± 0.0
20:4($n-3$)	0.8	0.6 ± 0.3	0.4 ± 0.1	0.5 ± 0.1
20:5($n-3$)	12.7	13.0 ± 0.8	11.3 ± 1.2	13.8 ± 2.6
22:1($n-11$)	0.2	0.2 ± 0.0	1.1 ± 1.5	0.3 ± 0.1
22:1($n-9$)	0.3	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.1
22:5($n-3$)	0.6	0.6 ± 0.0	0.7 ± 0.1	0.8 ± 0.2
22:6($n-3$)	16.3	18.5 ± 2.4	21.8 ± 3.9	26.4 ± 1.3

Number of samples (n).

cycle (Böer et al., 2005). In this study, *C. limacina* was caught in summer (end of June), and therefore most of the specimens were lower in storage lipids compared to spring and autumn animals which is in accordance with the seasonal lipid dynamic. Lipid-poor specimens had probably utilised their lipids for development and reproduction (Böer et al., 2005). Some animals were still rich in storage lipids, indicating that they were in a phase before gonad development and spawning. Because it is not possible to differentiate *C. limacina* visually into lipid-rich and lipid-poor individuals, specimens with different storage lipid levels were subjected to both experiments.

4.1. Feeding

Specimens of the monophagous predator *C. limacina* were fed with one specimen each of *L. helicina* to follow quantitatively the conversion of food during digestion. The increase in dry mass of *C. limacina*, determined after 5 days of digestion, corresponds to about 80% of the ash-free dry mass of *L. helicina*. This high assimilation rate supports the very effective utilisation of food which is reported for carbon and nitrogen to be close to 100% (Conover and Lalli, 1974). The lipid mass of *L. helicina*, however, is too low to explain the increase of lipids in *C. limacina* by

direct incorporation of dietary lipids. About two thirds of the lipids, accumulated by *C. limacina*, must be synthesised de novo from non-lipid constituents which are mostly proteins (70% to 77%) and some carbohydrates (2.5%) (Percy and Fife, 1981). The storage lipids TAG and DAGE increased significantly to highest levels after 5 to 7 days of digestion. This trend is slightly but not significantly different to the trend of dry mass which was highest at day 5. In contrast to storage lipids, the amount of PL remained almost constant during feeding.

The increase of TAG is possible by restructuring of absorbed dietary fat (monoacylglycerol pathway) and de novo synthesis (glycerol phosphate and dihydroxyacetone phosphate pathways, DHAP) (Gurr and Harwood, 1991), whereas ether-linked glycerols (DAGE) are formed via the DHAP pathway (e.g. Snyder, 1972; Hajra, 1995). Results from studies with cell-free systems of dogfish liver (*Squalus acanthias*) show that the biosynthesis of TAG exceeded greatly that of DAGE (Malins and Sargent, 1971). In later studies, Malins and Robisch (1974a,b) reported that acyl moieties are incorporated faster into TAG and DAGE than alkyl moieties into DAGE. The production of DAGE is probably slower than that of TAG because the biosynthesis of DAGE involves the replacement of the acyl against the alkyl moiety. Nevertheless, the biosynthesis of lipids and fatty acids by *C. limacina* within 5 to 7 days was very fast. A similar rapid accumulation and production of lipids was found for herbivorous *Calanus* species from high latitudes, where, for example, lipids in *C. hyperboreus* were completely exchanged by dietary uptake and de novo production within 11 days, as revealed from feeding experiments with ^{13}C labelled microalgae. In *C. hyperboreus*, the most lipid-dependent species, the de novo biosynthesis of fatty alcohols was as fast as the incorporation of dietary fatty acids, and the fatty alcohols were immediately esterified to wax esters (Graeve et al., 2005).

The predominant alkyl moiety in *C. limacina* was 16:0 which may derive from the dietary 16:0 fatty acid. However, due to the low amount of dietary lipids this alkyl moiety had to be produced de novo. The second important alkyl moiety was 15:0 which had to be synthesised also de novo since odd-chain fatty acids are absent in *L. helicina* (e.g., Gannefors et al., 2005). The dominance of these two alkyl moieties of DAGE was also found in *C. antarctica* from the Southern Ocean (erroneously named *C. limacina* in Phleger et al., 1997, 2001).

All fatty acids of *L. helicina* may be incorporated by *C. limacina* since it is well established that fatty acids are transferred unchanged within the food web (Graeve et al., 1994, 2005; Dalsgaard et al., 2003). Despite the distinct food chain of phytoplankton–*Limacina*–*Clione*, a clear relationship between the fatty acid compositions of both species has not been found (Kattner et al., 1998; Böer et al., 2005). However, the increase in polyunsaturated fatty acids in *C. limacina* is almost equal to the amount of the same fatty acids available through ingestion of *L. helicina*. This confirms the general rule that animals are not able to synthesise polyunsaturated fatty acids de novo. The increase in TAG and DAGE in excess of the dietary lipid input was mainly due to de novo lipid biosynthesis, especially of the monounsaturates 16:1($n-7$), 17:1($n-8$) and 18:1($n-7$), 18:1 ($n-9$) as well as 16:0 and the alkyl moieties 16:0 and 15:0. This is the first experimental evidence that these fatty acids are biosynthesised by *C. limacina* which has been already hypothesised by Kattner et al. (1998) and Böer et al. (2005). The generally excepted opinion, that 16:1($n-7$) originates from feeding on diatoms and that 18:1($n-7$) is then produced by chain elongation, is not valid for *C. limacina*. Although the clearly defined food chain relationship involves possible feeding on diatoms, the mass balance calculation applied to lipid components reveals that less than 10% of 16:1($n-7$) can originate from food uptake.

As obtained from field studies, odd-chain fatty acids occur preferentially in DAGE, whereas dietary fatty acids in TAG. However, odd-chain fatty acids were also found in TAG and are part of PL (Kattner et al., 1998). For *C. limacina*, experimental evidence is still missing for the biosynthesis of odd-chain fatty acids. It is assumed that the propionyl-moiety of dimethylsulfoniopropionate (DMSP) is involved in the synthesis because DMSP is strongly accumulated by *L. helicina* via phytoplankton uptake (Ackman and Hingley, 1965; Levasseur et al., 1994) and thus ingested by *C. limacina*.

The decrease in dry mass and lipid mass, which started after 5 to 7 days of digestion, showed the rapid utilisation of the ingested and converted food. In some animals, the storage lipid content decreased

to initial values, whereas others had reduced amounts but still high proportions of TAG and DAGE. Since summer is the main spawning period of *C. limacina* (Mileikovsky, 1970), animals may use food and lipids for maturation and spawning. Reproduction, which has a high energy demand, may start immediately when enough food is available. On the other hand, specimens, which were using TAG preferentially, are probably still in the process of development and growth (Böer et al., 2005). The group, which still had high proportions of TAG and DAGE, seems to be less active in development, although a considerable part of TAG had been utilised. This supports the hypothesis that TAG serves for short-term energy demand.

4.2. Starvation

At the beginning and during the first days of the starvation experiment, some individuals of *C. limacina* were especially rich in storage lipids and others lipid-poor. The high lipid content could be due to a recent feeding event. However, as the feeding experiment shows, lipids of *C. limacina* were only temporarily affected by ingestion of *L. helicina* (at least in the studied specimens from summer) since the very efficient uptake and incorporation of food was followed by a similar fast utilisation. *C. limacina* might be also storage lipid-rich because they could just be in the phase of sexual maturation since lipid content and composition is especially depended on the ontogenetic stage and reproduction. Lipid-rich specimens were found in early summer before they start to utilise their lipid stores for development, maturation and reproduction (egg production) (Böer et al., 2005). During starvation, the lipid-rich *C. limacina* probably continued maturation or egg production (if fertilised) since a considerable portion of TAG was utilised. It might be possible that a certain low limit of TAG or, more general, lipid content triggers an interruption of maturation and a reduction of metabolic activity to save energy during starvation.

The animals, which had only very small lipid deposits at the beginning of the starvation experiment, had probably already spawned. Lowest lipid contents were found in *C. limacina* during or after maturation and egg production which occurs in Svalbard waters in July/August. They normally replenish their lipid stores after reproduction and were again lipid-rich in late summer to autumn before overwintering (Böer et al., 2005). In addition or alternatively, feeding may not have occurred for some time. Due to starvation, and consequently insufficient energy reserves at collection, they might have been in a phase of low metabolic activity. Conover and Lalli (1974) reported that a starved *C. limacina* can be 20 times less metabolically active then a fed one. This was also reported for many invertebrates as a possible mechanism to cope with food limited periods (e.g., Ansell, 1973; Dall and Smith, 1986; Christiansen and Diel-Christiansen, 1993; Torres et al., 1994; Hervant et al., 1997). Despite the low initial lipid content, lipid-poor *C. limacina* specimens were still able to survive a period of 100 days without food.

In contrast to TAG, DAGE were metabolised more slowly in the lipid-rich animals and thus retained for long-term storage. The fact that the use of storage lipids is lipid-class specific to a certain extent, is also known from other zooplankton species. TAG were utilised more rapidly than the fatty alcohol containing wax esters by the copepods *Euchaeta japonica* (Lee, 1974) and *Gaussia princeps* (Lee and Barnes, 1975). Because the oil sac of copepods contains pure wax esters (Benson et al., 1972), TAG is located at a different anatomical site and could be therefore mobilised separately from wax esters (Sargent, 1976). In contrast, in *C. limacina* both TAG and DAGE were found in the digestive gland and in the integuments being rich in oil droplets (M. Böer, unpublished data) as well as in the gonads (Böer et al., 2005). Therefore, it can be excluded that different rates of utilisation of lipid classes are regulated by storage in different body compartments.

The differences in the fatty acid compositions of *C. limacina* support the classification in lipid-rich and lipid-poor specimens. Although the amount of all fatty acids decreased according to total lipid, the composition within the two groups only changed marginally which indicates a well-balanced catabolism of all fatty acids. The initial proportions of fatty acids, which have been attributed to TAG (14:0, 18:4($n-3$)) and DAGE (16:1($n-7$), 17:1($n-8$), 18:1($n-7$) and 15:0) (Böer et al., 2005), were lower in the lipid-poor animals than in the lipid-rich ones which is in accordance with the differences in the lipid classes. The clear difference between these fatty acids sug-

gests that they were already depleted in the lipid-poor animals some time before the onset of the experiment due to maturation or starvation. The selective utilisation, as found for TAG and DAGE, was not obvious for the individual fatty acids which were assigned to these lipid classes.

If we combine the results and interpretations from the storage lipid-rich and lipid-poor specimens, we are able to develop a model about how long *C. limacina* may be able to withstand periods without food. It seems reasonable to assume an exponential decrease of the storage lipids with starvation time. In Fig. 5, the exponential fit of TAG of the lipid-rich animals is extrapolated to close to zero. This fit overlaps with that of the lipid-poor animals beginning at about day 70 which results in a gap of 20 days. This means that the lipid-rich animals have to starve for these 20 days in addition to the 50 days of starvation to reach the initial conditions of the lipid-poor animals. Hence, TAG stores may suffice for almost 170 days of starvation. The same extrapolation was done for DAGE. The gap between the lipid-rich and lipid-poor specimens is the same as for TAG which also results in a depletion of DAGE after 170 days (Fig. 5). If we, however, extrapolate DAGE of the lipid-rich animals to the final DAGE concentration of the lipid-poor specimens (0.007 mg), then animals might be able to survive 260 days. Both scenarios result in animals, in which lipids are totally reduced to the membrane constituents, phospholipids and sterols.

It has to be kept in mind that dry and lipid mass of *C. limacina* decreased simultaneously which results in the constant lipid percentage of dry mass. Thus, not only are lipids utilised during starvation but also other body components which results in body shrinkage. *C. limacina* has more than twice the amount of proteins than lipids (Percy and Fife, 1981) which shows that proteins are an important source of energy during starvation. The utilisation of the typical membrane fatty acids 22:6($n-3$), 20:5($n-3$) and 16:0 supports the conclusion of body shrinkage which involves a reduction in cells and cell volume. This process is also known for *Euphausia superba* under starving conditions (McGaffin et al., 2002). Thus, body shrinkage in *C. limacina* in combination with lipid utilisation is presumably a strategy, to cope with long-term food scarcity or absence. The concept of body shrinkage during food limitation is well known for *E. superba* (e.g. Ikeda and Dixon, 1982; Nicol et al., 1992) and also suggested for *C. limacina* by Conover and Lalli (1974). Ikeda and Dixon (1982) reported a considerable reduction in metabolic activity of starved *E. superba* compared to wild species, but they did not find changes in the chemical composition (C and N). They therefore concluded that both body lipid and protein are used as energy sources by starved krill. Similar results have been found during long-term starvation of *E. superba* (Virtue et al., 1997).

5. Conclusion

Monophagy requires eco-physiologically adaptation to periods of food scarcity and when available, an ability to utilise food very efficiently. Due to the

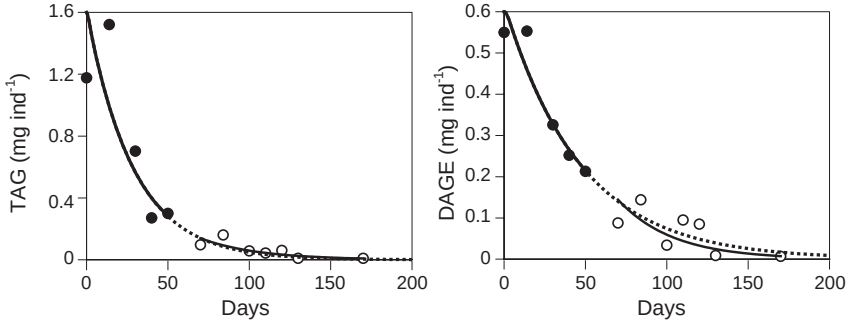

Fig. 5. Model of the potential time period which *C. limacina* may survive without food. The exponential fits of TAG and DAGE of the lipid-rich animals are extrapolated to close to zero (dashed line). These fits represent those of the lipid-rich animals from days 0 to 50 (dots and bold line) and are overlaid by those of the lipid-poor animals at day 70 for TAG and DAGE (open circles and bold line).

large capacity of de novo lipid synthesis combined with rapid utilisation, we conclude that lipids in *C. limacina* are essential energy sources for short-term energy demanding processes like reproduction. During long-term starvation, lipids are also important because of their high energetic value. For survival of long periods of food scarcity, all body constituents, including storage lipids and phospholipids, are utilised. This overall utilisation results in body shrinkage. The combination of shrinkage with a strongly reduced metabolism including a very slow utilisation of lipids seems to be the key factor to withstand such extremely long periods of food absence as calculated from the lipid data.

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