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Lipids and fatty acids in *Clione limacina* and *Limacina helicina* in Svalbard waters and the Arctic Ocean: trophic implications

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Abstract Lipid class and fatty acid compositions were determined in *Limacina helicina* and *Clione limacina* from an Arctic fjord and the marginal ice zone around Svalbard. *C. limacina* had higher levels of neutral lipids, including both alkyldiacylglycerols (ADG) and triacylglycerols (TAG), than *L. helicina*, which contained mainly TAG. However, considerable heterogeneity in the lipid classes and their fatty acids/alcohols were observed in *C. limacina* in that only two out of the seven specimens analysed were lipid-rich and contained both ADG and TAG, the others having only low percentages of TAG. In specimens of *C. limacina* containing ADG, 15:0 and 17:1*n*-8 were prominent fatty acids in both ADG and TAG. The fatty acids of the TAG of *L. helicina* were variable but 15:0 and 17:1*n*-8 were absent. We consider the heterogeneity in the fatty acid compositions of *L. helicina* to reflect temporal and spatial variability in the animals' predominantly phytoplanktonic and particulate diet, which occasionally includes small copepods. We further consider *L. helicina* to be the prime food for *C. limacina* and the noticeable amounts of 22:1 found in one sample of *C. limacina* to reflect significant input of *Calanus* either directly or indirectly through their prime food, *L. helicina*. We view the heterogeneity in the fatty acid compositions of both *L. helicina* and *C. limacina,* as well as the ability of *C. limacina* to biosynthesise WE, ADG, 15:0, and 17:1*n*-8, as adaptations to a large variation of food availability that enables *C. limacina* to synthesise lipids rapidly and flexibly. Thus, the lipid biochemistry of *C. limacina* is important in enabling the species to thrive in strong pulses in polar systems.

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Introduction

Arctic marine pelagic systems are characterised by large variations in abiotic environmental parameters including pronounced seasonal oscillations in incident global radiation, and also pronounced climatic variations on hourly to decadal and even longer time scales which are reflected in dramatic changes in the ice regime (Romanov 1995; Proshutinsky et al. 1999; Falk-Petersen et al. 2000a; Vinje 2000). The major components of polar zooplankton, the pelagic copepods and euphausiids, have adapted to these variations by developing biochemical pathways enabling them to accumulate large lipid stores, predominantly wax esters (Lee 1975; Falk-Petersen 1988; Hagen 1988; Sargent and Falk-Petersen 1988; Falk-Petersen et al. 2000b). Other, less well-studied members of the polar zooplankton include the pelagic pteropods that are particularly interesting because they occur in pulses with high abundance and in close association with oscillations of their main food or prey source. The pteropods have developed high feeding and growth rates and rapid reproductive responses as an adaptation to the fluctuating polar environment (Lalli and Gilmer 1989; Gilmer and Harbison 1991).

Limacina helicina and *Clione limacina* are prominent Arctic pteropods, being found in mass occurrences during late summer and autumn (Smidt 1979). The thecosomate *L. helicina* has been described as a pure herbivore (Gilmer 1974) but studies by Gilmer and Harbison (1991) have shown that the species is an omnivore, with small copepods and juvenile forms of *L. helicina* accounting for up to half the food mass in its digestive tract. Previous studies have strongly indicated that the pteropod *C. limacina*, an active predator with a fast-strike feeding response

(Hermans and Satterlie 1992), feeds exclusively on *L. helicina* (Conover and Lalli 1974; Lalli and Gilmer 1989) The present study is part of a large programme investigating energy transfer and trophic relationships in marine food webs in Svalbard waters and the Arctic Ocean (Falk-Petersen et al. 2000a) that includes applying lipid and fatty acid analyses to illuminate trophic relationships. We were motivated to undertake the present study because previous analyses of *C. limacina* revealed the presence of high levels of relatively unusual alkyldiacylglycerols and the very unusual fatty acid 17:1*n*-8 (Pfleger et al. 1997; Kattner et al. 1998).

Materials and methods

Sampling

The present study was carried out as a part of the NP ICE-BAR and BIODAFF programmes and UNIS (University Courses of Svalbard) cruises to the Arctic Ocean (Polhavet).

In 1997, sampling was carried out in Kongsfjorden, Svalbard (N78°57′ E11°50′). The samples were collected from 24 August to 22 September 1997 in small beakers from the surface, using a small boat. Individual animals were kept alive in cold water during transportation, transferred directly into chloroform:methanol (2:1 v/v) on arrival a few hours later in the laboratory at Ny-Aalesund, and stored at −20 °C. There was no autumn bloom in 1997 but phytoplankton and food availability were good, as indicated by the abundance of faecal pellets in the samples. Samples coded 172, 173 and 192 were collected in the inner part of the fjord while samples coded 164, 165, 166 and 186 were collected in the outer part of the fjord.

In 1998, the UNIS cruise to the Arctic Ocean was undertaken by R/V *Jan Mayen* of the University of Tromsø from 9 to 22 September. Samples were taken in Kongsfjorden (K), in the Arctic Ocean at ice station 1 (N81°30′ E29°12′) and at ice station 2 (N80°55′ E15°03′). A late autumn phytoplankton bloom was observed at ice station 1, while there were very low phytoplankton levels at ice station 2 and in Kongsfjorden. Pelagic zooplankton was collected at each station with a WP-3 net (120 cm opening diameter, 1,000 μm mesh size) or a Tucker Trawl (1 m^3 opening, 1,000 μm mesh size) in the upper 50 m. Live animals for lipid analyses were immediately identified to species, dropped into chloroform:methanol (2:1, v/v) contained in Teflon-capped glass vials and stored at −20 °C.

Laboratory analyses

Total lipid was extracted from the samples stored in chloroform:methanol (2:1, v/v) by the method of Folch et al. (1957), dried under nitrogen and then under vacuum for 24 h, and weighed. The class composition of the total lipid was measured by quantitative thin-layer chromatography (TLC) and densitometry, as described by Olsen and Henderson (1989). Triacylglycerols and wax esters were separated on TLC silica gel plates using hexane:diethyl ether:acetic acid (90:10:1, v/v/v). The resultant lipid classes, as well as total lipid from each sample, were supplemented with a known amount of the fatty acid 21:0 as internal standard and transmethylated in methanol containing 1% sulphuric acid with toluene for 16 h at 50 °C. The reaction products were extracted into ether, dried under nitrogen and subjected to TLC in hexane:diethyl ether:acetic acid (70:30:1,v/v/v) to separate fatty acid methyl esters and free fatty alcohols. These were recovered from the plates, and the fatty alcohols converted to acetate esters by reacting with acetic anhydride in pyridine (Farquhar 1962). Fatty acid methyl esters and fatty alcohol acetates were identified and quantified by capillary gas-liquid chromatography, by comparison with the internal standard, as detailed previously (Falk-Petersen et al. 1999). Peaks were identified by reference to samples of known composition and by gas chromatography-mass spectrometry (GC-MS) using a Fisons MD 800 fitted with a DB-5MS column (15 m × 0.25 mm i.d.; J&W Scientific) using helium as a carrier gas. Fatty acids were also characterised by GC-MS after producing dimethyl disulphide adducts of monounsaturated fatty acids (Nichols et al. 1986) and diethylamide derivatives of polyunsaturated fatty acids (Nilsson and Liljenberg 1991).

Results

Four samples of *L. helicina* were collected from four stations in Kongsfjorden in 1997, and four from one station in Kongsfjorden and two ice stations in 1998. *C. limacina* was collected from three of the stations in Kongsfjord in 1997, and from the one station in Kongsfjord and the two ice stations in 1998 (Table 1).

Limacina helicina

Lipid classes

Three of the *L. helicina* samples collected in Kongsfjord in 1997 were very similar in having polar lipid (PL) as their major lipid with modest amounts of triacylglycerols (TAG) and traces of wax esters (WE). The fourth sample of *L. helicina* collected in Kongsfjord in 1997 differed from the other three in having small but significant amounts of alkyldiacylglycerols (ADG), as well as modest amounts of TAG (Table 1). This sample was collected from the outer regions of the fjord, whereas the other samples were collected from the inner fjord. Of the four samples of *L. helicina* collected in 1998, one collected in Kongsfjord and the one collected at ice station 2 were similar to each other, and also to the majority of the samples collected in 1997, in having modest amounts of TAG. The other two were quite different. Thus, one sample collected at ice station 1 had 57% of its total lipid as TAG; the other collected in Kongsfjord had ADG as its major neutral lipid (23% of the total). In general, the levels of neutral lipids were higher in the samples collected in 1997 than in 1998, although considerable variation occurred in both years.

Clione limacina

Lipid classes

Few individuals of *C. limacina* were available for analyses but, even so, the variability in lipid class analyses in this species was clearly greater than in *L. helicina* (Table 1). Thus, of the three individuals of *C. limacina* collected in Kongsfjord in 1997, one contained circa 20% each of TAG and ADG, one had circa 20% of TAG and only 6% of AGD, and the other had only 10% TAG and very little ADG. All three individuals

Table 1 Lipid class compositions (% total lipid) of *Limacina helicina* and *Clione limacina* (*TL* total lipid; *PL* polar lipid; *TAG* triacylglycerols; *ADG* alkyldiacylglycerols; *WE* wax esters; *tr* trace). Sites and number of specimens analysed (e.g. $n=5$) are specified below species headings in the *left hand column*. Data are means ± SD

Species	Code	PL	Sterols	TAG	ADG	WE
1997						
Limacina helicina						
$n=5$	172	57.3	9.8	9.3	6.6	0.9
L. helicina						
$n=4$	192	71.9	11.5	8.3	0.0	1.5
L. helicina						
$n=5$	164	71.2	10.6	11.3	0.0	1.3
L. helicina						
$n=5$	186	75.6	10.6	6.3	0.0	0.0
Mean ± SD		69.1 ± 8.1	10.6 ± 0.7	8.8 ± 2.1	1.6 ± 3.3	0.9 ± 0.6
1998						
L. helicina						
$n=5$	314	31.4	14.5	11.6	23.0	2.4
L. helicina						
$n=5$	316	63.8	10.2	13.3	0.0	0.0
L. helicina						
Ice 1, $n=1$	364	19.2	7.1	57.0	0.0	0.0
L. helicina						
Ice 2, $n=1$	418	60.4	8.7	9.0	tr	0.0
Mean ± SD		43.7 ± 21.9	10.1 ± 3.2	22.7 ± 22.9	7.7 ± 13.3	0.6 ± 1.2
1997						
Clione limacina						
$n=1$	173	47.6	9.9	23.3	6.0	4.0
C. limacina						
$n=1$	165	31.4	7.6	20.2	22.7	8.6
C. limacina						
$n=1$	166	52.9	12.2	10.4	1.3	6.8
Mean ± SD		44 ± 11.2	5.9 ± 2.3	17.9 ± 6.7	10 ± 11.2	6.5 ± 2.3
1998						
C. limacina						
$n=2$	318	55.9	13.9	14.6	0.0	0.0
C. limacina						
Ice 1, $n=1$	333	56.8	12.0	10.2	0.0	0.0
C. limacina						
Ice 2, $n=1$	417	22.0	5.5	32.7	31.4	tr
Mean ± SD		44.9 ± 19.8	10.4 ± 4.4	19.1 ± 11.9	10.5 ± 18.1	0.0

had small amounts of WE, with the highest level (9%) present in the individual rich in ADG. In 1998, the individual from ice station 2 had equal and high amounts (circa 30%) of TAG and ADG, while individuals from ice station 1 and Kongsfjord had only modest levels of TAG and no detectable ADG. None of the individuals collected in 1998 had WE. In general, the *C. limacina* individuals were more lipid-rich than the *L. helicina* samples, as evidenced by their total lipid containing higher percentages of neutral lipids. Overall, however, the most striking feature in the data in Table 1 is the large variation in neutral lipid, especially ADG, between individual specimens.

Fatty acids

Fatty acid analyses were carried out on PL, TAG, ADG and WE. For PL, only the means ± SD for all the samples over the 2 years for each species are shown because, as evidenced by the small SD in Table 2, only small variations occurred between individuals of a given species, irrespective of site or year of sampling. This was expected since the fatty acid compositions of cellular phospholipids are relatively little influenced by diet, being very largely determined genetically. In passing, the marked similarity between individual samples, especially for the large numbers analysed for *L. helicina*, confirms the precision and reproducibility of the sampling, processing and analytical methods used. Less expected was the marked similarity between the PL of the two species. Thus, both have the same percentage of the major fatty acids, 22:6*n*-3 and 20:5*n*-3 in a ratio of somewhat less than 3:2.

Limacina helicina

The fatty acid compositions of the TAG from the various samples of *L. helicina* are shown in Table 3. The four samples analysed in 1997, all from Kongsfjorden, were generally similar. Thus, all were consistently rich in *n*-3 polyunsaturated fatty acids (PUFA) (circa 40% of the total), with 18:4*n*-3, 20:5*n*-3 and 22:6*n*-3 all being

Table 2 Mean fatty acid compositions of polar lipid from *Limacina helicina* and *Clione limacina*. Data are means of weight % ± SD of total fatty acids, with $n = 4$ for *L. helicina* and $n = 3$ for *C. limacina*. The individual samples analysed were samples coded 172 ($n = 5$), 192 ($n = 4$), 164 ($n = 5$) and 186 ($n = 5$) of *L. helicina* and samples coded 173 ($n = 1$), 165 ($n = 1$) and 166 ($n = 1$) of *C. limacina* in Table 1

Fatty acid	*L. helicina*	*C. limacina*
14:0	3.3 ± 0.1	0.9 ± 0.6
16:0	12.9 ± 0.7	9.6 ± 0.9
16:1*n*-7	1.0 ± 0.1	1.8 ± 0.7
18:0	2.0 ± 0.1	4.8 ± 0.7
18:1*n*-9	1.7 ± 0.1	1.9 ± 0.3
18:1*n*-7	1.0 ± 0.1	2.2 ± 0.5
18:2*n*-6	1.5 ± 0.1	0.6 ± 0.2
18:3*n*-3	1.0 ± 0.9	0.6 ± 0.4
18:4*n*-3	4.4 ± 0.5	1.6 ± 0.9
20:1*n*-9	2.8 ± 0.4	2.5 ± 0.2
20:1*n*-7	4.1 ± 0.7	3.0 ± 0.1
20:2*n*-6	2.2 ± 0.1	1.6 ± 0.1
20:4*n*-6	0.7 ± 0.1	1.5 ± 0.2
20:4*n*-3	1.9 ± 0.2	0.7 ± 0.2
20:5*n*-3	21.5 ± 0.6	22.3 ± 1.8
22:5*n*-3	0.9 ± 0.1	0.9 ± 0.1
22:6*n*-3	28.2 ± 2.6	28.6 ± 0.6
sat	21.2 ± 0.7	21.9 ± 0.3
mono	11.4 ± 1.3	15.0 ± 1.7
n-6	5.2 ± 0.7	4.2 ± 0.2
n-3	58.7 ± 1.9	55.0 ± 1.9

prominent. The major saturated and monounsaturated fatty acids were, respectively, 16:0 and 18:1*n*-9, with 18:1*n*-7 being relatively minor. These analyses are consistent with the *L. helicina* in 1997 consuming substantial amounts of phytoplankton composed mainly of flagellates and to lesser extent diatoms.

The samples of *L. helicina* analysed in 1998 are more heterogeneous than those sampled in 1997 (Table 3). Thus, of the two samples taken from the same site in Kongsfjorden and separated by only a few hours, one is much richer in monounsaturated fatty acids, largely due to high percentages of 16:1*n*-7 and 18:1*n*-9, while the other is richer in *n*-3 PUFA, due to elevated percentages of 22:6*n*-3, 20:5*n*-3 and 18:4*n*-3. Interestingly, both samples have notable amounts of 20:1*n*-9 and 22:1*n*-11, which are considered markers of calanoid copepods. The sample from ice station 1 is the richest in 20:5*n*-3 of all the samples of *L. helicina* analysed and it also has notable amounts of 18:4*n*-3 and C16 PUFA, features well accounted for by the diatom-dominated bloom occurring at ice station 1. The sample from ice station 2 has elevated levels of 22:6*n*-3, 20:5*n*-3 and 18:4*n*-3, together with the highest percentages of 18:0 and 18:2*n*-6 recorded of all the samples. This sample is possibly the least nutritionally replete of all the *L. helacina* samples analysed.

Clione limacina

Fatty acid analyses of the TAG in *C. limacina* show even more variation than found for *L. helicina* (Table 4). Thus, the three samples of *C. limacina* from Kongsfjord in 1997 all differ notably. First, the specimen rich in both TAG and ADG is striking in having large percentages of both 15:0 and 17:1*n*-8, and also a small but notable percentage of 17:0. However, *n*-3 PUFA are relatively minor in this sample with 22:6*n*-3 and 20:5*n*-3 (in a ratio of circa 3:2), but not 18:4*n*-3, being well represented. Second, the specimen with a modest level of ADG lacks 15:0 and 17:1*n*-8 but has higher percentages of 22:6*n*-3 and 20:5*n*-3 (again in a ratio of circa 3:2) In addition, this specimen has notable amounts of the *Calanus* markers 20:1*n*-9 and 22:1*n*-11 in a ratio of circa 2:1. Third, the specimen with very small amounts of ADG lacks 15:0 and 17:1*n*-8 but is particularly rich in *n*-3 PUFA, including 22:6*n*-3, 20:5*n*-3 and 18:4*n*-3. This specimen is substantially richer in fatty acid markers of mixed diatoms and flagellates (including *Phaeocystis*). The TAG in the specimens of *C. limacina* from 1998 again show heterogeneous fatty acid compositions (Table 4). The specimen from ice station 2, which is rich in both TAG and ADG, is striking in containing high percentages of 15:0 and 17:1*n*-8 and a significant percentage of 17:0. The specimen of *C. limacina* from Kongsfjord in 1998 with zero ADG is particularly rich in 22:6*n*-3 and 20:5*n*-3 in a ratio of 3:2, these two fatty acids accounting for more than one-third of the total; 18:4*n*-3 is a minor fatty acid in this sample, which contains small amounts of 20:1*n*-9 but little 22:1*n*-11. Finally, the TAG of *C. limacina* from ice station 1 in 1998 has notably higher percentages of both 18:1*n*-9 and 16:1*n*-7, notably lower percentages of 20:5*n*-3 and 22:6*n*-3, and small amounts of 20:1*n*-9 and 22:1*n*-11.

Table 5, showing the fatty acid composition of ADG and WE, reveals that the unusual combination of 15:0, 17:0 and 17:1*n*-8 found in the TAG of the two *C. limacina* samples rich in both TAG and ADG, applies also to the fatty acids of the ADG and the traces of WE. The fatty acids of the WE but not the ADG in the *C. limacina* from ice station 2 contain notable amounts of 20:1*n*-9 and 22:1*n*-11 in a ratio of circa 2:1. However, the WE and especially the ADG contain quite high percentages of 22:6*n*-3 and, to a lesser extent, 20:5*n*-3. The fatty acids of the ADG of *L. helicina* contain only very small percentages of 15:0 and 17:0 and no 17:1*n*-8 (Table 5). The chief fatty acids in the ADG of *L. helicina* are 16:0, 20:1*n*-9 and 22:6*n*-3, of which only 22:6*n*-3 is abundant in the ADG of *C. limacina*.

Discussion

Kattner et al. (1998) unequivocally identified large amounts of the unusual fatty acid 17:1*n*-8 in *C. limacina* from both the Arctic and the Antarctic. These authors proposed that 17:1*n*-8 is biosynthesised by a Δ-9 fatty acid desaturase acting on 17:0, which is formed by chain-elongating 15:0, which is biosynthesised de novo by the animal from an unidentified 3C (propionyl) initiator. The present study confirms the finding of Kattner

Table 3 Fatty acid composition of triacylglycerols of *Limacina helicina*. Data are weight % of total fatty acids. Codes and stations are as detailed in Table 1 (*No*. numbers of animals pooled for analyses at individual stations). *Mean* refers to numbers of stations analysed in each year

	1997					1998				
Code	172	192	164	186		314	316	364	418	
Station No.	5	4	5	5	Mean ± SD	5	5	Ice1 1	Ice2 1	Mean ± SD
14:0	3.4	3.2	1.3	3.6	2.9 ± 1.1	10.2	5.0	5.4	3.5	6.0 ± 2.9
16:0	13.8	13.1	13.0	16.2	14.0 ± 1.5	17.4	20.0	10.9	17.7	16.5 ± 3.9
16:1*n*-9	0.0	0.8	0.7	1.0	0.6 ± 0.4	0.4	0.6	0.0	5.7	1.7 ± 2.7
16:1*n*-7	6.1	4.9	5.5	5.6	5.5 ± 0.5	18.1	7.8	33.9	5.3	16.3 ± 13.0
C16 PUFA	0.8	0.0	0.0	0.0	0.2 ± 0.4	0.5	0.4	5.7	0.4	1.8 ± 2.6
17:0	1.3	0.7	1.1	1.7	1.2 ± 0.4	0.0	0.9	0.0	2.7	0.9 ± 1.3
17:1*n*-8	0.0	0.0	0.0	0.0	0.0 ± 0.0	0.0	2.2	0.2	4.8	1.8 ± 2.2
18:0	1.7	2.1	2.5	3.2	2.4 ± 0.6	2.7	3.5	0.2	5.0	2.9 ± 2.0
18:1*n*-9	7.9	7.6	10.8	11.8	9.5 ± 2.1	16.0	0.0	1.4	11.2	7.1 ± 7.7
18:1*n*-7	2.7	2.9	3.6	3.4	3.2 ± 0.4	3.5	3.5	2.4	3.0	3.1 ± 0.5
18:2*n*-6	4.4	4.6	4.4	5.7	4.8 ± 0.6	2.6	5.5	1.2	7.4	4.2 ± 2.8
18:3*n*-3	4.4	3.7	4.2	4.3	4.1 ± 0.3	0.7	1.6	1.0	0.9	1.0 ± 0.4
18:4*n*-3	18.9	14.1	13.8	13.5	15.1 ± 2.6	1.0	5.3	11.1	0.5	4.5 ± 4.9
20:1*n*-9	3.9	3.8	6.1	4.2	4.5 ± 1.1	8.3	8.2	0.5	2.6	4.9 ± 4.0
20:1*n*-7	1.4	5.1	2.2	1.3	2.5 ± 1.8	2.6	1.7	0.0	1.8	1.5 ± 1.1
20:4*n*-3	1.3	1.1	1.4	1.2	1.3 ± 0.1	0.2	0.7	0.5	0.0	0.4 ± 0.3
20:5*n*-3	9.9	12.6	11.8	8.7	10.8 ± 1.8	3.9	8.3	19.0	5.4	9.2 ± 6.8
22:1*n*-11	0.0	0.3	1.1	0.3	0.4 ± 0.5	2.8	5.4	0.0	0.6	2.2 ± 2.4
22:1*n*-9	0.3	0.3	0.6	0.0	0.3 ± 0.2	0.8	0.9	0.0	0.8	0.6 ± 0.4
22:5*n*-3	0.5	0.5	0.5	0.4	0.5 ± 0.1	0.2	0.5	0.2	0.4	0.3 ± 0.1
22:6*n*-3	9.5	10.6	9.9	7.7	9.4 ± 1.3	5.1	9.1	3.1	9.9	6.8 ± 3.3
Sat.	23.7	22.0	20.4	26.3	23.1 ± 2.5	30.9	34.8	18.0	33.7	29.3 ± 7.7
Mono.	24.3	27.7	32.3	31.1	28.8 ± 3.6	53.1	31.8	39.3	37.3	40.4 ± 9.1
n-6	5.5	5.7	5.4	6.9	5.9 ± 0.7	4.4	7.2	1.8	10.2	5.9 ± 3.6
n-3	45.0	43.1	42.0	35.8	41.5 ± 4.0	11.1	25.5	34.9	17.2	22.2 ± 10.3

et al. (1998) that the unusual 17:1*n*-8 is present together with 15:0 in both the TAG and ADG of *C. limacina*, and extends that finding by showing that both of these fatty acids are present in the small amounts of WE present in the species. It follows that 15:0 and 17:1*n*-8 biosynthesised de novo by *C. limacina* are esterified into WE within the animal. Both ADG and WE require fatty alcohols as well as fatty acids for their biosynthesis, to form the alkyl units in the ether and ester linkages, respectively, in these lipids. Pfleger et al. (1997), who first identified substantial amounts of ADG in *C. limacina*, established that the alkyl groups of the ADG in the species were rich in 15:0 and 16:0 but not 17:1*n*-8 units. Therefore, *C. limacina* is capable of biosynthesising its ADG de novo, a deduction that applies equally to its WE. Given that *C. limacina* is relatively lipid-rich, it clearly has an extensive ability to biosynthesise both acyl and alkyl-containing neutral lipids de novo. ADGs were also detected in minor to moderate amounts in two of the *L. helicina* samples analysed here. However, neither the ADG nor the TAG of these samples of *L. helicina* contained 15:0, 17:0 or 17:1*n*-8 fatty acids in significant amounts. ADGs were not detected in the various samples of Arctic and Antarctic *L. helicina* analysed by Kattner et al. (1998).

C. limacina is considered to feed predominantly, if not exclusively, on *L. helicina* (Conover and Lalli 1974; Lalli and Gilmer 1989) so that a close correspondence between at least some of the lipids of the two species may be expected. This is certainly the case for the PL of the two species whose fatty acid compositions show a very close correspondence (Table 2). However, there is little or no correspondence between the fatty acid compositions of the neutral lipids of those specimens of *C. limacina* rich in ADG and the mainly polar lipid of *L. helicina*. This can be accounted for by the marked ability of *C. limacina* to biosynthesise neutral lipids, by definition from sources other than lipid in its diet and presumably mainly from dietary protein or carbohydrate. However, only two of the seven specimens of *C. limacina* analysed in the present study contained substantial amounts of ADG (and TAG) (Table 1). Those specimens of *C. limacina* lacking ADG still had significant amounts of TAG (Table 1) whose fatty acid compositions were surprisingly variable (Table 3). Thus, the TAG of one of the specimens (from Kongsfjord in 1997) had noticeable amounts of 20:1*n*-9 and 22:1*n*-11, conventionally considered biomarkers for calanoid copepods (Sargent and Whittle 1981; Falk-Petersen et al. 1990), indicating a significant dietary input of these animals. This also applies, albeit to a lesser extent, to the TAG of one specimen (with zero ADG) from Kongsfjord in 1998, to the TAG of the specimen from ice station 1 in 1998 (Table 3) and to the traces of WE in the ADG-rich specimen from ice station 2 in 1998. In addition, the TAG of the specimen with zero ADG from

Table 4 Fatty acid compositions of triacylglycerols of *Clione limacina*. Data are weight % of total fatty acids. Codes and stations are as detailed in Table 1 (*No*. numbers of animals pooled for analyses at individual stations). *Mean* refers to numbers of stations analysed in each year

	1997				1998			
Code	173	165	166		318	333	417	
Station No.	1	1	1	Mean ± SD	2	Ice1 1	Ice2 1	Mean ± SD
14:0	3.4	2.1	2.8	2.7 ± 0.6	1.4	4.5	2.7	2.8 ± 1.6
15:0	2.3	13.8	1.3	5.8 ± 7.0	0.8	1.2	7.9	3.3 ± 4.0
16:0	18.0	10.5	16.6	15.0 ± 4.0	19.7	16.9	11.5	16.0 ± 4.1
16:1*n*-9	4.0	0.0	0.0	1.3 ± 2.3	0.0	1.6	0.0	0.5 ± 0.9
16:1*n*-7	7.6	10.3	8.6	8.8 ± 1.4	3.7	14.3	11.3	9.8 ± 5.5
C16 PUFA	0.0	0.0	0.0	0.0 ± 0.0	0.0	1.2	0.0	0.4 ± 0.7
Iso17:0	0.8	0.7	0.8	0.8 ± 0.1	4.1	0.5	2.7	2.4 ± 1.8
17:0	2.8	5.2	2.9	3.6 ± 1.3	0.0	0.0	3.5	1.2 ± 2.0
17:1*n*-8	2.1	20.3	3.0	8.5 ± 10.3	0.0	0.0	13.4	4.5 ± 7.7
18:0	3.9	0.9	0.0	1.6 ± 2.0	4.2	5.2	1.3	3.6 ± 2.0
18:1*n*-9	10.0	3.3	6.9	6.7 ± 3.4	7.0	14.8	3.7	8.5 ± 5.7
18:1*n*-7	4.1	5.4	4.0	4.5 ± 0.7	2.7	4.0	4.5	3.7 ± 0.9
18:2*n*-6	1.7	0.3	2.0	1.4 ± 0.9	2.2	6.3	0.7	3.1 ± 2.9
18:3*n*-3	0.8	1.8	2.1	1.6 ± 0.7	1.4	1.0	1.4	1.3 ± 0.2
18:4*n*-3	1.9	0.4	7.6	3.3 ± 3.8	2.5	2.0	1.2	1.9 ± 0.6
20:1*n*-9	7.1	2.1	2.7	3.9 ± 2.7	4.5	4.8	2.6	4.0 ± 1.2
20:1*n*-7	2.7	2.5	2.2	2.5 ± 0.2	3.1	1.7	3.1	2.6 ± 0.8
20:2*n*-6	1.1	1.3	1.2	1.2 ± 0.1	1.3	0.4	1.2	1.0 ± 0.5
20:4*n*-6	0.0	0.0	0.0	0.0 ± 0.0	0.4	0.3	0.6	0.4 ± 0.2
20:4*n*-3	0.8	0.3	1.5	0.9 ± 0.6	1.0	0.4	0.4	0.6 ± 0.4
20:5*n*-3	7.5	5.5	12.9	8.6 ± 3.9	14.2	6.5	9.0	9.9 ± 3.9
22:1*n*-11	3.7	0.0	0.0	1.2 ± 2.1	1.2	1.8	0.0	1.0 ± 0.9
22:1*n*-9	0.0	0.0	0.0	0.0 ± 0.0	0.7	0.0	0.0	0.2 ± 0.4
22:5*n*-3	0.4	0.4	0.6	0.5 ± 0.1	0.5	0.3	0.5	0.4 ± 0.1
22:6*n*-3	10.9	9.1	15.1	11.7 ± 3.1	22.0	5.1	11.7	13.0 ± 8.5
Sat.	31.2	33.3	26.7	30.4 ± 3.3	30.2	30.0	32.1	30.8 ± 1.1
Mono.	42.6	46.4	29.5	39.5 ± 8.9	24.1	45.8	40.2	36.7 ± 11.3
n-6	3.2	1.6	3.2	2.6 ± 0.9	4.1	7.6	2.7	4.8 ± 2.5
n-3	23.1	18.7	40.6	27.5 ± 11.6	41.6	15.4	24.3	27.1 ± 13.3

Kongsfjord in 1998 was very rich in *n*-3 PUFA, mainly 22:6*n*-3 and 20:5*n*-3. The specimen from Kongsfjord in 1997 (with low ADG) was equally rich in *n*-3 PUFA but in this case 22:6*n*-3, 20:5*n*-3 and 18:4*n*-3 were all well represented.

We believe that the observed heterogeneity in the fatty acid compositions of the neutral lipid in *C. limacina* can be accounted for by variations both in the quantity and quality of its dietary lipid. Thus, *C. limacina* containing both ADG and TAG is clearly rich in neutral lipids that are extensively biosynthesised de novo from non-lipid dietary precursors, which requires an abundance of prey. All the *C. limacina* analysed by Kattner et al. (1998) were lipid-rich and contained both TAG and ADG rich in 15:0 and 17:1*n*-8 fatty acids. Although the biosynthetic precursors of these odd chain fatty acids remain unknown, there is no reason to believe that their precursors do not originate in *L. helicina*. Therefore, we propose that the presence of high densities of *L. helicina* enable *C. limacina* to accumulate large, unusual neutral lipid reserves mainly through de novo biosynthesis. In contrast, when low densities of *L. helicina* are present, insufficient nutrients are available for extensive de novo lipid biosynthesis by *C. limacina*. Under these conditions *C. limacina* is not rich in neutral lipid and we propose that the fatty acids in its modest levels of TAG are then derived predominantly from dietary lipid. Copepods containing 20:1*n*-9 and 22:1*n*-11 can on occasions contribute to such dietary lipid, accounting for the presence of these fatty acids in some specimens of *C. limacina*. This could occur directly, or indirectly via *L. helicina*, which on occasions contains significant amounts of 20:1*n*-9 and 22:1*n*-11. *L. helicina*, even in low densities, will contribute dietary lipids to *C. limacina*, in which case the modest levels of TAG deposited by *C. limacina* will be rich in *n*-3 PUFA. However, the *n*-3 PUFA present in the TAGs deposited will vary reflecting their varying abundances in *L. helicina*. For example, the *L. helicina* studied here contained on average 42% of their total fatty acids as *n*-3 PUFA in 1997, with 18:4*n*-3 the major PUFA, accounting for an average of 15% of the total (Table 3). In contrast, the *L. helicina* in 1998 contained an average of only 22% of their fatty acids as *n*-3 PUFA, with 20:5*n*-3 the major PUFA accounting for an average of 9% of the total, and 18:4*n*-3 for an average of only 5% (Table 3). Furthermore, a sample of *L. helicina* containing 50 pooled individuals from Kongsfjord analysed in 1997 contained 19% of its total

Table 5 Fatty acid compositions of alkyldiacylglycerols and wax esters from *Clione limacina* and of alkyldiacylglycerols from *Limacina helicina*. Data are weight % of total fatty acids. Codes and stations are as detailed in Table 1 (*No*. numbers of animals pooled for analyses)

	Alkyldiacylglycerols		Wax esters *C. limacina*
	C. limacina	*L. helicina*	
Code	417	314	417
Station	Ice2		Ice2
No.	1	5	1
14:0	1.3	8.2	1.0
15:0	6.1	1.3	7.7
Iso16:0	2.2	0.3	0.0
16:0	6.5	21.8	9.6
16:1*n*-7	15.5	2.7	7.6
17:0	4.0	2.0	5.2
17:1*n*-8	19.9	0.0	16.5
C16 PUFA	1.0	1.4	0.0
18:0	0.6	3.3	2.4
18:1*n*-9	3.8	3.4	6.5
18:1*n*-7	3.9	2.6	3.7
18:2*n*-6	0.4	2.1	1.0
18:4*n*-3	0.6	5.3	0.0
20:1*n*-9	2.5	11.4	10.3
20:2*n*-6	0.6	1.5	1.1
20:4*n*-3	0.3	1.3	0.2
20:5*n*-3	6.3	9.7	5.2
22:1*n*-11	0.6	0.3	3.1
22:6*n*-3	17.0	12.9	9.0
Sat.	21.1	37.7	26.7
Mono.	49.0	21.9	53.3
n-6	1.2	4.3	2.5
n-3	26.0	31.7	15.7

lipid as TAG (and no ADG), which contained 21% of its total fatty acids as 18:4*n*-3 (S. Falk-Petersen, J.R. Sargent, S. Kwasniewski, B. Gulliksen, R-M. Millar, data not shown). Such differences will undoubtedly be reflected in the fatty acid compositions of TAG deposited by predators of *L. helicina*, not least *C. limacina*. They are readily accounted for by temporal and spatial differences in the abundance and composition of the phytoplankton and particulates that form the major diet of *L. helicina*, with C16 PUFA and 20:5*n*-3 originating mainly from diatoms, and 22:6*n*-3 and 18:4*n*-3 originating mainly from flagellates, including *Phaeocystis* (Sargent and Whittle 1981; Sargent et al. 1985; Falk-Petersen et al. 1990; Hamm et al., in press). Thus, the prominence of both C16 PUFA and 20:5*n*-3 in *L. helicina* from ice station 1 in 1998 (Table 3) is readily explained by the diatom-dominated bloom that was occurring there. We propose, therefore, that the observed heterogeneity of the lipid analytical data for *C. limacina* reflects basically temporal variations, first in the abundance and species composition of phytoplankton. This influences the levels and composition of the neutral lipids, mainly TAG, in *L. helicina*, which is generally not a lipid-rich species and is predominantly a herbivore (Gilmer 1974). However, *L. helicina* can also ingest small copepods (Gilmer and Harbison 1991) resulting in the incorporation of some copepod fatty acid biomarkers into its neutral lipid. At low densities of *L. helicina*, the modest levels of TAG in *C. limacina* reflect the fatty acid composition of the ingested *L. helicina* lipids, which are predominantly PLs rich in *n*-3 PUFA derived directly from phytoplankton. It is only when high densities of *L. helicina* occur that *C. limacina* engages in extensive de novo biosynthesis of TAG, ADG and, to some extent, WE. Throughout all of these phases, the fatty acid compositions of the polar lipids of the two species remain virtually identical (Table 2), reflecting their very close predator-prey relationships.

Kattner et al. (1998) suggested that the production of ADG by *C. limacina* is a strategy to buffer a pulsed food supply similar to the wax ester storage of other zooplankton species. This is an attractive suggestion for a carnivore that has fast feeding and growth rates and rapid reproductive responses to a fluctuating food supply. It is possible also that ADG together with TAG may be accumulated by *C. limacina* as the animal develops towards sexual maturity, in which case the specimens of *C. limacina* with low and high levels of neutral lipid may be in early and late stages of maturation, respectively, linked to the development of the primary bloom and the subsequent bloom of *L. helicina*. Future studies on the developmental stages of these animals, especially *C. limacina*, may reveal the true role of the lipids in these animals and further illuminate their adaptive strategies and roles in polar ecosystems.

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