Marine Biology (2005) 147: 169–177
DOI 10.1007/s00227-004-1544-y

RESEARCH ARTICLE

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The Arctic sea butterfly *Limacina helicina*: lipids and life strategy

Received: 19 June 2004 / Accepted: 27 November 2004 / Published online: 10 February 2005

Abstract The sea butterfly *Limacina helicina* was collected from May to September 2001 in Kongsfjorden, Spitsbergen, to investigate population structure and body and lipid composition with regard to life cycle and reproductive strategy. Veligers and juveniles were only found in late autumn and spring, whereas females occurred from July to September. The size of the females increased until mid-August and decreased in September. Dry and lipid mass were closely related to size; dry mass increased exponentially and lipids linearly with size. The lipid content was highest in veligers (31.5% of dry mass) and juveniles (23.6%) but low in females (< 10%). Phospholipids were the dominating lipid class followed by triacylglycerols. Females, veligers, and egg ribbons, all from September, were richest in phospholipids. Juveniles contained the highest amounts of triacylglycerols, whereas females had low levels in July and the beginning of August. In mid-August, levels of triacylglycerols were variable and higher. This suggests that females were in their main spawning period and the high variability in triacylglycerols points to different stages within the spawning cycle. Enhanced amounts of free fatty acids in females from July may be related to gonad development. The 16:1(n-7) fatty acid was more dominant in spring whereas 18:4(n-3) increased in summer and autumn, which reflects a change in diet from diatom-dominated food items in spring to dinoflagellates in summer/autumn. Small amounts of long-chain monounsaturated fatty acids suggest ingestion of copepods, and the fatty acid composition of veligers feeding on particulate matter. *L. helicina* has a one-year life cycle with peak spawning in August and over-winters as veligers that may grow to juveniles during the winter period. They metamorphose into juveniles during spring, develop to males in early summer, and mature into females in July and August.

Communicated by M. Kühl, Helsingør

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Introduction

The sea butterfly *Limacina helicina* (Phipps, 1774) is the only thecosomatous pteropod found in the Arctic (Fig. 1). It has a bipolar distribution and can occur in high densities in polar regions as well as in sub-polar waters (Kerswill 1940; Kobayashi 1974; Lalli and Gilmer 1989a; Cooney et al. 2001). It is holoplanktonic and has evolved a pair of parapodia (“wings”) for swimming instead of a gastropod foot. *L. helicina* is described as a protandric hermaphrodite. It retains its largest known size in sub-Arctic Atlantic waters, being male at smaller sizes (4–5 mm) and developing into female as it becomes larger (< 5 mm). The eggs are arranged on a ribbon (Paranjape 1968; Lalli and Gilmer 1989a).

As a prominent member of the polar zooplankton community, *L. helicina* is exposed to extreme environmental conditions. The marginal seas of the Arctic are characterised by a pronounced seasonality in light conditions, temperature, and ice cover resulting in short and intensive blooms of primary producers (Sakshaug 2003). Hence, zooplankton is faced with an environment of highly variable food abundance.

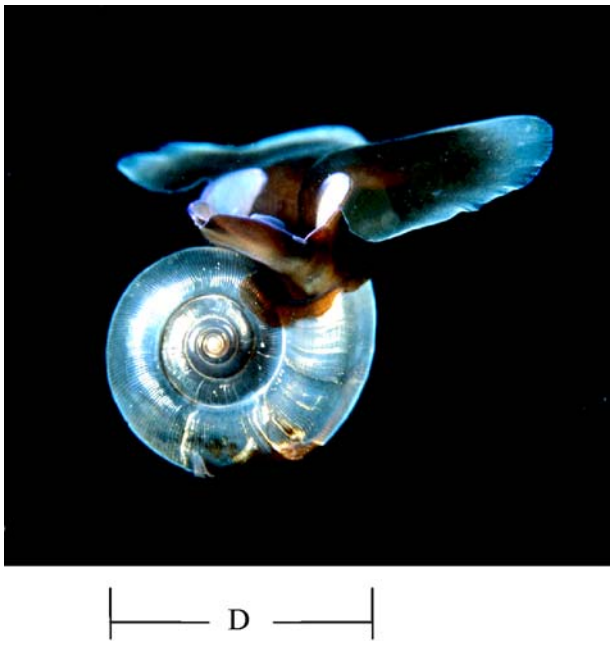

Fig. 1 The pteropod *Limacina helicina* with measurement of diameter. Photo: Erling Svensen

L. helicina is omnivorous and feeds by excreting a mucous web, in which its prey gets entangled (Gilmer 1972, 1990; Harbison and Gilmer 1992). *L. helicina* is food for other organisms, such as larger carnivorous zooplankton, for example, ctenophores (Larson and Harbison 1989), the only prey of *Clione limacina* (Conover and Lalli 1972), and it is also preyed upon by sea birds (Meisenheimer 1905) and several species of fish, for example, polar cod (*Boreogadus saida*; H. Hop, personal communication).

L. helicina has only moderate lipid content of $<20\%$ of dry mass (Percy and Fife 1981; Kattner et al. 1998). Some data concerning lipid classes and fatty acids of *L. helicina* are available from the Greenland Sea and the Svalbard area (Falk-Petersen et al. 2001; Kattner et al. 1998) and from Antarctica (Kattner et al. 1998; Phleger et al. 1997). These studies were strongly focused on the feeding relationship between *Clione limacina* and *L. helicina* since *C. limacina* is monophagous and feeds exclusively on *L. helicina* (Lalli 1970; Conover and Lalli 1972; Lalli and Gilmer 1989b; M. Böer, personal communication). The data collected in earlier investigations are generally from one period (often summer), and individuals analysed were not separated into different sizes or developmental stages. No previous effort has been made to describe the lipid composition with regards to ontogenetic or seasonal changes.

The goal of this study was to fill the gap in knowledge concerning *L. helicina* by (1) investigating its population structure and body composition, (2) elucidating its lipid biochemistry with regard to seasonality of total lipid, lipid class, and fatty acid compositions, and (3) relating the seasonal variations in population structure and biochemical composition to their life strategy and feeding behaviour.

Materials and methods

Study area

This study was carried out in Kongsfjorden (79°N, 12°E), West Spitsbergen, Svalbard (Fig. 2) from 20 May to 17 September 2001. Western Svalbard generally has a milder climate compared to other areas at the same latitude due to the influence of the West Spitsbergen Current, a branch of the North Atlantic Current that carries relatively warm and saline Atlantic water along the west coast of Spitsbergen (Svendsen et al. 2002). Kongsfjorden is an open fjord without a sill. Water masses originating from warm and saline Atlantic Water and cold and less saline Arctic Water enter the fjord as Transformed Atlantic Water ($T>1$°C, $S>34.7$; Svendsen et al. 2002).

Sampling

Veligers and juveniles of *Limacina helicina* were collected with WP-2 (opening 0.250 m^2, mesh size 180 µm) and WP-3 (opening 0.781 m^2, mesh size 1,000 µm) nets, and undamaged individuals were selected for the analyses. *L. helicina* has a thin and fragile shell that is often damaged and broken during sampling, and the thin parapodia are easily torn or ripped off when collected in nets (Hamner et al. 1975; Gilmer 1990). In addition, single undamaged adults were collected from the surface with a sieve (20 cm diameter) on a rod or with 1-l plastic jars from a Zodiac. For transportation, organisms were kept in 10-l buckets. Samples were immediately sorted according to size and frozen at −20°C. Egg ribbons were collected from the females during morphometric measurements.

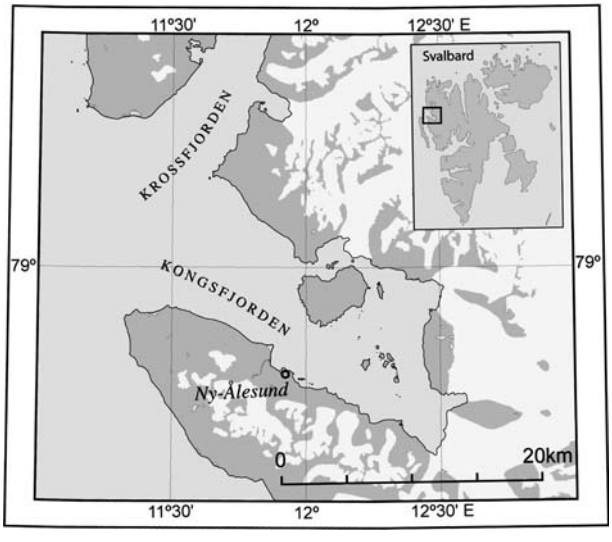

Fig. 2 Map of Kongsfjorden and Spitsbergen

Shell diameter was measured on site prior to freezing (Fig. 1) according to Kobayashi (1974). Differentiation of ontogenetic stages was based on sizes obtained from Lalli and Wells (1978): veligers <0.3 mm, juveniles 0.3 to 4 mm, males 4 to 5 mm, and females >5 mm. Samples collected for body composition were frozen in fours at –20°C. For lipid analyses, samples were preserved immediately after sampling and size measurement in chloroform:methanol (2:1 v/v) with 0.01% antioxidant (Butylated Hydroxy Toluene, BHT), in scintillation vials with PTFE-sealed screw cap and consecutively frozen at −20°C. Adults were individually preserved in fours, whereas 40–205 individuals of veligers and 2–4 juveniles were pooled per sample.

Laboratory analyses

For determination of body composition, wet, dry, and lipid mass of approximately 250 individuals were measured gravimetrically. Measurements of size (in millimetres) and wet mass were done on frozen individuals, and dry mass was measured after drying individuals for approximately 24 h at 55°C. Ash mass was determined after burning for 8 h at 540°C in a muffle furnace and cooling inside the oven before weighing the next day. Total lipid mass was determined by extraction with chloroform:methanol (2:1 v/v) according to Folch et al. (1957). After purification, the lipid extracts were dried in a water bath at 50°C for 12 h before weighing.

For lipid class and fatty acid analyses, dry mass (DM) was determined after removing the solvent by aerating the sample to dryness with nitrogen. Large amounts of solvent, that is, when several samples were combined, were evaporated in a Rotavapor. Samples were then placed in a lyophilisator (Christ) for 48 h and immediately weighed. Total lipid (TL) was extracted from the freeze-dried samples by the method of Folch et al. (1957) and measured gravimetrically. Lipid classes were analysed by high performance thin layer chromatography (HPTLC) densitometry, following the method described by Olsen and Henderson (1989). Pre-coated HPTLC silica gel 60 plates (20×10 cm) were preconditioned in hexane:diethylether (1:1 v/v). Lipid extracts were spotted on the plates by using a Camag-Linomat IV applicator. The following standards were used for calibration: phospholipids (phosphatidylcholine, PL), sterols (cholesterol, ST), free fatty acids (FFA), triacylglycerols (TAG), 1-O-alkyldiacylglycerol ethers (DAGE), and wax esters (WE), each at concentrations from 0.1 to 15 µg. After developing, lipid classes were stained with manganese (II)-chloride · 4 H_2O, methanol and sulphuric acid reagent, dipped in a Camag immersion device for 5 s, followed by combustion at 120°C for 20 min. Identification and quantification were performed with standards at 550 nm with a Camag TLC Scanner 3.

For fatty acid quantification, an internal standard of 19:0 fatty acid was added before total lipid extraction. Total lipid extracts were hydrolyzed and fatty acids converted into 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 on a gas chromatograph (SGE HP 6890) on a capillary column (30 m×0.25 mm i.d.; film thickness: 0.25 µm; liquid phase: DB-FFAP), using temperature programming (60–180°C) and helium as carrier gas. The analyses, as well as identification and quantification of fatty acids, were done according to the method of Kattner and Fricke (1986).

Statistics

A general linear model type III for unequal sample size was used in the statistical analysis (GLM, Statistica, version 6 for Windows, StatSoft. Inc.). To obtain normality, values were log $(x+1)$ transformed. The expo-

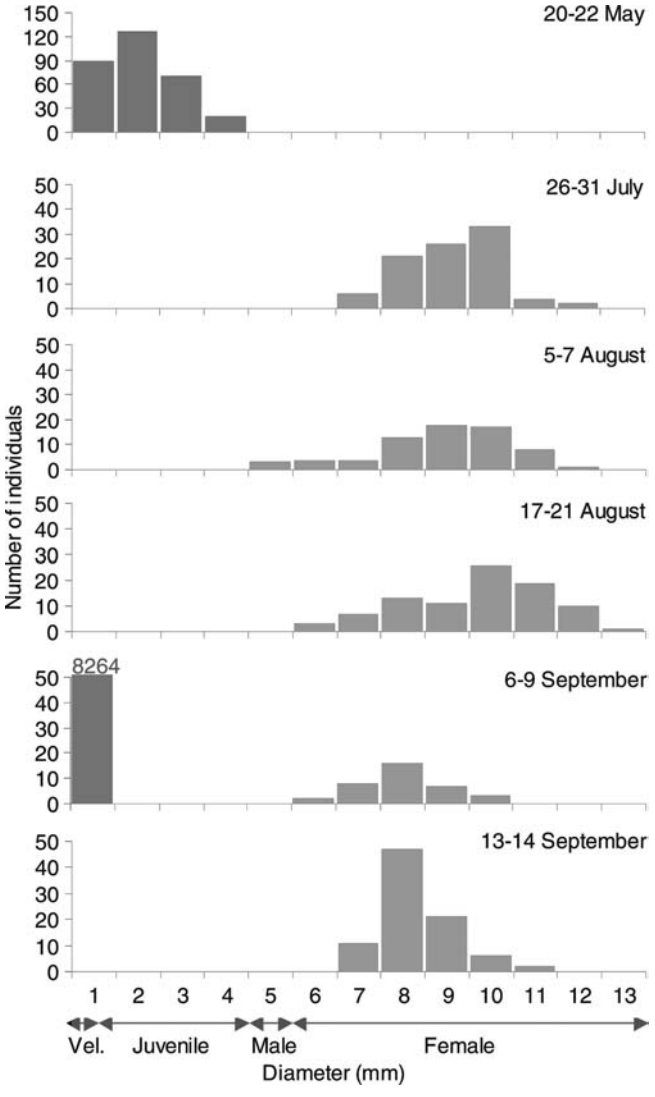

Fig. 3 *L. helicina* from Kongsfjorden. Size distributions from six time periods from May to September 2001. Individuals were collected with WP-2 and WP-3 nets (*black columns*) or sampled from the surface (*grey columns*). *Vel* veliger

nential trends were double-log transformed prior to analysis. To determine significant differences between the time periods investigated with regard to the size–weight relationship, a post hoc Tukey's honest significant difference test (HSD for unequal n) with α-level 0.05 was used. Significant differences in size between time periods for TL were determined by an analysis of variance model (ANOVA type III), and pairwise comparison were done with Tukey's HSD.

Results

Population structure and body composition

In spring (May), only veligers and juveniles of *Limacina helicina* were present (0.2–4 mm, Fig. 3). High numbers of veligers also occurred during September. A few veligers were also found at the end of July (W. Walkusz, personal communication), but from late July to the middle of August mainly females occurred. During summer, females slightly increased in size from 8.8 to 9.6 mm on average, but were smaller (7.9 mm) in September (ANOVA, $P < 0.001$, post hoc unequal n HSD; Table 1), and their abundance had significantly decreased (Fig. 3).

Juveniles had a higher proportion of dry mass (DM) in relation to wet mass (on average 19%) compared to females (11%). Ash content varied between 35 and 40% of DM (Table 1) and did not change between seasons or developmental stages. Wet and dry mass differed significantly (GLM type III, $P < 0.001$) between the time periods; both were highest in summer and decreased towards the autumn. The trend was similar for total lipid (ANOVA, $P < 0.001$), but more variable during summer (Table 1, Fig. 4).

Dry mass of *L. helicina* increased exponentially with diameter (D; $\mathrm{DM} = 0.257D^{2.141}$; $r^2 = 0.93$; $P = 0.00$; $n = 260$; Fig. 4a) and showed a lower allometrical exponent than the "normal" value of 3. The surface–volume ratio of the "ball-shaped" pteropod could be influenced by its use of energy during maturation, reproduction, and spawning and hence lower the ash free dry mass even though the shell (ash mass) stays the same.

The total amount of lipid (TL) showed a significant exponential increase with size ($\mathrm{TL} = 0.19D^{0.281}$; $r^2 = 0.68$, $P < 0.001$, $n = 260$; Fig. 4b). The total lipid relative to dry mass ($R = \mathrm{TL/DM}$) was highest in veligers, decreasing in juveniles, and lowest in females (Fig. 4c). The relative values showed an exponential decrease ($R = 35.45D^{-0.650}$) with increasing size ($r^2 = 0.51$, $P < 0.001$, $n = 260$). The high lipid content of the juveniles exhibited the highest variability ranging from 10 to 40% of DM (24% on average), with large juvenile specimens being less lipid-rich than the smaller ones. The low lipid content of females (< 10% of DM) was almost constant during summer and autumn (Table 1). The egg ribbons, released by females during size measurements,

Table 1 *Limacina helicina*. Diameter (in millimetres), wet mass (*WM*), dry mass (*DM*), total lipid (*TL*) mass, ash mass (*AM*), DM percentage of WM, TL percentage of DM, and AM percentage of DM during May to September 2001. All mass values (in milligrams) are presented as means ± SD; n sample size

Date	Stage	Diameter (mm)		WM (mg/ind.)		DM (mg/ind.)		AM (mg/ind.)		TL (mg/ind.)		DM (% of WM)		AM (% of DM)		TL (% of DM)	
		Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
20–22 May	Juvenile	2.4 ± 0.7	31	30.9 ± 30.7	29	1.8 ± 1.2	31	0.3 ± 0.0	9	1.3 ± 0.8	31	19.4 ± 21.6	29	38.6 ± 6.4	9	23.5 ± 10.3	31
26–31 July	Female	8.7 ± 1.2	50	259.5 ± 97.8	60	28.2 ± 8–2	47	10.2 ± 2.7	20	2.4 ± 1.0	50	12.3 ± 4.2	47	34.3 ± 4.4	20	8.2 ± 3.2	47
5–7 August	Female	9.2 ± 1.2	53	293.7 + 77.3	40	33.0 ± 9.8	50	–		2.3 ± 0.7	53	10.5 ± 3.2	40	–		7.5 ± 2.9	50
17–21 August	Female	9.4 ± 1.5	48	332.5 ± 149.8	71	33.9 ± 12.8	48	14.9 ± 5.1	29	3.4 ± 1.7	53	11.7 ± 2.6	48	39.1 ± 7.3	29	10.5 ± 4.3	48
6–9 September	Female	7.8 ± 0.9	33	173.3 ± 69.5	32	19.9 ± 5.6	33	–		2.1 ± 1.1	31	12.1 ± 1.7	32	–		11.3 ± 2.7	31
13–14 September	Female	8.0 ± 0.8	61	202.6 ± 62.5	52	19.3 ± 3.7	49	7.9 ± 1.3	18	2.2 ± 0.7	61	7.7 ± 4.2	49	40.0 ± 3.3	18	10.5 ± 3.3	49

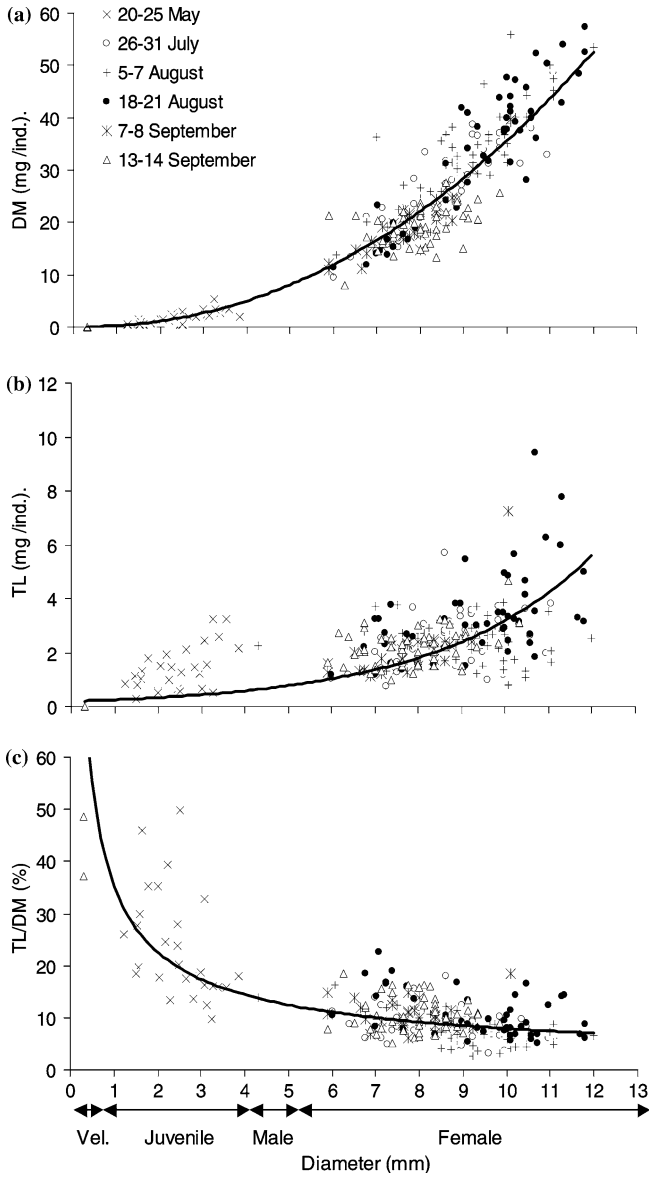

Fig. 4a–c *L. helicina*. Dry mass (*DM*), total lipid mass (*TL*), and lipid percentage of DM (*% TL/DM*) in relation to size from May to September 2001

had an even lower lipid content of about 5% of DM (Table 2).

Lipid composition

Phospholipids (PL) were predominant in all developmental stages during all seasons ranging from 31 to 94% of total lipid. PL in juveniles was less variable, accounting for 62%, which was similar to that in females in July. In females, the highest proportions of PL were found in mid-August and September. However, PL proportions were highly variable in mid-August (31 to 93%). PL in the veligers accounted for 87.7 ± 8.8%, and the egg ribbons were almost entirely composed of PL (Table 2).

Table 2 *L. helicina*. Dry mass (*DM*), total lipid mass (*TL*), TL percentage of DM, and lipid class composition percentage of TL; phospholipids (*PL*), sterols (*ST*), free fatty acids (*FFA*), triacylglycerols (*TAG*), alkyldiacylglycerol ethers (*DAGE*), and wax esters (*WE*) during 20 May–17 September 2001

Date	Stage	*n*	Diameter (mm) Mean ± SD	DM (mg/ind.) Mean ± SD	TL (mg/ind.) Mean ± SD	TL (% of DM) Mean ± SD	PL (% TL) Mean ± SD	ST (% TL) Mean ± SD	FFA (% TL) Mean ± SD	TAG (% TL) Mean ± SD	DAGE (% TL) Mean ± SD	WE (% TL) Mean ± SD
20–22 May	Juvenile	6	2.4 ± 0.6	2.9 ± 1.4	0.6 ± 0.2	23.6 ± 11.3	62.4 ± 9.5	1.7 ± 0.4	1.1 ± 0.6	33.6 ± 9.2	1.1 ± 1.7	0.4 ± 0.5
26–31 July	Female	5	7.2 ± 0.8	16.4 ± 6.0	1.5 ± 0.4	9.9 ± 2.9	65.4 ± 7.8	5.2 ± 0.5	19.6 ± 4.1	8.9 ± 8.0	0.7 ± 0.3	0.1 ± 0.1
5–7 August	Female	8	9.3 ± 1.7	36.8 ± 10.1	2.7 ± 0.6	7.5 ± 1.2	64.0 ± 6.1	9.0 ± 1.1	10.3 ± 3.7	10.4 ± 8.0	5.5 ± 3.2	0.8 ± 1.1
17–21 August	Female	5	8.6 ± 1.6	30.2 ± 16.4	2.7 ± 1.6	9.0 ± 1.3	63.7 ± 25.9	5.2 ± 1.1	4.5 ± 2.7	24.5 ± 24.7	2.1 ± 1.6	0.1 ± 0.2
6–14 September	Female	5	8.4 ± 0.5	18.5 ± 3.6	1.5 ± 0.1	8.2 ± 1.3	77.0 ± 64.4	7.5 ± 1.9	6.8 ± 2.7	2.8 ± 1.5	5.2 ± 2.0	0.0 ± 0.1
6–14 September	Veliger	2	0.3 ± 0.0	0.03 ± 0.01	0.01 ± 0.00	42.9 ± 8.1	87.7 ± 8.8	5.1 ± 4.6	1.8 ± 1.5	3.1 ± 1.3	0.0 ± 0.0	2.5 ± 1.5
6–14 September	Egg ribbon	1	–	22.9	1.2	4.9	97.9	2.1	–	–	–	–

Table 3 *L. helicina*. Fatty acid composition (mass, percent) of total lipid, during 20 May–17 September 2001. Means ± SD are percentage values of total fatty acids. Veligers have SD based on only two replicates of 40 and 60 individuals, respectively

Date	20–22 May	26–31 July	5–7 August	17–21 August	6–14 September		
Stage (*n*) Fatty acids	Juvenile (7) Mean ± SD	Female (4) Mean ± SD	Female (8) Mean ± SD	Female (5) Mean ± SD	Female (7) Mean ± SD	Veliger (2) Mean ± SD	Egg ribbons (l)
14:0	3.7 ± 0.9	3.0 ± 0.3	4.0 ± 0.5	3.8 ± 1.1	3.4 ± 1.0	2.9 ± 0.2	4.6
15:0	0.9 ± 0.6	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	1.0 ± 0.3	2.7 ± 0.5	1.2
16:0	13.3 ± 2.2	15.3 ± 2.3	14.7 ± 2.4	15.0 ± 1.2	13.9 ± 1.8	17.0 ± 0.1	16.9
16:1(n-7)	8.9 ± 3.6	1.0 ± 0.1	1.6 ± 0.7	3.7 ± 1.6	1.3 ± 0.6	4.7 ± 0.2	1.6
16:2(n-4)	0.7 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.6 ± 0.8	1.9 ± 0.4	0.4
16:3(n-4)	1.4 ± 1.4	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.9	0.4 ± 0.2	0.2
16:4(n-1)	0.6 ± 0.5	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0
18:0	2.1 ± 0.8	2.0 ± 0.4	3.1 ± 3.4	2.2 ± 0.4	3.0 ± 1.7	10.3 ± 3.8	4.4
18:1(n-9)	2.7 ± 0.8	3.5 ± 0.7	6.0 ± 10.9	2.3 ± 0.4	2.2 ± 0.9	12.1 ± 0.6	3.4
18:1(n-7)	2.1 ± 0.4	0.9 ± 0.1	1.4 ± 0.2	1.9 ± 0.3	1.2 ± 0.4	2.9 ± 0.4	1.8
18:2(n-6)	2.2 ± 0.6	1.5 ± 0.3	2.7 ± 0.5	2.4 ± 0.4	1.7 ± 0.3	4.7 ± 0.1	3.8
18:3(n-3)	1.1 ± 0.4	1.6 ± 0.1	3.1 ± 3.4	2.3 ± 0.4	1.6 ± 0.3	1.2 ± 0.6	1.9
18:4(n-3)	3.8 ± 1.1	2.8 ± 0.8	5.2 ± 2.5	7.5 ± 3.9	5.0 ± 1.6	1.5 ± 0.4	8.6
20:0	0.6 ± 0.4	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.4
20:1(n-9)	1.8 ± 0.5	3.5 ± 0.6	3.0 ± 0.5	2.8 ± 0.5	3.1 ± 0.6	2.2 ± 0.8	3.7
20:1(n-7)	2.4 ± 1.1	2.7 ± 0.2	2.6 ± 0.8	3.2 ± 0.6	2.9 ± 0.3	1.7 ± 0.2	1.5
20:4(n-6)	1.2 ± 0.8	1.0 ± 0.4	0.8 ± 0.4	0.5 ± 0.3	0.8 ± 0.5	0.7 ± 0.1	0.4
20:3(n-3)	0.4 ± 0.4	1.1 ± 0.2	0.8 ± 0.2	0.5 ± 0.4	0.7 ± 0.6	0.0 ± 0.0	0.0
20:4(n-3)	1.4 ± 0.6	1.6 ± 0.3	1.7 ± 0.7	2.0 ± 0.3	2.3 ± 0.5	1.7 ± 1.4	1.7
20:5(n-3)	22.5 ± 3.0	20.0 ± 1.3	20.7 ± 1.9	23.6 ± 1.6	18.8 ± 2.4	9.4 ± 0.6	17.2
22:1(n-11)	0.6 ± 0.8	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0
22:1(n-9)	0.4 ± 0.5	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0
22:5(n-3)	1.7 ± 0.9	3.0 ± 2.6	1.4 ± 0.6	1.7 ± 0.9	2.1 ± 1.1	4.1 ± 3.9	1.0
22:6(n-3)	19.5 ± 6.3	29.6 ± 3.9	26.9 ± 3.3	21.1 ± 5.4	30.8 ± 2.2	15.9 ± 0.7	25.2

The second important lipid class was triacylglycerol (TAG) (Table 2). The proportions of TAG were highly variable throughout all stages and seasons. Juveniles were the richest in TAG (mean 33.6 ± 9.2%). Females in summer exhibited extremely variable TAG proportions. They were low in July (8.9 ± 8.0%) and strongly increased towards mid-August (24.5 ± 24.7%). In September, female TAG was nearly depleted (2.8% on average). TAG in the veligers and egg ribbons was similarly low.

Free fatty acids (FFA) were more abundant than TAG in females from July, accounting on average for nearly 20%. During the other seasons, FFA were lower, being lowest in juveniles and veligers. Sterols were present in all stages with lowest proportions in juveniles and highest proportions in females from early August (Table 2). In addition, small amounts of diacylglycerol ethers (DAGE) were found in most of the animals. Moderate proportions of DAGE occurred in females in early August and September. Wax esters (WE) were negligible in all specimens.

The fatty acid compositions of all samples were dominated by three major fatty acids that included the saturated 16:0, and the polyunsaturates 20:5(n-3) and 22:6(n-3), accounting for about 15, 20, and 30% of total fatty acids, respectively (Table 3). The highest proportions of 22:6(n-3) were found in the PL-rich females in July, early August, and September. In females from mid-August, the 18:4(n-3) fatty acid occurred in proportions of up to 7.5%, and 16:1(n-7) accounted for up to 4%. Females from September were rich in 18:4(n-3), but not in 16:1(n-7). In the juveniles from May, the proportion of 16:1(n-7) was higher than that of 18:4(n-3), exhibiting levels of up to 14.8% (mean of 8.9%) and 3.8%, respectively. The two 20:1 isomers had proportions of about 2 to 3% each and were found in higher amounts in females than in the younger stages. The fatty acid composition of the egg ribbons was almost identical to that of the females from September, at the time when ribbons were released. While 16:0, 20:5(n-3), and 22:6(n-3) were the predominant fatty acids in all stages, 18:4(n-3) was an important fatty acid in females during August, accounting for 5–8% of the total fatty acids (Table 3). The fatty acid composition of the veligers was different from all other samples although the lipid class composition was very similar to that of the females in September. This was due to considerable amounts of fatty acids with 18 carbon atoms, especially of 18:1(n-9), 18:0, and 18:2(n-6), which accounted for 12.1, 10.3, and 4.7%, respectively.

Discussion

Limacina helicina is an important member of the zooplankton community in Arctic and sub-Arctic regions appearing in large swarms or forming aggregates (Kerswill 1940; Percy and Fife 1985; Lalli and Gilmer 1989a). High densities were found in the Greenland Sea, in the area around Svalbard, and in the northern Barents Sea

(Gilmer and Harbison 1991; Falk-Petersen et al. 1999). It is also described as numerous in Kongsfjorden (Weslawski et al. 2000) but appeared only in pulses of low density during our study, which might be due to their patchy distribution (Kerswill 1940; Omori and Hamner 1982; van der Spoel and Dadon 1999).

The population structure changed from veligers and juveniles during spring to adults in July. The size of females increased until late summer and decreased towards autumn. In September, small veligers were again found, indicating spawning in late summer/autumn. High numbers of veligers and juveniles have been observed in Svalbard waters during late autumn and winter observations from October, March, and December (S. Falk-Petersen, H.J. Hirche, and J.E. Søreide, personal communication for the respective months). The size range of *L. helicina* is in accordance with that from studies in other sub-Arctic regions (Paranjape 1968; Lalli and Wells 1978; Gilmer and Harbison 1991), but contrary to specimens found in the Arctic Ocean, which were 2 to 3 times smaller (Kobayashi 1974). Dry mass was variable in females, but increased exponentially with size as did the lipid accumulation. Lipid content was about 2 to 3 times higher in juveniles and veligers than in females, while females showed a relatively constant and low lipid content during summer. Their lipid content was low compared to results by Percy and Fife (1981) who measured values twice as high, but without any additional data on size and dry mass a direct comparison is difficult.

After spawning, the veligers hatch within 2 to 6 days and start feeding immediately (Paranjape 1968; Lalli and Gilmer 1989a). The eggs contain lipids mostly in the form of phospholipids, which are essential for membranes and cell differentiation. A major portion of the phospholipid is certainly associated with membranes, but part of it may be also in the lipovitellins in which phosphatidylcholine is the major lipid component (Lee 1991). Due to their ability to feed directly after hatching, veligers are probably not dependent on neutral lipids provided by the eggs. Instead they immediately utilise food for growth and accumulation of lipids. This is in contrast to, for example, copepods at the same high latitudes, such as C *alanus hyperboreus*, that do not start feeding until late naupliar stages and, thus, the first stages rely on internal lipid reserves (Conover and Huntley 1991; Hirche and Niehoff 1996). On the other hand, the eggs of *L. helicina* might have been released not fully developed, as a reaction to stress during collection and measurements. The egg ribbons in our study had a much lower number of eggs compared to observations by Paranjape (1968).

Fatty acids have been used as qualitative markers to trace or confirm predator–prey relationships through food webs in the marine environment (Falk-Petersen et al. 1990; Graeve et al. 1994; Dalsgaard et al. 2003). In veligers, saturated and unsaturated fatty acids with 18 carbon atoms were present in clearly enhanced amounts as compared to the other stages. These fatty acids indicate the dietary input of particulate organic matter (Kattner et al. 1998; Scott et al. 2002a) and are more resistant to decomposition in the water column than, for example, polyunsaturates (Hama 1999). The high amount of particulate fatty acids marker shows that *L. helicina*, especially the veligers, has the ability to incorporate and utilise particulates as a major food source, which is supported by Kobayashi (1974). We further suggest that veligers develop into young juveniles during winter and towards spring due to this food source.

Juveniles, found in May, were the lipid-richest of all analysed specimens, except veligers. The high amount of TAG was likely caused by feeding on the spring diatom bloom, which occurs at that time in Kongsfjorden (H. Hodal, unpublished). The utilization of this food source was clearly reflected in the fatty acid composition. High levels of the 16:1(n-7) and enhanced 20:5(n-3) fatty acid are unequivocal indicators for feeding on diatoms. Even higher proportions of these fatty acids were reported for specimens of *L. helicina* from the Greenland Sea that were especially enriched in TAG (Kattner et al. 1998). High levels of the 22:6(n-3) fatty acid were found in all *L. helicina* samples, and they were highest in the phospholipid-rich specimens. This fatty acid generally occurs in high proportions in marine phospholipids (Albers et al. 1996). Lee (1974, 1975) also reported that several species of Arctic zooplankton have high levels of 22:6(n-3) in their polar lipid [e.g. *Themisto abyssorum* (24%), *Pareuchaeta glacialis* (40%), and *Calanus hyperboreus* (31–43%)].

The development of juveniles into mature females requires continuous input. Thus, the high lipid deposits in juveniles are most likely utilised for growth and maturation, which explains the clearly reduced TAG deposits in females from July. However, considerable amounts of FFA were detected. This fraction may be accumulated from TAG cleavage, but also from the diet. High amounts of FFA were also found in *L. helicina* from Antarctica in summer (Phleger et al. 1997). As a possible reason these authors suggested increased lipase activity, due to a prolonged time of sorting and counting in a heated van prior to freezing. Because this was not the case in our study, we propose that the occurrence of FFA is a regular process during gonad development. Similarly high FFA proportions were found in *Clione limacina* during seasonal studies (Falk-Petersen et al. 2001; Böer et al., submitted) but do not seem to be a specific process in pteropods only. There are some reports concerning the occurrence of FFA in different species during various stages of development, but the role of FFA in the metabolic processes is not sufficiently resolved.

The accumulation of TAG in females during August shows that TAG is an essential energy source for egg production. The TAG proportions are extremely variable, which suggests that August is the peak spawning period, displaying females in different stages of egg production and spawning, as well as spawned-out females. The same high variability in TAG was found by

Falk-Petersen et al. (2001). The spawning period was almost completed in September, deduced from the low levels of neutral lipids in the females. The lipid profile and also the disappearance of the larger females are clear indications that females generally die shortly after spawning, although some will survive autumn and may over-winter.

The dietary input during the summer and autumn was clearly reflected in the fatty acid compositions of the females of *L. helicina*, which were characterised by higher proportions of 18:4(n-3) and a deficiency in 16:1(n-7) as compared to juveniles from spring. This change shows a shift from a diatom-dominated diet in May, to a diet containing summer plankton communities, such as dinoflagellates, in Kongsfjorden (Hop et al. 2002). The occurrence of dietary fatty acids is more clearly reflected in the fatty acid composition of the triacylglycerols as already shown by Kattner et al. (1998) and Falk-Petersen et al. (2001). The latter found high variations in 18:4(n-3) accumulation between animals collected in two different years. The fatty acid composition of the storage lipids reacts fast to changes in diet (Graeve et al. 1994). The 18:4(n-3) was also found in higher proportions in the egg ribbons, which show that dietary fatty acids may also be directly used for egg production.

The occurrence of typical phytoplankter fatty acids is, however, no argument for feeding exclusively on phytoplankton. As described by Gilmer and Harbison (1991), a considerable part of the diet of *L. helicina* during mid-summer in the Arctic consisted of zooplankton and juvenile *L. helicina*. Thus, the fatty acid composition should also reflect the ingestion of these larger species. The small amounts of 20:1 isomers in females may indicate feeding on calanoid copepods. These 20:1 isomers are most abundant in *Calanus finmarchicus* and *C. glacialis* (Kattner and Hagen 1995; Scott et al. 2002b), which were the dominant copepods in Kongsfjorden (Kwasniewski et al. 2003). Similar proportions of these monounsaturates were also found in *L. helicina* from Arctic and Antarctic waters (Kattner et al. 1998; Falk-Petersen et al. 2001). However, the relatively low amount of these fatty acid markers contradicts previous findings by Gilmer and Harbison (1991) and Falk-Petersen et al. (2001) and could suggests that calanoid copepods are not as important a part of the diet as previously assumed. It is also possible that *L. helicina* is unable to store these long chain monounsaturates, but rather catabolises them for energy demands.

Conclusions

The amounts and changes in lipids in *L. helicina* are a result of developmental stage, reproductive status, and food availability. Based on the fatty acid composition, we suggest that *L. helicina* is a true omnivore feeding on available particulates, ranging from a phytoplankton-based diet in spring and summer to degraded organic material in late autumn and winter. Our results reveal that *L. helicina* has adapted to the polar environment of Kongsfjorden by developing a reproductive strategy with peak spawning during late summer and autumn. During autumn, lipid-rich veligers develop and over-winter as veliger and/or juveniles. This strategy differs from other polar zooplankton species, which more often tend to over-winter as lipid-rich eggs, well-developed stages, or females, to enable spawning prior to the spring bloom with hatching timed to the seasonal bloom. We propose a 1-year life cycle for *L. helicina* in the coastal regions around Svalbard, in contrast to previous estimates of 1.5 to 2 years (Kobayashi 1974). This conclusion is especially based on the strong depletion of lipids in females and the relative high presence of lipids in veligers, but also on the disappearance of larger specimens in late September.

Acknowledgements We thank the captain and crew of "R.V. Lance", "R.V. Håkon Mosby", and "R.V. Oceania", the staff at Kings Bay Research Facility for their professional support, and Audun Igesund for providing the map. The work was supported by the Personnel Exchange Programme between the Norwegian Research Council and Deutscher Akademischer Austauschdienst (DAAD).

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