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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 alklydiacylglycerols (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 17:1n-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 17:1n-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 17:1n-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.

**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 alkyl-diacylglycerols and the very unusual fatty acid 17:1n-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-Alesund, 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 m² 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 Kongsfjorden in 1997, and from the one station in Kongsfjorden and the two ice stations in 1998 (Table 1).

**Limacina helicina**

**Lipid classes**

Three of the *L. helicina* samples collected in Kongsfjorden 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 Kongsfjorden in 1997 differed from the other three in having small but significant amounts of alkyl-diacylglycerols (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 Kongsfjorden 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 Kongsfjorden 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 Kongsfjorden in 1997, one contained circa 20% each of TAG and ADG, one had circa 20% of TAG and only 6% of ADG, and the other had only 10% TAG and very little ADG. All three individuals