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RESEARCH ARTICLE

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The Arctic pteropod *Clione limacina*: seasonal lipid dynamics and life-strategy

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Abstract During various seasons from May 2001 until July/August 2003, the lipid dynamics of the pteropod *Clione limacina* from Kongsfjorden, Svalbard, were investigated with respect to ontogenetic development and life-cycle. Polytrochous larvae, which were dominant in spring (April, May), composed the lipid-richest specimens of the population, with total lipid of about 50% of dry mass (%DM). Major lipid classes were triacylglycerols (TAG) and 1-O-alkyldiacylglycerol ethers (DAGE), accounting on average for 53.1 and 21.9% of total lipid, respectively. Until summer, larvae grew to adults by utilising their storage lipids. In July/August, lipids were depleted to about 10%DM due to maturation and reproduction. Almost all animals in autumn (September) were mature and able to replenish their lipid deposits by accumulating DAGE (26.7%) and TAG (39.6%). This is probably the prerequisite for successful overwintering.Principal component analysis (PCA), based on the fatty acid compositions, revealed ontogenetic differences between polytrochous larvae, and small and full-grown adults. Higher proportions of 18:4(*n*-3) and 14:0 were found in polytrochous larvae and smaller adults during spring. Both fatty acids were highly significantly correlated with the proportions of TAG, which were used for growth and development because they are presumably easier to metabolise. PCA also divided *C. limacina* specimens into DAGE-rich and DAGE-poor. We suggest that DAGE are a long-term energy store and hypothesise that they are necessary during periods of food scarcity, but may also serve as an energy source for reproduction. The fatty acids 17:1(*n*-8), 15:0, 16:1(*n*-7) and 18:1(*n*-7) were significantly correlated with the proportion of DAGE but not with TAG. These fatty acids, which do not originate from their only prey, *Limacina helicina*, are synthesised de novo. Their abundance reflects an efficient lipid production by *C. limacina*. Based on the results of lipid biosynthesis and accumulation in combination with the population structure, we suggest that *C. limacina* has at least a 2-year life-cycle in Svalbard waters.

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Introduction

The pteropod *Clione limacina* is the most abundant gymnosome of the pelagic food web in temperate and polar waters. It is recognised as an important food for baleen whales and some fish species (Lalli and Gilmer 1989). In Arctic waters, *C. limacina* is found throughout the central Arctic Ocean, and its distribution extends southwards into sub-Arctic waters in both the North Atlantic and the North Pacific to approximately 30–40°N. Two races of *C. limacina* with different maximum size are generally recognised: a northern, cold-water race, with a length of 70–85 mm, and a smaller, southern form, which reaches a length of 12 mm (Lebour 1931).

Clarke (1983) emphasised that the dominant factor to which polar ectotherms have to adapt was not low temperature, but extreme seasonality in food supply. For example, herbivorous copepods and euphausiids buffer the pulsed food availability by accumulating large lipid stores (e.g. Lee 1975; Falk-Petersen 1981; Sargent and Henderson 1986; Hagen 1988; Falk-Petersen et al. 2000). However, omnivorous and carnivorous zooplankton are not as affected by seasonality because they have a more stable food supply. The present knowledge concerning feeding modes of gymnosomes suggests that all are highly specialised carnivores. Although the diet is known only for a dozen species, all of them feed on

thecosomes (Lalli and Gilmer 1989). The order Gymnosomata is unique, because it comprises a number of species, which are monophagous feeders. Feeding studies have revealed that *C. limacina* feeds exclusively on the thecosome *Limacina helicina* in polar waters, and on *L. retroversa* in temperate oceans (Meisenheimer 1905; Lalli 1970; Conover and Lalli 1972; Hopkins 1985, 1987). Almost all developmental stages of *C. limacina* in polar waters are dependent on the availability of *L. helicina*.

In the North Atlantic, sub-Arctic and North Pacific oceans, spawning of *C. limacina* is correlated with the spring/summer period and peak abundance of phytoplankton, which serves as food for the earliest larval stages (veligers) and early polytrochous larvae. Spawning then declines sharply, but still remains at low intensity during autumn and winter (Mileikovsky 1970). Little is known about the life-cycle of *C. limacina* and even less about adaptations due to its extreme specialisation in food uptake. This monophagous feeding mode may result in long periods of starvation when the single food source is scarce.

Our knowledge about structure, function and biosynthesis of lipids in *C. limacina* is rather limited, based on few studies during the past three decades. Ikeda (1972) reported on the lipid content of *C. limacina* from the North Pacific, and some publications have dealt with lipid and fatty acid compositions in Arctic and Antarctic specimens (Lee 1974, 1975; Phleger et al. 1997, 2001; Kattner et al. 1998; Falk-Petersen et al. 2001). Besides triacylglycerols (TAG), *C. limacina* contains considerable amounts of 1-O-alkyldiacylglycerol ethers (DAGE), an unusual depot lipid in marine zooplankton. Until now, the function of DAGE in *C. limacina* has not been known. However, Sargent (1989) and Kattner et al. (1998) suggested that DAGE are produced for long-term energy storage, and Phleger et al. (1997) proposed a buoyancy function.

Another exceptional feature is the high proportion of odd-chain length fatty acids in lipids of *C. limacina*, which may account for up to one-third of the total fatty acids. Kattner et al. (1998) proposed that the biosynthesis of odd-chain fatty acids results from the ingestion of dimethylsulfoniopropionate (DMSP), which can have high concentrations in many phytoplankton species (Keller et al. 1989). DMSP is also accumulated by *L. helicina* via phytoplankton ingestion (Ackman and Hingley 1965) and further into Atlantic cod (*Gadus morhua*) through ingestion of "blackberry feed" (Levasseur et al. 1994). The propionate moiety of DMSP would serve as starter molecule for the biosynthesis of odd-chain fatty acids. However, the biosynthetic pathway for the production of such high amounts of odd-chain fatty acids, as well as their function, has not been validated and needs to be further clarified.

This paper presents a comprehensive study on ontogenetic development in combination with seasonal lipid dynamics for *C. limacina*. Our results on the size distribution in relation to changes in lipid and fatty acid compositions will improve the knowledge about its life-cycle and lipid metabolism. This linkage between lipids and population structure also aims to elucidate the biosynthesis and utilisation of lipids by *C. limacina* in view of its extraordinary lipid storage mode.

Materials and methods

Study area

Kongsfjorden, a glacial fjord in the Arctic, is located on the west coast of Svalbard at about 79°N, 12°E (Fig. 1). The area is well studied with respect to overall ecosystem properties (Hop et al. 2002). Kongsfjorden is an open fjord with no sill at the entrance, and the outer areas are strongly influenced by oceanic processes on the surrounding shelf areas. Transformed Atlantic water, entering the fjord, is a mixture of warm, saline Atlantic water and colder, less saline Arctic water masses (Svendsen et al. 2002). In addition, the fjord contains locally formed water masses, including those formed by convection, winter cooling and glacial input, as well as melting during the spring and summer.

Sampling

C. limacina specimens were collected quantitatively and qualitatively at different sampling sites in Kongsfjorden (Fig. 1). In spring (May 2001 and April 2002), larvae and small adults were collected with a WP-2 plankton net (opening 0.25 m^2, mesh size 180 µm) and larger specimens with a WP-3 net (opening 0.78 m^2, mesh size 1,000 µm) from RV "Lance" or from a small boat. Additionally, in April, visually detected large adults were caught by scuba-diving through a hole in the ice. During the other sampling campaigns, animals were

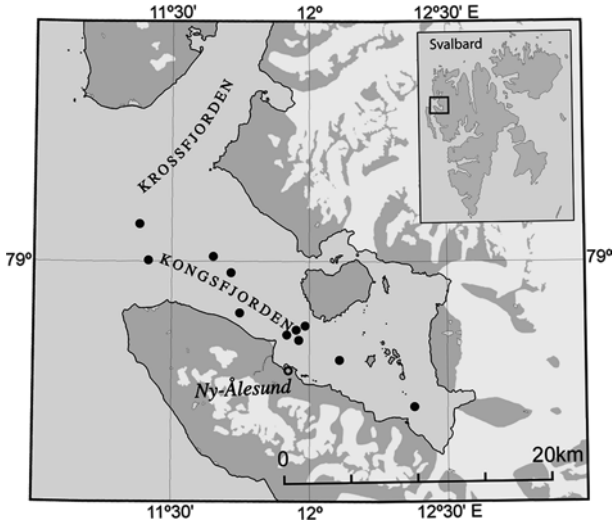

Fig. 1 Map of Kongsfjorden and Svalbard with sampling sites

collected with a WP-3 plankton net from RV "Oceania", by scuba-diving or from the surface with a sieve (20 cm diameter) on a rod.

Live specimens were transported in 10-l plastic buckets filled with cold seawater to the laboratory in Ny-Ålesund. For investigation of the population structure, the body lengths of 371 specimens were measured after relaxation (for a few minutes) in a petri dish filled with cooled seawater. According to Conover and Lalli (1972), the length of *C. limacina* was determined from the base of the anterior tentacles to the posterior end of the trunk. Polytrochous larvae and adults were differentiated by length: larvae were defined from about 1–15 mm length based on the existence of ciliary bands in specimens up to about 15 mm long (Pelseneer 1887; Lalli and Gilmer 1989). Larger specimens were separated into small and large adults.

Immediately after body-length measurement, the specimens from April, May, August and September were dropped into glass vials filled with chloroform:methanol (2:1 by vol.) including 0.01% antioxidant (butylhydroxy-toluene, BHT) and stored at −20°C. Some specimens from July 2001 were immediately frozen in liquid nitrogen after length determination and stored at −20°C in Ny-Ålesund. For lipid analysis, samples were transported frozen to the home laboratory in Bremerhaven.

During summer 2003 (July and August), adult *C. limacina* were sampled from Kongsfjorden and kept in aquaria. Specimens with conspicuous orange gonads were selected, frozen and transported home. In the home laboratory, specimens were defrosted, and the gonads were dissected from the trunk for separate analyses.

Laboratory analyses

A representative subset of 52 samples was taken for the determination of dry mass (DM) and lipid analyses. Samples, stored in chloroform:methanol (2:1 by vol.), were evaporated with nitrogen to dryness. Then, all samples, including the frozen samples, were transferred to pre-weighted vials, lyophilised for 48 h, and then DM was determined gravimetrically. Total lipid (TL) was extracted from the freeze-dried samples using dichloromethane:methanol (2:1 by vol.), essentially after Folch et al. (1957), and measured gravimetrically.

Lipid classes were determined by high-performance thin layer chromatography (HPTLC) densitometry (Olsen and Henderson 1989). Pre-coated HPTLC silica gel 60 plates (20×10 cm, Merck) were pre-developed in hexane:diethylether (1:1 by vol.) to remove impurities. The plates were then dried in a vacuum desiccator for 1 h and spotted with samples and standards. For calibration, the following standards were used in concentrations of 0.1, 1, 2, 5, 10 and 15 μg/μl: phospholipids (phosphatidylcholine, PL), sterols (cholesterol, ST), free fatty acids (oleic acid, FFA), triacylglycerols (triolein, TAG), wax esters (oleic acid palmityl ester, WE) (all from Sigma) and 1-O-alkyldiacylglycerol ethers (DAGE) that were isolated from shark-liver oil by preparative TLC. Five microlitres of sample extracts and standard solutions were spotted on the HPTLC plates with a CAMAG-Linomat 4. The separation of lipid classes was performed in a CAMAG horizontal chamber with hexane:diethylether:acetic acid (80:20:2 by vol.). Thereafter the plate was dried in a desiccator under vacuum for 30 min. Lipid classes were visualised by submerging the plate in manganese (II)-chloride (4 H_2O), methanol and sulphuric acid reagent in a CAMAG immersion device for 5 s followed by combustion at 120°C for 20 min. The quantification was performed with a TLC Scanner (CAMAG 3) combined with winCATS software. The measurement was carried out with a wolfram lamp at 550 nm.

The fatty acid compositions were analysed by gas-liquid chromatography according to Kattner and Fricke (1986). Fatty acids of the total lipid extracts were converted to their methyl esters by transesterification in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h. After extraction with hexane, fatty acid methyl esters were analysed with a Hewlett-Packard 6890 Series gas chromatograph with a DB-FFAP fused silica capillary column (30 m×0.25 mm inner diameter, 0.25 μm film thickness) using temperature programming (160–240°C at 4°C min^{-1}, hold 15 min). For recording and integration, Class-VP software (4.3) (Shimadzu, Germany) was used. Fatty acids were identified with standard mixtures and, if necessary, additional confirmation was obtained by gas chromatography-mass spectrometry (Kattner et al. 1998). The determination of double bond positions was performed by GC-MS after 4,4-dimethyloxazoline (DMOX) derivatisation (Fay and Richli 1991).

Statistical analyses

The fatty acid compositions of all samples were subjected to principal component analysis (PCA) using the program "Primer" (Clarke and Warwick 2001). The main purpose of PCA is to condense the information contained in a large number of original variables into a smaller set of new composite dimensions, with a minimum loss of information (McCarigal et al. 2002). According to Mayzaud et al. (1989), a distortion of the PCA results occurs if variances are heteroscedastic. Homoscedasticity (i.e. homogeneity of variances) can be obtained by appropriate transformation, and in our case the percentage values of the fatty acids were transformed by the formula: log%/(100−%) (McCullagh and Nelder 1989). In PCA, the first principal axis explains the maximum amount of variation possible in a single dimension (e.g. ontogenetic development), whereas the second principal axis explains the maximum remaining variation not explained by the first (e.g. change in storage lipids). Principal components are derived from a secondary matrix computed from the transformed data. The loading matrix is used in the derivation of scores;

thus variables with high loadings are the most influential in the calculation. Loadings contain information about relationships among variables (e.g. fatty acids), whereas scores show relationships between samples. Regression curves were best-fitted by exponential, polynomial or linear functions. The test of significance was performed with the "JMP" software (SAS Institute, Cary, NC).

Results

For the investigation of the life-cycle of *C. limacina*, specimens collected during 2001 were combined with those from April 2002, which were taken to describe the early spring population, since we expect that the life-cycle is recurrent during different years. Animals from July/August 2003, from which trunk and gonads were analysed separately, were included to investigate the lipid metabolism during maturation.

Body-length frequency

The body-length frequency distribution of *C. limacina* exhibited three different length groups (cohorts C1–3) in April 2002, but no clear differentiation between developmental stages during 2001 (Fig. 2), including the specimens from July/August 2003. In April 2002, very small polytrochous larvae with mean length of 1.7 mm occurred, as well as larger larvae (13.0 mm) and small adults (22.4 mm). From May until July/August 2001, the population consisted simultaneously of larvae, and small and large adults. In May, the length of *C. limacina* showed a high variability; the population comprised polytrochous larvae with a minimum length of 2 mm and the largest adults up to 52 mm. In July/August, larger larvae were found with a length of 14 mm together with adults of a maximum length of 45 mm. In September, only adults with a length range of 19–51 mm were present.

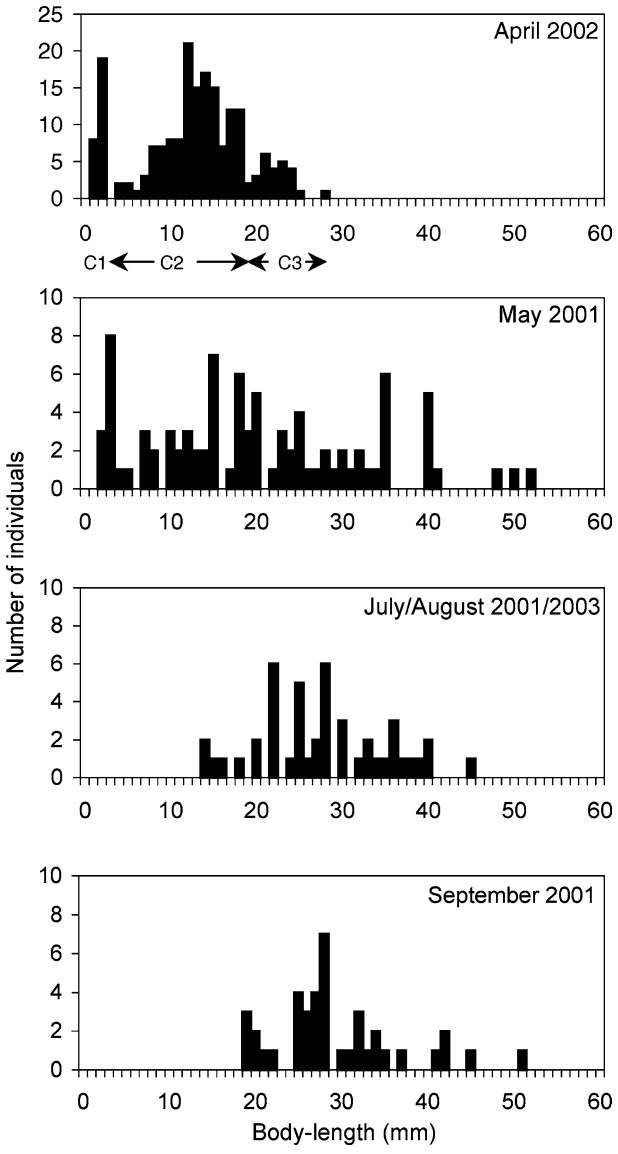

Fig. 2 *Clione limacina*. Body-length frequency distribution during six periods from April (2002) and May to September (2001) including summer specimens from 2003. *C1* to *C3* as definition for cohorts

Body composition

The dry mass of *C. limacina* increased significantly and exponentially with body-length ($r^2 = 0.746$; $P < 0.001$; $n = 57$). Polytrochous larvae with length up to 15 mm, which mostly occurred in April and May, showed only little variability in dry mass, whereas dry mass of adults > 15 mm clearly increased, being highly variable in all seasons (Fig. 3a). Total lipid also increased exponentially with length ($r^2 = 0.633$; $P < 0.001$; $n = 57$), but the increase was less pronounced than for dry mass. Specimens up to 15 mm had relatively similar lipid levels. Total lipid of adults > 15 mm slightly increased and was more variable. Large adults > 35 mm showed the highest variability in total lipid, with some specimens from autumn being particularly rich in lipid (Fig. 3b). The proportion of lipid per dry mass (%DM) decreased strongly polynomially (second order) from more than 50% in polytrochous larvae to about 10–30% in small and large adults ($r^2 = 0.471$; $P < 0.001$; $n = 57$).

In spring 2002 (April), the smallest specimens of *C. limacina* (1.7 mm) were found to be very rich in lipid (48.5%DM). The lipid content decreased with body-length in the larger animals from 23.4 to 20.2%DM. In May 2001, the smallest specimens again exhibited the highest lipid content (50.4%DM). Like the animals from April, the lipid content also decreased with increasing length. The lipid content strongly decreased in July/August (7.7 to 10.2%DM) and in small specimens in September (13%DM). In the largest individuals from September, the lipid content ranged to 25%DM (Fig. 3c). The separately analysed gonads of large adults (25–48 mm) from summer 2003 had a mean dry mass of

3.5 mg and a lipid mass of 0.8 mg (i.e. 22.9%DM). However, values calculated for the complete organism were comparable to the summer specimens from 2001 with lipid levels of 9.6%DM.

Seasonal and ontogenetic changes of lipid composition

For a detailed and comprehensive assessment of the seasonal and ontogenetic development of *C. limacina* based on changes in lipids, the large data set (52 analyses) of the fatty acid compositions of all specimens from the different seasons was subjected to a principal component analysis. For the classification, 28 fatty acids were taken as variables. Two principal components (PC) explained 54.5% of the total variance in the data set; PC 1 accounted for 30.9% and PC 2 for 23.6%. PC 3 explained another 9.2%, but did not add additional information and was therefore not included in the data interpretation. The PCA produced four distinct "multivariate groups" in the two-dimensional score plot (Fig. 4a).

Along PC 1, polytrochous larvae and small adults from spring (April and May) are distinguished from larger adults of midsummer and autumn. PC 2 mainly separates specimens from April and September from those of May and July/August. The PC loading plot (Fig. 4b) demonstrated that a-17:0 and 17:0, as well as 14:0, 18:4(*n*-3) and i-15:0, were the most important fatty acids for the differentiation of samples along PC 1. Important fatty acids along PC 2 were 18:0, 20:5(*n*-3) and 20:4(*n*-6), as well as 15:0, 17:1(*n*-8), 16:1(*n*-7) and 18:1(*n*-7).

For the four groups, all parameters were compiled and presented as means in Tables 1 and 2. Major lipid classes of *C. limacina* were phospholipids, triacylglycerols, 1-O-alkyldiacylglycerol ethers, sterols and free fatty acids. Wax esters occurred only in small amounts.

Group I comprised the smallest specimens, being polytrochous larvae and small adults from April. One large specimen (42 mm) from May was also classified in

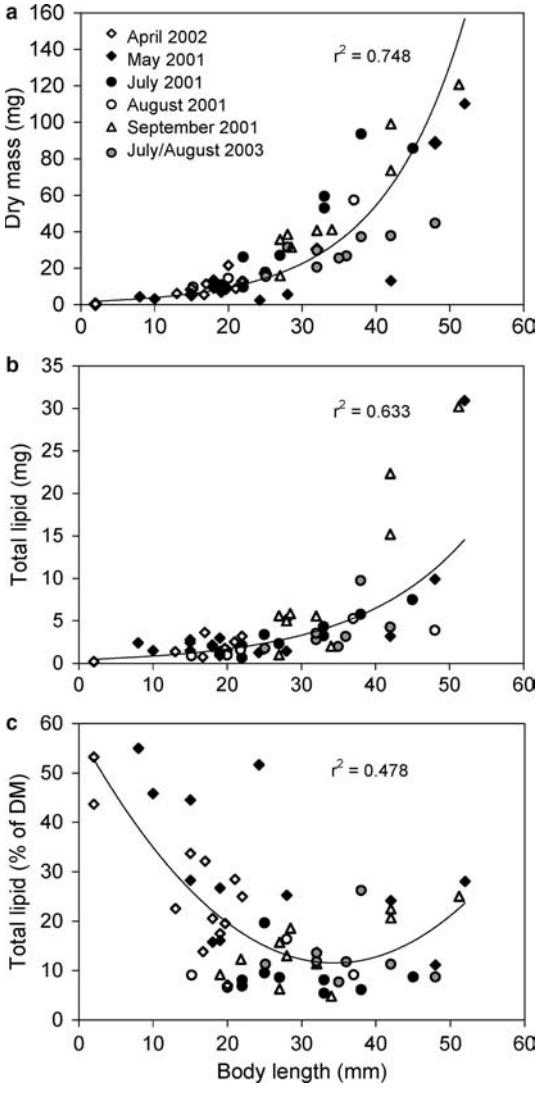

Fig. 3 *Cliona limacina*. **a** Dry mass (*DM*) with exponential fit ($y = 1.6146^{e0.088x}$), **b** total lipid with exponential fit ($y = 0.4401^{e0.0673x}$), and **c** total lipid in %DM with polynomial fit ($y = 0.0393x^2 - 2.7029x + 58.04$, second order) from April (2002), May to September (2001) and July/August (2003)

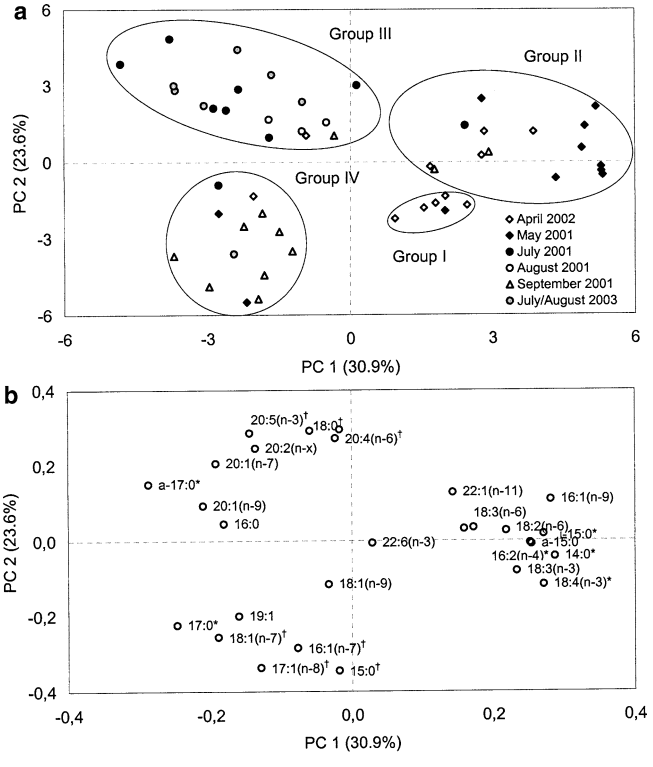

Fig. 4 *Cliona limacina*. Principal component analysis (PCA) based on the fatty acid compositions during six periods from April (2002) and May to September (2001) and during July/August (2003). The last comprises specimens with fully developed gonads. **a** Principal component plot. **b** Loading plot of fatty acids and their contribution to the spread along PC 1 and PC 2. For clarity, only the most important fatty acids are designated (for symbols see Table 2)

this group, but due to its different size it was not included in the calculation of means for group I (Table 1). The mean dry and lipid masses of the small specimens accounted for 6.2 and 1.8 mg, respectively, resulting in a lipid content of 36.8%DM. This is in accordance with high proportions of TAG, ranging from 42.9 to 60.4% of total lipid and DAGE from 18.6 to 28.9%. PL accounted on average for 23.8%. The large specimen from May also had high proportions of total lipid (24.2%DM) and storage lipids (57.8% of TAG; 21.9% of DAGE).

Group II consisted of larger polytrochous larvae and small adults from May, as well as some small adults from other seasons. Compared to group I, these specimens were larger (mean 18.1 mm) and exhibited higher dry masses (8.5 mg). Total lipid accounted for 1.5 mg and lipid levels for 23.9%DM. The composition of storage lipids still showed high proportions of TAG (49.5%), but lower proportions of DAGE (10.8%). PL averaged out at 30.3%.

The large adults from July/August 2001 of group III were almost twice as long as those of group II (mean 29.8 mm) and about 4 times their dry mass (32.3 mg). These specimens had low lipid contents (10.4%DM), which is in accordance with low proportions of TAG (4.9%) and DAGE (2.7%) and very high levels of PL (73.2%). Two adults, one from September and one from April, also belonged to this group. The adults from summer 2003, with fully developed gonads, were also assigned to group III. To include the data in the PCA, lipid compositions of the total organisms were calculated from the separate analyses of gonads and trunks. Dry mass of the gonads was, on average 3.5 mg, which corresponds to 12.3% of the total dry mass of the organism. Total lipid of the gonads and trunks was 0.8 and 2.2 mg, respectively, and thus the lipid content of the gonads was clearly higher (mean 24.7%DM) than that of the trunks (8.9%DM). PL dominated the lipid class composition of gonads (82.4%) and trunks (72.0%), which is in accordance with the summer adults from 2001 (Table 1).

Group IV consisted mainly of the largest adults (mean 37.1 mm) from autumn and a few very large specimens from the other seasons with length up to 52 mm. According to the body-lengths, these specimens had highest dry masses (61.8 mg). Despite moderate lipid contents (19.0%DM), these large specimens exhibited the high proportions of TAG (39.6%) and highest levels of DAGE, ranging from 11.5 to 42.7% (mean of 26.7%). PL accounted for 22.2% (Table 1).

The fatty acid compositions of all groups, as well as for gonads and trunks (Table 2), were dominated by three major fatty acids, which were 16:0, varying between 10.1 and 14.4% of total fatty acids, and the polyunsaturates 20:5(*n*-3) accounting for 11.4–19.9%, and 22:6(*n*-3) accounting for 16.0–24.5%. In general, the PC 1 decisive fatty acids for groups I and II exhibited higher proportions than for groups III and IV. The 14:0 fatty acid accounted for 3.4 and 3.8% for groups I and II and 1.8 and 1.7% for groups III and IV, respectively. The 18:4(*n*-3) proportions were 9.1 and 7.6% (groups I and II), and 1.9 and 3.0% (groups III and IV). For the fatty acids along PC 2, the DAGE-rich groups I and IV had higher levels of 17:1(*n*-8), accounting for 4.8 and 10.8%, whereas only 1.7 and 1.8% occurred in group II and III specimens. The odd-chain fatty acid 15:0 exhibited 3.0 and 4.4% in groups I and IV, whereas in groups II and III the proportions were lower with 1.8 and 1.3%. Higher amounts of 16:1(*n*-7) occurred in groups I and IV (7.3 and 9.0%), in addition to 18:1(*n*-7) (2.6 and 4.2%). Correspondingly, in groups II and III lower proportions were found for 16:1(*n*-7) (3.8 and 3.9%) and also for 18:1(*n*-7) (1.7 and 2.2%). The highest proportions of 22:6(*n*-3) and 20:5(*n*-3) were mostly found in group II and in the phospholipid-rich adults of group III, whereas in groups I and IV the proportions were the same (16.2%).

Some differences were obvious between the fatty acid compositions of gonads and trunks from the summer 2003 specimens. The 22:6(*n*-3) fatty acid exhibited considerably higher proportions in the trunks (24.5%) than in the gonads (16.0%). Other minor differences were

Table 1 *Clione limacina*. Body-length (*BL*) in millimetres, dry mass (*DM*) in milligrammes, total lipid (*TL*) in milligrammes, total lipid in %DM and lipid class composition of groups I–IV based on PCA. Data of gonads and trunks from summer 2003 specimens are added; data of complete specimens are part of group III; phospholipids (*PL*), sterols (*ST*), free fatty acids (*FFA*), triacylglycerols (*TAG*), 1-O-alkyldiacylglycerol ethers (*DAGE*) and wax esters (*WE*); number of samples (*n*)

	Group I[a] (5) Mean ± SD	Group II (15) Mean ± SD	Group III (18) Mean ± SD	Group IV (13) Mean ± SD	Gonads (8) Mean ± SD	Trunk (8) Mean ± SD
BL (mm)	12.4 ± 9.9	18.1 ± 5.2	29.8 ± 9.0	37.1 ± 10.7	–±–	35.7 ± 7.5
DM (mg)	6.2 ± 5.5	8.5 ± 4.9	32.3 ± 22.1	61.8 ± 35.8	3.5 ± 1.7	25.2 ± 8.8
TL (mg)	1.8 ± 1.5	1.5 ± 0.6	2.9 ± 1.4	11.9 ± 9.8	0.8 ± 0.4	2.2 ± 0.7
TL (%DM)	36.8 ± 11.6	23.9 ± 15.6	10.4 ± 4.1	19.0 ± 7.4	24.7 ± 10.4	8.9 ± 1.3
PL	23.8 ± 3.7	30.3 ± 15.4	73.2 ± 12.2	22.2 ± 9.4	82.4 ± 25.6	72.0 ± 24.6
ST	1.2 ± 0.7	4.6 ± 2.8	12.3 ± 4.7	5.9 ± 2.9	5.3 ± 2.8	13.2 ± 7.5
FFA	0.1 ± 0.1	2.8 ± 3.6	6.1 ± 9.0	2.6 ± 5.5	2.8 ± 7.6	0.5 ± 0.5
TAG	52.2 ± 7.1	49.5 ± 16.6	4.9 ± 5.5	39.6 ± 11.0	4.2 ± 7.7	9.1 ± 19.5
DAGE	21.9 ± 4.5	10.8 ± 4.5	2.7 ± 4.8	26.7 ± 7.7	3.2 ± 6.9	3.9 ± 9.5
WE	0.8 ± 0.3	2.1 ± 2.5	0.7 ± 1.0	3.0 ± 2.9	2.0 ± 5.6	1.3 ± 1.6

[a]Large specimen from May (42 mm) is not included in the means

Table 2 *Clione limacina*. Fatty acid compositions of groups I–IV, as well as data of gonads and trunks. Mean ± standard deviation (*SD*) in mass percent of total fatty acids. Number of samples (*n*); fatty acids used for PCA analysis (*z*); main fatty acids that defined PC 1 (*); main fatty acids that defined PC 2 (†)

		Group I (6) Mean ± SD	Group II (15) Mean ± SD	Group III (18) Mean ± SD	Group IV (13) Mean ± SD	Gonads (8) Mean ± SD	Trunk (8) Mean ± SD
Fatty acids							
14:0	z*	3.4 ± 0.5	3.8 ± 0.7	1.8 ± 0.9	1.7 ± 0.4	1.9 ± 0.6	1.4 ± 0.9
i-15:0	z*	0.7 ± 0.1	0.8 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
a-15:0	z	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
15:0	z†	3.0 ± 0.3	1.8 ± 0.5	1.3 ± 0.7	4.4 ± 1.2	1.9 ± 1.9	1.2 ± 1.3
i-16:0		0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
a-16:0		0.2 ± 0.0	0.4 ± 0.3	0.5 ± 0.4	0.6 ± 0.3	0.3 ± 0.4	0.4 ± 0.4
16:0	z	11.9 ± 1.1	10.1 ± 1.6	12.7 ± 1.7	12.0 ± 1.7	14.4 ± 1.5	12.0 ± 1.0
16:1(*n*-9)	z	0.5 ± 0.3	1.0 ± 0.3	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
16:1(*n*-7)	z†	7.3 ± 0.9	3.8 ± 1.2	3.9 ± 2.1	9.0 ± 2.4	9.2 ± 4.4	4.6 ± 3.4
i-17:0		1.1 ± 0.2	0.8 ± 0.2	0.7 ± 0.3	0.5 ± 0.3	0.6 ± 0.2	0.5 ± 0.1
a-17:0	z*	1.3 ± 0.1	1.1 ± 0.2	2.2 ± 0.5	1.5 ± 0.2	2.0 ± 0.5	2.3 ± 0.4
16:2(*n*-4)	z*	0.7 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
17:0	z*	2.0 ± 0.5	1.2 ± 0.3	1.9 ± 0.4	3.3 ± 0.6	2.1 ± 0.9	2.3 ± 0.6
17:1(*n*-8)	z†	4.8 ± 2.1	1.7 ± 0.8	1.8 ± 0.9	10.8 ± 3.9	2.1 ± 1.4	2.4 ± 4.0
18:0	z†	1.5 ± 0.2	2.6 ± 1.1	3.6 ± 1.0	1.7 ± 0.5	2.7 ± 0.7	3.9 ± 1.1
18:1(*n*-9)	z	3.2 ± 0.6	3.0 ± 1.9	3.0 ± 1.1	3.7 ± 0.8	4.0 ± 1.0	2.8 ± 1.4
18:1(*n*-7)	z†	2.6 ± 0.3	1.7 ± 0.4	2.2 ± 0.7	4.2 ± 1.0	3.9 ± 1.2	2.4 ± 1.0
18:2(*n*-6)	z	1.4 ± 0.2	3.2 ± 3.4	1.3 ± 1.0	1.0 ± 0.2	1.3 ± 0.6	0.8 ± 0.3
18:2(*n*-x)	z	0.4 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1
18:3(*n*-6)	z	0.7 ± 1.0	1.1 ± 1.6	0.3 ± 0.3	0.2 ± 0.1	0.6 ± 0.5	0.3 ± 0.3
19:0		0.7 ± 0.2	0.4 ± 0.2	0.2 ± 0.2	0.5 ± 0.6	0.7 ± 0.4	0.4 ± 0.4
19:1	z	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	1.0 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
18:3(*n*-3)	z	2.0 ± 0.4	1.9 ± 0.5	0.8 ± 0.5	1.1 ± 0.4	0.8 ± 0.4	0.5 ± 0.2
18:4(*n*-3)	z*	9.1 ± 2.1	7.6 ± 2.3	1.9 ± 1.3	3.0 ± 1.6	2.1 ± 1.0	1.0 ± 0.7
20:0	z	0.2 ± 0.0	0.5 ± 0.2	0.7 ± 0.4	0.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.4
20:1(*n*-9)	z	2.4 ± 0.4	1.7 ± 0.4	2.6 ± 0.5	2.2 ± 0.4	2.6 ± 0.6	2.8 ± 0.4
20:1(*n*-7)	z	1.8 ± 0.1	1.7 ± 0.3	2.4 ± 0.5	1.8 ± 0.4	2.3 ± 0.5	2.6 ± 0.5
20:2(*n*-6)	z	0.5 ± 0.0	0.5 ± 0.2	0.8 ± 0.3	0.5 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
20:4(*n*-6)	z†	0.5 ± 0.1	0.8 ± 0.2	1.0 ± 0.4	0.5 ± 0.3	0.7 ± 0.2	1.1 ± 0.4
20:4(*n*-3)		1.1 ± 0.1	1.2 ± 0.3	0.7 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	0.5 ± 0.1
20:5(*n*-3)	z†	12.4 ± 0.3	12.0 ± 1.9	16.9 ± 3.1	11.4 ± 2.0	17.6 ± 2.9	19.9 ± 3.1
22:1(*n*-11)	z	0.3 ± 0.1	0.6 ± 0.3	0.4 ± 0.3	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.4
22:1(*n*-9)		0.4 ± 0.1	0.6 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.2	0.1 ± 0.3
22:5(*n*-3)		0.7 ± 0.2	2.1 ± 1.0	1.3 ± 1.0	0.9 ± 0.4	1.0 ± 0.4	1.1 ± 0.2
22:6(*n*-3)	z	16.2 ± 0.9	21.6 ± 4.2	26.1 ± 5.4	16.2 ± 4.1	16.0 ± 4.7	24.5 ± 5.9

found in the proportions of 16:1(*n*-7), and C_{18} fatty acids being higher in the gonads. The proportions of the odd-chain fatty acids 15:0 and 17:1(*n*-8) were low in both gonads and trunks.

A clear relationship was found between fatty acids and lipid classes ($P < 0.001$; $n = 52$; Fig. 5). Both 14:0 and 18:4(*n*-3), which are decisive for PC 1, correlated significantly with the proportions of TAG, but not with DAGE. In contrast, the fatty acids 15:0 and 17:1(*n*-8), as well as 16:1(*n*-7) and 18:1(*n*-7), were significantly correlated with the proportions of DAGE, but not with TAG. The correlation coefficients were highest for the odd-chain fatty acids. The fatty acids 20:5(*n*-3) and 22:6(*n*-3) were correlated with PL.

Discussion

On the basis of body-length frequency distributions, age-groups can generally be separated and growth expressed as change of mean length over time (Falk-Petersen 1985). The length-frequency distribution of *C. limacina* revealed no unequivocal population structure because larvae and adults of different lengths occurred simultaneously during most sampling periods. In Kongsfjorden, the year-round presence of most of the stages can be explained by the spawning behaviour of *C. limacina*, with main spawning in summer, but also low spawning during the other seasons (Mileikovsky 1970). The overall population structure, however, showed a clear change from larvae in spring to adults in summer, with the largest specimens occurring in autumn. Since a differentiation of the various growth stages by cohort analysis was not possible, we applied PCA to explain the seasonal and ontogenetic development of *C. limacina*. Thus, the large data set was arranged into four distinct "multivariate groups", and data were combined according to their ontogenetic development (PC 1) and their changes in storage lipids (PC 2), both on a seasonal basis.

Group I represented the smallest larvae of *C. limacina* found in April with the highest lipid content of all specimens. These larvae had developed from veligers probably hatched during autumn or winter. They presumably overwintered without notable growth and at

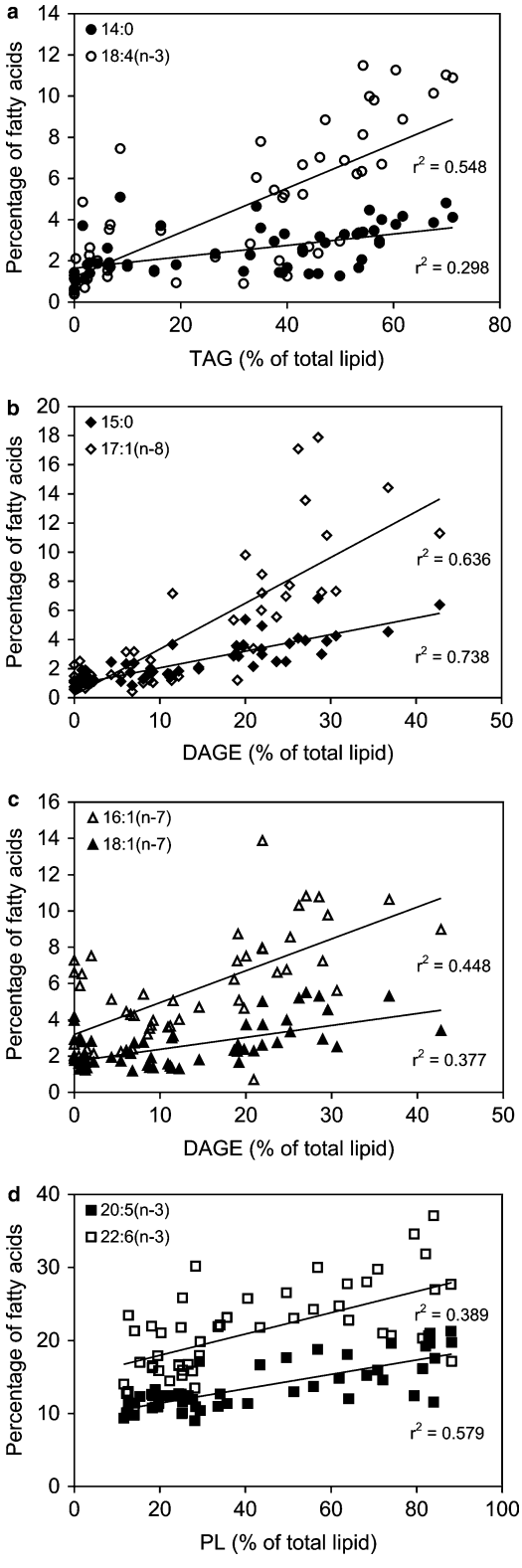

Fig. 5a–d Correlation between triacylglycerols (*TAG*) and the fatty acids 14:0 and 18:4(*n*-3), between alkyldiacylglycerol ethers (*DAGE*) and 15:0, 17:1(*n*-8), 16:1(*n*-7) and 18:1(*n*-7), and between phospholipids (*PL*) and 20:5(*n*-3) and 22:6(*n*-3)

reduced metabolism, or had developed from smaller larval stages during winter by feeding on early stages of *L. helicina*. Specimens of this group had a mean length of 12.4 mm except for one large animal from May. This individual probably represents the specimens that had overwintered as adults.

Until May, *C. limacina* grew to larger larvae or small adults assigned to group II. The reduced lipid content compared to group I specimens is a consequence of this development. The development of *C. limacina* larvae coincided with a high abundance of lipid-rich veligers and juveniles of *L. helicina* (Gannefors et al. 2005) as abundant food source. This may explain their still high lipid content. Besides specimens from May, this group also included some small adults from April and September due to similar fatty acid compositions. Thus, all specimens combined in this group may have fed on *L. helicina*, which were grown during late autumn and winter, at a time when they exhibited a typical autumn fatty acid signature.

Although specimens of group III (July/August) nearly doubled their length and increased fourfold in dry mass, they were lipid-poor because they had invested most of their lipids in growth, maturation and subsequent reproduction during the main spawning period in summer (Mileikovsky 1970). In September (group IV), most adults were large mature specimens with enhanced lipid levels. A few lipid-rich adults from spring and summer were also classified in group IV due to similarity of the fatty acid signatures, which coincides with higher levels of DAGE. These specimens had probably survived the winter season. Their high lipid content might be due to good feeding conditions in autumn or recent feeding events. September specimens of group IV were obviously able to replenish their lipid stores after spawning. These adults accumulated lipids strongly, especially by enhancing DAGE, in preparation for overwintering. The ability to seasonally accumulate high amounts of lipids suggests that *C. limacina* has at least a 2-year life-cycle.

Higher proportions of the abundant fatty acids defining PC 1, 18:4(*n*-3) and 14:0, were found in smaller and larger larvae from spring (groups I and II). These fatty acids correlated with the proportions of TAG. The accumulation of the dietary fatty acid 18:4(*n*-3) should reflect the recent use of food for the production of TAG-rich lipid deposits since TAG are produced more quickly during high food supply than other storage lipids, as known from studies on wax-ester-producing copepods (e.g. Sargent and Henderson 1986). It is well established that fatty acids can be channelled unchanged through the food web (Graeve et al. 1994; Dalsgaard et al. 2003). Therefore, the compositions of total fatty acid of *C. limacina* were compared with those of different developmental stages of *L. helicina* from Kongsfjorden (Gannefors et al. 2005) during the same investigation period using PCA. In May and July/August, only minor similarities were found between the total fatty acid compositions of both species, whereas in September no

relationship occurred. The marker fatty acid 18:4(*n*-3) was more abundant in *L. helicina* from autumn, whereas for *C. limacina* the highest proportions were detected in spring and summer specimens. It may be possible that 18:4(*n*-3) originated from feeding on early juvenile stages of *L. helicina*, which had developed during winter and still carried the fatty acid signature of autumn specimens. The limited use of 18:4(*n*-3) as dietary marker fatty acid for this trophic relationship has also been noted by Kattner et al. (1998).

PC 2 arranged *C. limacina* specimens according to the differences in storage lipids. To group I (polytrochous larvae and young adults from early spring) and group IV (large adults from autumn), some large specimens from other seasons were assigned. They may have overwintered and belong to another generation. The specimens of group I and IV were extremely rich in lipids, with high proportions of TAG and DAGE, which shows that larvae also strongly accumulate lipids, perhaps during autumn, but also during winter and early spring. We suggest that DAGE function generally as a long-term energy storage, independent of stage and that this is necessary during food scarcity, when corresponding stages of *L. helicina* are absent. However, DAGE are probably most important as an energy source for growth and reproduction since our data show that they are almost depleted in the adults from summer. Food scarcity might be possible since Gannefors et al. (2005) reported pulses of low density of *L. helicina* in Kongsfjorden. They also found that spawning of *L. helicina* was almost finished by September and larger females disappeared, which is in accordance with the proposed 1-year life-cycle. Only some adults will survive and overwinter as females. However, during late autumn and winter, high numbers of veligers and juveniles of *L. helicina* have been observed in Svalbard waters. This potential food supply in winter may be especially helpful for the larvae of *C. limacina* to survive the winter as lipid-rich stages because they are dependent on larvae of *L. helicina* as their only prey due to their extreme co-development.

Group II and III specimens were different from those of group I and IV because they exhibited lower proportions of the PC 2 decisive fatty acids. Group II is composed mostly of specimens from May and a few larger specimens from April exhibiting lower lipid levels, as well as lower proportions of DAGE compared to group I specimens, although the proportions of TAG were still high. TAG are usually considered as a short-term energy store (Lee et al. 1971; Sargent and Henderson 1986). However, for development to maturation, a considerable amount of energy is necessary, which is available in the form of very high proportions of TAG in both group I and II specimens. We suggest that TAG are more easily metabolised for growth and development than DAGE.

Specimens of group III comprised mostly adults from summer. Because the summer specimens, of which gonads and trunks were separately analysed, fit into the same group, we assume that the specimens from summer 2001 also had well-developed gonads. In addition to the high level of PL, some of these adults showed higher proportions of FFA. This was also observed for *C. limacina* and *L. helicina* from the Southern Ocean (Phleger et al. 1997) and *L. helicina* from the Arctic during summer (Falk-Petersen et al. 2001; Gannefors et al. 2005). We hypothesise that higher proportions of FFA may occur during gonad development of pteropods because enhanced levels in both species were only found during summer. The gonads were extremely rich in PL, which must result from a conversion of storage lipids into PL via FFA. PL are accumulated in gonads for egg production since eggs have yolks with lipovitellin, a lipoprotein with approximately equal amounts of protein and phospholipids (mainly phosphatidylcholine), as known from other zooplankton species (Lee 1991).

Odd-chain fatty acids [17:1(*n*-8), 15:0] were highly significantly correlated with the proportion of DAGE, but not with TAG. They are especially enriched in DAGE, less in TAG and occur only in traces in PL (Kattner et al. 1998). The depletion of DAGE is associated with a reduction of these fatty acids, and in some specimens analysed by Falk-Petersen et al. (2001), DAGE and 17:1(*n*-8) are totally lacking. These odd-chain fatty acids are products of de-novo biosynthesis since they were not found or existed only as traces in *L. helicina*. The biosynthesis of odd-chain fatty acids in *C. limacina* is still unclear, but a likely hypothesis is the utilisation of dimethylsulfoniopropionate (DMSP), from which the propionate moiety is the starter molecule for the biosynthesis, as proposed by Kattner et al. (1998). DMSP is strongly accumulated by *L. helicina*, a mucus trapper, due to feeding on detritus and phytoplankton (Gilmer and Harbison 1991; Levasseur et al. 1994).

The fatty acids 16:1(*n*-7) and 18:1(*n*-7), which are generally accepted as typical diatom marker fatty acids (Dalsgaard et al. 2003), are probably also de novo synthesised by *C. limacina* since their occurrence is not correlated with that in *L. helicina*. The highest proportions of these two fatty acids were found in the autumn specimens of *C. limacina*, but also in spring specimens of *L. helicina* (Gannefors et al. 2005). The importance of these monounsaturates, especially that of 17:1(*n*-8), suggests that *C. limacina* may possess a very active Δ9 desaturase, which preferentially acts on 17:0 and 16:0 fatty acids. The 18:1(*n*-7) results then from elongation of 16:1(*n*-7). These possibilities of biosynthesis reflect the high ability of de novo production of lipids by *C. limacina*. Both acyl- and alkyl-containing neutral lipids are extensively produced.

Conclusions

C. limacina is monophagous and therefore dependent on the occurrence of its only prey *L. helicina*. Its specialisation in lipid biosynthesis shows that it has developed a very unusual and extraordinary life-strategy to cope

with a likely food limitation. In contrast to *L. helicina* with a proposed life-cycle of 1 year (Gannefors et al. 2005), we conclude that *C. limacina* has at least a 2-year life-cycle or even longer. This assumption is based on the seasonal lipid accumulation, de novo lipid biosynthesis and a population structure defined by the fatty-acid-based PCA. The utilisation of lipid stores for maturation, production of gonads and spawning during summer, followed by the replenishment of lipids during late summer and autumn, clearly indicate a life-cycle of more than 1 year. The biosynthesis of DAGE, as long-term storage energy, supports the perennial life-cycle and is probably important during periods of food scarcity, but also for reproduction. Enhanced TAG accumulation, however, might be a prerequisite for a successful growth and development. The fatty acids, correlating with the proportion of TAG, may originate preferentially from dietary input, whereas de-novo synthesised odd-chain fatty acids were dominant moieties of DAGE. This synthesis, probably via DMSP, confirms that *C. limacina* is very effective in utilising its specific food supply, and shows that this is part of its metabolic efficiency (Conover and Lalli 1974). The overwintering strategy of *C. limacina* is still unknown although it is likely that the production of large lipid deposits helps it survive possible food scarcity during winter.

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