Wax ester composition of the dominant calanoid copepods of the Greenland Sea/Fram Strait region*

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The composition of the intact wax esters of Calanus hyperboreus, C. finmarchicus, and C. glacialis from the Fram Strait area of the Greenland Sea was studied. In addition the fatty acid and alcohol composition of the wax esters was analysed. All copepods were rich in wax esters, especially C. hyperboreus in which long-chain wax esters were generally more abundant than in the other species.

The wax ester composition was species dependent but strongly overlapped by spatial variabilities. Because phytoplankton fatty acids were partially incorporated directly into wax esters, the composition of the wax esters was widely influenced by the phytoplankton community which itself varied considerably in the Fram Strait region. Thus, the wax esters of the polar species, collected in the North East Water Polynya near Greenland, were dominated by diunsaturated compounds in the range from C_{12} to C_{18}. The shorter chain components resulted from a combination of palmitoleic acid which is typical of diatom lipids with long-chain alcohols. In the marginal ice zone, higher levels of polyunsaturated wax esters were detected due to the presence of the 18:4 fatty acid, typical of a summer phytoplankton population.

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Introduction

The herbivorous copepods are an important group in the plankton community of the Greenland Sea (Smith 1988). Their life in high latitudes is dependent upon the seasonal availability of food and on the storage of lipids.

The high lipid content of copepods in Polar regions is mostly a result of an increase in wax esters, which partially account for more than 90% of the total lipid (Lee 1974, 1975; Sargent et al. 1981; Tande & Henderson 1988; Kattner et al. 1989). This lipid store enables copepods to survive long periods of food shortage or even periods lacking food; it also enables them to preserve energy for reproduction.

Wax esters are molecules in which a long-chain fatty acid is esterified with a long-chain fatty alcohol. The alcohols are biosynthesised de novo by calanoid copepods (e.g. Sargent et al. 1976), whereas the fatty acids partly reflect their phytoplanktonic dietary input. The principal alcohols are 20:1 and 22:1 with smaller amounts of 16:0; the fatty acids are much more variable in composition.

Until now, methods of determining intact wax esters have provided only an inadequate separation (Lee et al. 1971, 1974; Boon & de Leeuw 1979; Wakeham & Frew 1982; Itabashi & Takagi 1984). In the present study the compositions of intact wax esters of Calanus hyperboreus, C. finmarchicus and C. glacialis are presented. The results are based on a recent method which allows the separation of wax esters according to their degree of unsaturation (Kattner et al. 1990). With this method it is possible to study, for example, the influence of species variabilities and diet on the wax esters by the examination of intact molecules.

Material and methods

The calanoid copepods Calanus hyperboreus, C. finmarchicus, and C. glacialis were caught in the Fram Strait area between Greenland and Spitsbergen during the 1984 MIZEX cruises of the R/V POLARSTERN and R/V VALDIVIA. The different species collected at the various stations are indicated in Fig. 1. The copepods were collected by vertical hauls from the upper 100 m with a bongo net (300 and 500 µm mesh). Immediately after sampling, individuals were sorted into species and stages, transferred into glass tubes containing...
Fig. 1. Fram Strait between Greenland and Spitsbergen with location of stations where copepods were collected. The solid line represents the marginal ice zone, which is indicated by 0°C isotherm at 5 m depth. The numbers refer to sampling stations. The broken line indicates the North East Water Polynya during the MIZEX cruise.
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The copepods were homogenised in the storage solution using a Potter homogeniser (Braun, Melsungen, Germany). From an aliquot of the lipid extract, wax esters were separated by thin-layer chromatography on silica gel 60 with hexane:diethylether:acetic acid (90:10:1). The bands were scraped off after visualising with 0.1% dichlorofluorescein in methanol, eluted in chloroform, evaporated to dryness, and redissolved in hexane.

Analyses of the intact wax esters were carried out in a gas chromatograph (Model 5370 Mega Series, Carlo Erba Instruments, Milano, Italy) on a 25 m x 0.25 mm i.d. wall-coated open tubular bonded, fused silica column coated with a 0.10 µm film of a Triglyceride Analysis Phase (TAP, Chrompak, Mühlheim, FRG) using on column injection and temperature programming. The details of the method are described elsewhere by Kattner et al. (1990). The fatty acids and alcohols were analysed according to the method of Kattner & Fricke (1986).

Results

The total lipids of all the copepods studied were rich in wax esters (Fig. 2). *Calanus hyperboreus* had the highest proportion of wax esters in total lipids, with the proportion increasing from the copepodid Stage IV to females. In *C. finmarchicus*, the proportion was less in all stages than in *C. hyperboreus*. The variability was higher in *C. finmarchicus* and the females contained less wax esters than the copepodid Stage V. Generally the wax ester content decreased from adults to the young copepodid stages as reported for *C. finmarchicus* from the North Sea (Fig. 2). The wax ester content of *C. finmarchicus* was similar in both areas. *C. glacialis* females, collected in the Polynya contained about 73% of wax esters in total lipid, but under the pack ice, levels of more than 90% were detected.

The wax ester compositions, together with the composition of their fatty acid and alcohol moieties, of the three calanoid copepod species are presented in Figs. 3 and 4. The wax esters were different within the species and varied moreover due to the different sampling locations. Their chain length range was from C30 to C44. Saturated compounds were generally not detected, although their existence might be possible since saturated fatty acids and fatty alcohols were found after wax ester hydrolysis (Figs. 3 and 4).

North East Water Polynya

In the cold water of the East Greenland Current, *Calanus hyperboreus* and *C. glacialis* were collected, being typical species for this region. Sampling was carried out in or near the North East Water Polynya at Station 245. As shown in Fig. 3, diunsaturated wax esters accounted for the major portion of the wax ester composition of these two species. In both species the 36:2 (number of carbon-atoms:number of double bonds) and 38:2 wax esters were dominant as a combination of the 16:1 acid and the 20:1 or 22:1 alcohols. The other diunsaturated wax esters with chain length of 40-44 C-atoms are most likely combinations of the 20:1 and 22:1 acids with their corresponding alcohols.

In *C. glacialis* the wax esters with shorter chain length were more abundant than in *C. hyperboreus*. Also monounsaturated wax esters accounted for higher levels in *C. glacialis* in which the major chain length was 36 C-atoms. The different composition may result from different possible combinations of fatty acids and alcohols in wax esters. The relatively high proportion of the 14:0 acid, in contrast to *C. hyperboreus*, may contribute to this pattern (Fig. 3).

The polyunsaturated wax esters were generally low in both copepods in the Polynya region. Only...
the 20:5 acid accounted for higher levels in the wax esters which results in a small proportion of wax esters with 5 to 6 double bonds. Also the chain lengths of the polyunsaturated wax esters of *Calanus glacialis* were shorter than those of *C. hyperboreus*.

**Marginal ice zone**

Further west in the marginal ice zone (MIZ) of the Fram Strait, *Calanus hyperboreus* and *C. finmarchicus* were found; *C. glacialis* was not present here. Comparing the wax ester compositions of *C. hyperboreus* from both sampling sites, differences became obvious (Figs. 3 and 4). In all wax esters, independent on the degree of unsaturation, the chain lengths were longer than in the Polynya region. The sum of diunsaturated wax esters was similar despite the change to longer chain lengths. About half of the total wax esters...
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Fig. 4. Calanus hyperboreus and C. finmarchicus from the marginal ice zone. Composition of wax esters together with their fatty acid and alcohol moieties. Numbers in the open bars correspond to the number of double bonds of the main polyunsaturated component.

are combinations of the 20:1 and 22:1 acids with their corresponding alcohols, which is much more than in the Polyna.

The proportion of monounsaturated wax esters was also low, but the polyunsaturated were much more abundant. This results from a dramatic change in the fatty acids, being now dominated by the 18:4 acid. The 18:4 acid accounted for about 20% of total wax ester fatty acids. The combination of this acid with the 22:1 alcohol was the dominant contributor to the polyunsaturated wax esters of C. hyperboreus from this region (Fig. 4).

Comparison of the wax ester compositions of Calanus hyperboreus and C. finmarchicus from the same region (the MIZ) resulted in widely different compositions (Fig. 4). C. finmarchicus was dominated by the 34:1 and 36:1 wax esters. Due to high amounts of the 14:0 acid, the combination again with the 20:1 or 22:1 alcohol was
the major contributor; but the combination of 16:0 acid and 20:1 alcohol, and conversely, may also contribute to the 36:1 wax ester. The diunsaturated wax esters were similarly distributed according to chain length but accounted only for half of the diunsaturated wax esters of *C. hyperboreus*. The polyunsaturated wax esters were combinations of the two major alcohols with the 18:4 acid which was also one of the major acids of the lipids of *C. finmarchicus*.

**Discussion**

Calanoid copepods from high latitudes biosynthesise large amounts of wax esters as an energy reserve (Lee 1974; Sargent et al. 1981; Tande & Henderson 1988; Kattner et al. 1989). They are therefore able to survive the long periods of food scarcity in Polar regions and to preserve energy for reproduction. Wax esters have to be biosynthesised de novo since they are absent in the phytoplankton diet of the herbivorous copepods.

The species dependent differences in the wax ester composition of the copepods were strongly overlapped by the spatial variability. The major reason for this was the changing diet which, because of the development and succession of the phytoplankton, was very different throughout the Fram Strait area. The phytoplankton of the Polynya region represents a spring bloom dominated by diatoms (Baumann pers. commun.). The most striking fatty acids of diatoms are the 16:1 and 20:5 acids (e.g. Kates & Volcani 1966; Ackman et al. 1968; Kattner et al. 1983). The North East Water Polynya is an area of open water which occurs for several months on the East Greenland Shelf. It starts opening in the spring and reaches its maximum extension in August. The highest phytoplankton biomass and primary production were found in this region. Fig. 1 describes the Polynya area between 30 June and 4 July 1984. The phytoplankton community of the marginal ice zone represents a summer population, dominated by *Phaeocystis sp.* and dinoflagellates (Smith et al. 1987). Their “marker” fatty acid, especially of *Phaeocystis sp.*, is the 18:4 acid (Harrington et al. 1970; Sargent et al. 1985). Both fatty acids marked considerably the wax ester composition of the copepod species. Thus in the Polynya about half of the diunsaturated wax esters were short-chain esters due to the combination with the 16:1 acid. In the MIZ the transfer of the dietary 18:4 acid into the wax ester fraction was reflected by high levels of polyunsaturates. Nevertheless a considerable proportion of wax esters were still entirely de novo synthesised as combinations between the 20:1 and 22:1 moieties. Generally the 42:2 wax ester presents the highest proportion because of the two combination possibilities of 20:1 acid and 22:1 alcohol and conversely. There exists no clear indication to a preferred biosynthesis of special wax ester combinations. On the other hand copepods living under unfavourable conditions, e.g. food scarcity due to close ice cover, contain higher amounts of the long-chain diunsaturated wax esters which leads to the assumption that wax esters with dietary fatty acid moiety were preferentially used up during starvation.

Generally the wax ester proportion of total lipids increases in the copepodid stages (Kattner & Krause 1987), parallel with the amount of de novo biosynthesised long-chain wax esters. The production or accumulation of the 20:1 and 22:1 alcohols seems to be time-dependent so that only the developmental time of the older stages is long enough to accumulate large amounts of these energy-rich lipid reserves. This would explain the species dependent differences in wax esters which were clear in spite of the spatial variability. In *Calanus hyperboreus*, the long-chain wax esters are much more dominant than in the smaller copepods. *C. hyperboreus* is at least a biennial species and its spawning is not dependent on food (Conover 1967), but probably dependent on the wax ester reserves. *C. finmarchicus*, on the other hand, has one generation per year and its reproduction is synchronised with the onset of phytoplankton blooms (Marshall & Orr 1955). The life cycle of *C. glacialis*, annual or biennial, is still in discussion (Tande & Henderson 1988), but it seems to be somewhat between the other two species, which is also reflected in its wax ester composition.

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