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Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters

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ABSTRACT: The percentage (%) fatty alcohol and fatty acid compositions of the wax esters of large numbers of Stage V and females of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* taken in late August to late September from Arctic waters (Kongsfjord in Svalbard, 78° 57′ N, 11° 50′ E) are presented. The data reveal that these stages of development of the 3 species can be discriminated on the basis of the % of 22:1n-11 fatty alcohol in their large levels of wax esters, with *C. hyperboreus* having the highest % followed by *C. finmarchicus* and then *C. glacialis*. Equally, *C. hyperboreus* has the lowest % of 20:1n-9 fatty alcohol in its wax esters with *C. finmarchicus* having a higher % and *C. glacialis* the highest %. Relatively minor differences occur in the fatty acid compositions of the wax esters of the 3 species, which consisted principally of 20:1n-9 (15 to 18%) and 22:1n-11 (10 to 15%), together with the diatom-derived fatty acids 16:1n-7 (20 to 23%) and 20:5n-3 (11 to 13%). The flagellate-derived fatty acids, 18:4n-3 (3 to 6%) and 22:6n-3 (1 to 3%), were minor constituents. The fatty acid compositions of the small amounts of polar lipid in the 3 species were indistinguishable with 22:6n-3 (41 to 46%) and 20:5n-3 (22 to 24%) being the major components. We conclude that Stage V and females of the species can be distinguished in autumn on the basis of the different % of 22:1n-11 and 20:1n-9 fatty alcohols in their wax esters and that de novo lipid biosynthetic activity in the copepods increases in the order *C. finmarchicus* < *C. glacialis* < *C. hyperboreus*. We discuss the results in terms of the contributions of fatty acids and fatty alcohols biosynthesised de novo and fatty acids derived from the diet to the copepods' lipids, the role of 20:1 and 22:1 fatty alcohols and fatty acids as energy sources, and the possible role of 22:6n-3 in the copepods' physiology.

KEY WORDS: Calanus · Arctic · Wax esters · Phospholipids · Acids · Alcohols

INTRODUCTION

Calanus hyperboreus, *C. glacialis* and *C. finmarchicus* can all be abundant in Arctic waters. *C. hyperboreus* is primarily a deep-water species found especially in the Greenland Sea and the Arctic Ocean (Hirche 1991, Hirche & Mumm 1992, Thibault et al. 1999). *C. glacialis* is primarily an Arctic shelf species (Conover 1988, Hirche & Kwasniewski 1997). *C. finmarchicus* is primarily a North Atlantic species (Fleminger & Hülsemann 1977) which is exported to the Arctic (Hirche 1991). However, all 3 species can be found in the same location, as for example in the Arctic fjord studied in the present investigation. All 3 species accumulate large amounts of oil, which consists principally of wax esters (Lee et al. 1971a,b, Lee & Hirota 1973, Sargent et al. 1976, Sargent & Henderson 1986).

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The large *C. hyperboreus* and the intermediate-sized *C. glacialis* contain higher levels of oil per unit body mass than the small *C. finmarchicus* (Scott et al. 2000).

The fatty acid compositions of the wax esters of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* reflect the fatty acid compositions of their phytoplanktonic diet, particularly in their % of 16:1n-7 and various polyunsaturated fatty acids (PUFA), whereas the fatty alcohols of the esters are invariably dominated by 20:1n-9 and 22:1n-11 units not found in phytoplankton (Lee et al. 1971a,b, Lee & Hirota 1973, Sargent et al. 1976, Sargent & Henderson 1986, Sargent & Falk-Petersen 1988, Tande & Henderson 1988, Graeve et al. 1994, Albers et al. 1996). It has been proposed, therefore, that calanoid copepods accumulate large stores of wax esters by efficiently assimilating and retaining dietary, phytoplanktonic fatty acids and esterifying them with fatty alcohols biosynthesised de novo from dietary phytoplanktonic protein and carbohydrate (Sargent & Henderson 1986). Earlier literature suggests that Stage V and adult *C. hyperboreus* have a substantially higher % of 22:1n-11 than 20:1n-9 in their wax esters (Lee 1974, 1975). Comparable stages of *C. glacialis* have a substantially lower % of 22:1n-11 than 20:1n-9 (Clarke et al. 1987, Tande & Henderson 1988). Comparable stages of *C. finmarchicus* have only a slightly lower % of 22:1n-11 than 20:1n-9 (Kattner & Krause 1987, Falk-Petersen et al. 1987, Kattner 1989). One difficulty in elucidating inter-species differences from these studies is that they all considered only a single species at a given time, with the different species being taken from different locations. However, Kattner et al. (1989) analysed Stages IV and V and females of *C. hyperboreus* and *C. finmarchicus* in the Fram Strait in the summer of a single year. Albers et al. (1996) presented analyses of females of all 3 species taken in the Fram Strait between 78° and 80° N in the summer of 2 successive years. These more extensive analyses (Kattner et al. 1989, Albers et al. 1996) showed the same trends for differences in the % of 22:1n-1 and 20:1n-9 in the wax esters of the 3 species as found earlier.

Such results strongly suggest species differences in the fatty alcohol compositions of the wax esters of the 3 species, consistent with their having different propensities to biosynthesise long-chain fatty alcohols. This has implications for the levels and energy contents of the wax esters in the different species. However, possible species differences in the fatty alcohol compositions of calanoid wax esters have thus far not been investigated in the same stages of different species taken at the same location, and in late summer-autumn when the copepods have accumulated maximal levels of wax esters. Nor have possible differences in fatty alcohol composition been clearly related to differences in maximal levels of wax esters in the different species. We have recently determined levels of total lipid and wax esters in large numbers of Stage V and female *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* taken in late summer-autumn at a single station in Kongsfjord, Svalbard (Scott et al. 2000). We present here the fatty alcohol and fatty acid compositions of the wax esters, together with the fatty acid compositions of the phospholipids of the same specimens. We discuss the results in terms of the contributions of fatty acids and fatty alcohols biosynthesised de novo and fatty acids derived from the diet to the copepods' lipids, and the roles of 20:1n-9, 22:1n-11 and 22:6n-3 in the their physiology.

MATERIALS AND METHODS

The copepods were sampled during an expedition (BIODAFF 97) from Ny Ålesund Large Scale Facility in Kongsfjorden, Svalbard, in 1997 as previously described by Scott et al. (2000). Sampling was performed at Stn K3 in Kongsfjord (78° 57′ N, 11° 50′ E) from a 2 m metal dory with an outboard engine. The water column in the fjord was formed of local waters overlaying a mix of Spitzbergen shelf water and transformed Atlantic water with a layer of intermediate water in between. Hauls were taken with 2 conical WP-2 (Working Party 2, UNESCO) plankton nets to obtain samples every 48 h when conditions allowed, from 24 August to 20 September 1997. A WP-2 net of 57 cm opening diameter with a 180 µm mesh size and a WP-2 net of 57 cm opening diameter with a 500 µm mesh size were used. Both nets were towed vertically from 200 m depth to the surface at a rate of 45 m min^{-1} (UNESCO 1968).

Individual copepods were identified to species and to stage of development, and both Stage V individuals, i.e. the stage immediately preceding full sexual development to males or females, and females were selected. At least 10 individuals of each of these 2 stages from each of the 3 species were pooled to constitute a single sample. Total lipid was extracted from separate samples by the method of Folch et al. (1957) and fractionated into lipid classes by thin layer chromatography (Olsen & Henderson 1989). Wax esters and phospholipids were eluted from the plates, dried and transmethylated in methanol/toluene (2/1 v/v) containing 1% sulphuric acid for 16 h at 50°C. The reaction products were extracted into diethyl ether, dried under nitrogen and subjected to thin layer chromatography in hexane/diethyl ether/acetic acid (70/30/1 v/v/v) to separate fatty acid methyl esters and, for wax esters, free fatty alcohols. The fatty acid methyl esters and the free fatty alcohols were eluted from the plates and the fatty alcohols converted to fatty alcohol acetates by

reacting with acetic anhydride in pyridine (Farquhar 1962). The % compositions of fatty acid methyl esters and fatty alcohol acetates were determined in a Fisons GC8160 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica, wall-coated capillary column (30 m × 0.32 mm i.d., Chrompack UK) with an on-column injection system and flame ionization detection. Hydrogen was used as carrier gas with an oven thermal gradient from an initial 50 to 180°C at 40°C min^{-1}, and then to a final temperature of 235°C at 2°C min^{-1}. Individual components were identified by comparison with known standards, with a well-characterised fish oil and by reference to published data, as described previously by Tande & Henderson (1988), and were quantified using a PC directly linked to the detector and operating Chrom-Card Software (Thermo-Quest Italia). All solvents contained 0.01% w/v butylated hydroxytoluene as an antioxidant.

Significances of differences between mean values for % fatty alcohols and % fatty acids were determined by 1-way analysis of variance followed, where appropriate, by Tukey's multiple range test (Zar 1996).

RESULTS

Fatty alcohol compositions of the wax esters

There were no clear-cut differences between % compositions of fatty alcohols in wax esters of Stages V and females in any of the 3 species. Therefore, the data for samples of Stages V and females of a given species were pooled. The results (Table 1) show that each species contained minor % of 16:0 and 16:1n-7 alcohols and major % of 20:1n-9 and 22:1n-11 alcohols. The % of 20:1n-9 alcohol was significantly different in the 3 species with the highest % in *Calanus glacialis*. Likewise, the % of 22:1n-11 alcohol was significantly different in the 3 species with the highest % in *C. hyperboreus*. Thus, the ratio of (22:1n-11 + 22:1n-9)/(20:1n-9 + 20:1n-7) alcohols was highest in *C. hyperboreus* and lowest in *C. glacialis*. However, the sum of 20:1 alcohol isomers (n-9 + n-7) and 22:1 alcohol isomers (n-11 + n-9) was very similar in the 3 species, increasing progressively from a total of 75.4% in *C. finmarchicus* to 77.1% in *C. glacialis* and 82.6% in *C. hyperboreus*. This was paralleled by the % 16:1n-7 decreasing progressively from 6.5% to 3.8% and 2.6% in the 3 species, although the decrease from *C. glacialis* to *C. hyperboreus* was not significant. Differences between the remaining fatty alcohols in the 3 species were minor and, although differences for a given alcohol between a given species and the other 2 species were generally significant, in no case were the differences between all 3 species significant.

Fatty acid compositions of the wax esters

As for the fatty alcohols, the fatty acid data were pooled since there were no clear-cut differences between Stages V and females in any of the 3 species. The % compositions of fatty acids (Table 2) reveal that 16:1n-7 was the major fatty acid in all 3 species, with lesser % of 20:1n-9 and 22:1n-11. The clear-cut differences between 20:1 and 22:1 fatty alcohols in the wax esters (Table 1) were only faintly echoed in the corresponding fatty acids (Table 2). Thus, *Calanus hyperboreus* had the highest % of 22:1 fatty acid of the 3 species and, although the differences between this species and both *C. glacialis* and *C. finmarchicus* were significantly different, the difference between the latter 2 species was not. *C. hyperboreus* had correspondingly and significantly lower % of both 14:0 and 16:0 than the other 2 species, but the differences between the % of 14:0 and 16:0 in *C. glacialis* and *C. finmarchicus* was only significant for 14:0. *C. glacialis* had the highest % of 20:1, which was significantly different from *C. finmarchicus* but not from *C. hyperboreus*. In contrast to the situation for the fatty alcohols, the % of 20:1 fatty acid exceeded that of 22:1 fatty acid in all 3 species. However, the progressive increase in the sum of the % 20:1 and 22:1 fatty alcohols from *C. finmarchicus* to *C. glacialis* to *C. hyperboreus* (Table 1) occurred also in the sum of the % of 20:1 and 22:1 fatty acids, which increased progressively from 27.5% to 30.8% and thence to 35.6% in the species, respectively. The PUFA present in all 3 species in the same % was 20:5n-3. Smaller amounts of 18:4n-3 were present, in significantly higher % in both *C. hyperboreus* and

Table 1. Fatty alcohol compositions (mass %) of the wax esters of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*. Data are means ± SD; n = number of samples; V = Stage V; F = female. Values which share a superscript letter within a given row are not significantly different. Values with different superscript letters within a given row are significantly different ($p < 0.05$)

	C. finmarchicus (n = 13V + 12F)	*C. glacialis* (n = 9V +8F)	*C. hyperboreus* (n = 11V + 14F)
14:0	2.0 ± 0.7^{b}	1.8 ± 0.5^{b}	2.9 ± 0.9^{a}
16:0	8.2 ± 1.2	8.6 ± 1.4	7.5 ± 2.2
16:1n-7	6.5 ± 1.8^{a}	3.8 ± 1.4^{b}	2.6 ± 1.8^{b}
18:1n-9	1.8 ± 0.6^{a}	1.8 ± 0.6^{a}	0.4 ± 0.4^{b}
18:1n-7	2.4 ± 0.8^{a}	2.2 ± 0.4^{a}	1.0 ± 0.4^{b}
20:1n-9	35.5 ± 4.7^{b}	43.1 ± 4.3^{a}	27.2 ± 4.2^{c}
20:1n-7	1.5 ± 1.7^{b}	1.3 ± 0.7^{b}	2.9 ± 1.2^{a}
22:1n-11	37.8 ± 7.1^{b}	29.8 ± 4.8^{c}	51.0 ± 6.7^{a}
22:1n-9	0.6 ± 0.4^{c}	2.9 ± 0.8^{a}	1.5 ± 1.1^{b}
20:1* + 22:*1	75.4	77.1	82.6
22:1*/20:1*	1.04	0.74	1.74

*Both isomers

Table 2. Fatty acid compositions (mass %) of the wax esters of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*. Data are means ± SD; n = number of samples; V = Stage V; F = female. Values which share a superscript letter within a given row are not significantly different. Values with different superscript letters within a given row are significantly different ($p < 0.05$)

	C. finmarchicus (n = 11V + 11F)	*C. glacialis* (n = 8V + 7F)	*C. hyperboreus* (n = 14V + 12F)
14:0	9.1 ± 3.5^{a}	6.8 ± 1.5^{b}	3.3 ± 2.4^{c}
16:0	7.1 ± 3.2^{a}	5.5 ± 1.7^{a}	2.5 ± 1.6^{b}
16:1n-7	23.0 ± 3.1^{a}	20.3 ± 3.9^{ab}	20.6 ± 3.3^{b}
16:2	1.5 ± 0.4^{a}	1.1 ± 0.3^{b}	1.5 ± 0.3^{a}
16:3	1.5 ± 0.6^{ab}	1.1 ± 0.5^{b}	1.6 ± 0.4^{a}
16:4	1.0 ± 0.7^{b}	0.7 ± 0.5^{b}	1.9 ± 1.0^{a}
18:0	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
18:1n-9	2.6 ± 0.7^{b}	4.7 ± 1.4^{a}	2.4 ± 0.6^{b}
18:1n-7	1.5 ± 0.3^{a}	1.1 ± 0.2^{b}	1.5 ± 0.2^{a}
18:2n-6	0.8 ± 0.5	1.1 ± 0.5	1.1 ± 0.4
18:3n-6	0.9 ± 1.8	0.2 ± 0.1	0.3 ± 0.6
18:3n-3	0.6 ± 0.9	0.7 ± 0.4	0.6 ± 0.8
18:4n-3	2.7 ± 1.9^{b}	5.1 ± 2.1^{a}	6.0 ± 2.3^{a}
20:1n-9	14.5 ± 2.1^{b}	18.4 ± 3.8^{a}	16.2 ± 2.8^{ab}
20:1n-7	1.9 ± 1.6^{a}	0.6 ± 0.5^{b}	2.4 ± 1.2^{a}
20:4n-6	0.6 ± 0.6	0.3 ± 0.1	0.5 ± 0.8
20:4n-3	0.7 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
20:5n-3	11.4 ± 2.7	12.3 ± 3.0	12.6 ± 2.4
22:1n-11	9.7 ± 3.9^{b}	10.5 ± 2.0^{b}	14.9 ± 3.4^{a}
22:1n-9	1.4 ± 1.8	1.3 ± 0.6	2.1 ± 2.2
22:6n-3	1.4 ± 0.9^{b}	2.0 ± 0.8^{b}	3.0 ± 0.8^{a}
20:1* + 22:1*	27.5	30.8	35.6
22:1* / 20:1*	0.68	0.62	0.91

*Both isomers

Table 3. Fatty acid compositions (mass %) of the polar lipid of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*. Data are means ± SD; n = number of samples; V = Stage V; F = female

	C. finmarchicus (n = 2F)	*C. glacialis* (n = 2V)	*C. hyperboreus* (n = 2V)
14:0	1.6 ± 0.2	1.6 ± 0.2	1.9 ± 0.0
16:0	14.5 ± 1.7	14.6 ± 0.0	14.9 ± 1.3
16:1n-7	1.4 ± 0.2	0.9 ± 0.5	1.5 ± 0.6
18:0	1.3 ± 0.2	1.8 ± 0.5	1.1 ± 0.2
18:1n-9	2.7 ± 0.3	2.8 ± 0.1	2.5 ± 1.1
18:1n-7	1.3 ± 1.0	1.2 ± 0.1	1.9 ± 0.1
18:2n-6	0.7 ± 0.2	0.8 ± 0.0	0.7 ± 0.3
18:3n-3	0.5 ± 0.7	0.8 ± 0.4	0.2 ± 0.1
18:4n-3	2.7 ± 0.8	2.8 ± 0.0	1.2 ± 0.3
20:1n-9	1.2 ± 0.2	0.8 ± 0.5	0.6 ± 0.3
20:4n-3	0.8 ± 0.1	1.1 ± 0.4	0.7 ± 0.2
20:5n-3	24.0 ± 0.0	22.7 ± 1.3	22.3 ± 4.8
22:6n-3	40.6 ± 4.5	40.9 ± 1.3	46.1 ± 8.5
24:1	1.8 ± 0.1	2.0 ± 0.2	1.2 ± 1.7

C. glacialis than in *C. finmarchicus*. Minor amounts of C16 PUFA were present in all 3 species with the % of 16:4 being significantly higher in *C. hyperboreus* than in both *C. glacialis* and *C. finmarchicus*. Minor amounts of 22:6n-3 were also present in all 3 species with, once more, *C. hyperboreus* having a significantly higher % than both *C. glacialis* and *C. finmarchicus*. Thus, *C. hyperboreus* had the highest % of total PUFA of all the 3 species. Overall, however, the fatty acid compositions of the wax esters of the 3 species were very similar.

Fatty acid compositions of the polar lipids

The % fatty acid compositions of the polar lipids, largely phospholipids, in the 3 species are shown in Table 3, where data were only available for female *Calanus finmarchicus* and Stages V of *C. glacialis* and *C. hyperboreus*. Despite this limitation, the fatty acid compositions of the phospholipids of all 3 species were very similar, being dominated by 16:0, 20:5n-3 and 22:6n-3, with the % of 20:5n-3 + 22:6n-3 exceeding 60% of the total fatty acids and the ratio of 22:6n-3/20:5n-3 approaching 2:1. The only difference of note between the species is that *C. hyperboreus* had a higher but not significantly different % of 22:6n-3 than the other 2 species.

DISCUSSION

The Stage V and female specimens of the 3 *Calanus* species analysed here were all captured from the same site in Kongsfjord. The water structure at the site sampled is complex, being formed of local waters overlaying a mix of Spitzbergen shelf water and transformed Atlantic water with a layer of intermediate water in between. It is improbable that the 3 species had all developed from nauplii to Stages V and females in Kongsfjord and probable that they had different origins. For example, it is plausible that *C. finmarchicus* had been advected into the fjord from the south and *C. hyperboreus* advected in from the north. This, together with the different development times for the 3 species, makes it virtually certain that they had developed in different locations at different times and thus had experienced different phytoplankton regimes, whether qualitatively in terms of species composition or quantitatively in terms of species abundance. Moreover, the Stages V and females of the 3 species were cap-

tured in late summer-autumn when they had accumulated their highest levels of wax esters prior to overwintering. Thus, in relating the fatty alcohol and fatty acid compositions of the copepods' wax esters to dietary input, as is attempted here, it is the cumulative, long-term dietary input into the copepods and their maximal accumulated wax esters that are under discussion. Shorter-term dietary influences can only be assessed by analyses of all of the copepods' 6 developmental stages.

The results here establish that *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* have significantly different % of both 20:1n-9 and 22:1n-11 fatty alcohols in their wax esters. Similar differences can be deduced from various studies on single species (Lee 1974, 1975, Clarke et al. 1987, Falk-Petersen et al. 1987, Kattner & Krause 1987, Tande & Henderson 1988, Kattner 1989) but only Albers et al. (1996) analysed the 3 species simultaneously. It can be calculated from the data of Albers et al. (1996) that the ratios of 22:1n-11/20:1n-9 fatty alcohols in females of *C. hyperboreus*, *C. glacialis* and *C. finmarchicus* were, respectively, 1.98, 0.43 and 0.99. The ratios for Stages V and females of the 3 species in the present study were 1.74 for *C. hyperboreus* (lower than reported by Albers et al. 1996), 0.74 for *C. glacialis* (higher than reported by Albers et al. 1996) and 1.04 for *C. finmarchicus* (the same as reported by Albers et al. 1996). Such differences may reflect the samples in the 2 studies being taken at different times and at different places. The samples of females analysed by Albers et al. (1996) were captured in June and July-August in 2 successive years in the Fram Strait between 78° and 80° N. The samples of Stages V and females analysed here were captured between late August and late September in Kongsfjord at 78° 57' N. Thus, it is probable that copepods of a given species analysed by Albers et al. (1996) had different nutritional histories from copepods of the corresponding species analysed here, as well as being at a slightly earlier stage of development. Therefore, it cannot be concluded that copepods of the same species in the 2 studies had accumulated maximal levels of wax esters. The chain lengths of the alcohols of the wax esters of *C. hyperboreus*, *C. glacialis* and *C. finmarchicus* vary with the levels of wax esters in the copepods, i.e. with their stages of development (Lee 1974, Kattner & Krause 1987, Tande & Henderson 1988), with lower levels of wax esters generally being associated with shorter fatty alcohols. Moreover, as evidenced by the large standard deviations, there is considerable variation in the % of 22:1n-11 and 20:1n-9 fatty alcohols in wax esters between individuals of a given species in the present study (Table 1), as occurs also in the study of Albers et al. (1996). Nonetheless, the present results, based on analyses of very large numbers of samples, together with the findings of Albers et al. (1996), led to the conclusion that Stages V and females of the 3 species have different % of 22:1n-11 and 20:1n-9 fatty alcohols and, therefore, different ratios of these fatty alcohols in their wax esters. We conclude that, in copepods with maximal levels of wax esters, the % of 22:1n-11 and 20:1n-9 fatty alcohols in their wax esters is species-specific.

Wax esters are considered to be formed by copepods in response to short periods of plentiful food followed by long periods of food scarcity (Lee et al. 1971a, Lee & Hirota 1973), a situation that applies above all to herbivorous zooplankton in high latitudes. Further, the fatty alcohol moieties of zooplankton wax esters are considered to be biosynthesised by the copepods from the corresponding fatty acids, which are themselves biosynthesised de novo by the copepods, largely from protein and carbohydrate precursors in the diet (Sargent & Henderson 1986). Key evidence for this hypothesis is that 20:1 and 22:1 are not significant components of phytoplanktonic lipid (Sargent & Henderson 1986). Fatty acids and fatty alcohols are energy-rich molecules that are catabolised to CO_2 and H_2O to generate large amount of energy (ATP) for metabolic needs, the stoichiometry of oxidation of 1 mol (338 g) of 22:1 fatty acid being:

$$C_{22}H_{42}O_2 + 31.5\ O_2 \rightarrow 22\ CO_2 + 21\ H_20 + 176\ ATP$$

A longer-chain fatty alcohol (or fatty acid) is more chemically reduced and has a higher energy content per unit mass than a shorter chain fatty alcohol (or fatty acid). Therefore, the energy content of wax esters (or triacylglycerols) is maximised by increasing the chain lengths of their constituent fatty alcohols or fatty acids. Thus, *Calanus hyperboreus* with the highest % of 22:1n-11 alcohol in its wax ester is the most active of the 3 species in biosynthesising lipid de novo and accumulates wax esters with the highest energy content. This is consistent with *C. hyperboreus* being the most highly adapted of the 3 species, maximising formation of the longest-chain end-product of lipid biosynthesis, 22:1n-11, reflecting its main location in the most extreme environment, the Greenland Sea and the Arctic basin. Moreover, the sum of 20:1 and 22:1 fatty alcohols in the wax esters of the 3 species increases from *C. finmarchicus* to *C. glacialis* to *C. hyperboreus* (Table 1), as does the sum of 20:1 and 22:1 fatty acids in the wax esters (Table 2). That is, major de novo fatty acid/fatty alcohol biosynthetic activity increases progressively from *C. finmarchicus* to *C. glacialis* to *C. hyperboreus*.

As established previously (Scott et al. 2000), the large Arctic basin *Calanus hyperboreus* and the intermediate-sized Arctic shelf *C. glacialis* contain the same level of lipid per unit body mass (ca. 65%) and

contain the same % of wax esters in their total lipid (ca. 70%). However, *C. glacialis* has a lesser ability to maximise formation of end product 22:1n-11 and, therefore, accumulates wax ester reserves with a lower energy content per unit weight than those in the similarly sized reserves accumulated by *C. hyperboreus*. Consequently, *C. glacialis* is less adapted to extreme environments than *C. hyperboreus*, reflecting its main location in the less extreme Arctic shelf waters. Nonetheless, *C. glacialis* is more adapted to Arctic waters than the smaller, North Atlantic species *C. finmarchicus*, whose wax ester reserves account for only ca. 33% of its body mass in the waters studied here (Scott et al. 2000). The sum of the % of 20:1 and 22:1 alcohols (and acids) in the wax esters is greater in *C. glacialis* than in *C. finmarchicus*, in line with the former species having the higher levels of wax esters. However, the % of 22:1 alcohols in the wax esters of *C. finmarchicus* is greater than in *C. glacialis* (Table 1) so that the lower levels of wax esters in *C. finmarchicus* have a higher energy content than those in *C. glacialis*. It should also be noted that 22:1 has the highest phase transition point (melting point) of all the fatty alcohols in copepod wax esters. It is present in the highest % in the wax esters of *C. hyperboreus*, the species that is likely to experience the lowest ambient temperatures for the longest period. This is not compatible with the accumulation of long-chain fatty alcohols/acids in polar copepods being an adaptation to low ambient temperatures *per se*.

The species differences in fatty alcohol compositions (Table 1) are reflected, albeit to a much lesser extent, in the fatty acid compositions of the wax esters of the 3 species (Table 2). Thus, the highest % of 22:1 fatty acid is in *Calanus hyperboreus* and the highest % of 20:1 is in *C. glacialis*. Therefore, the ratio of 22:1/20:1 fatty acids is highest in *C. hyperboreus* and lowest in *C. glacialis*, as is the case for the fatty alcohols. However, the ratios of 22:1/20:1 fatty acids are all substantially less than the ratios for the corresponding fatty alcohols in all the species, reflecting a higher abundance of 22:1 relative to 20:1 in the alcohols as compared to the acids. Nonetheless 20:1 and 22:1 fatty acids comprise ca. one-third of the total fatty acids in the species and, as noted above, the sum of 20:1 and 22:1 acids increases progressively from *C. finmarchicus* to *C. glacialis* to *C. hyperboreus*. The abundance of these fatty acids in the wax esters, together with the dominance of the corresponding fatty alcohols (formed from the corresponding fatty acids), emphasises how highly active the 3 *Calanus* species are in lipid biosynthesis.

Other than 20:1n-9 and 22:1n-11, the dominant fatty acids in the wax esters were 16:1n-7 and 20:5n-3, each present in the same % in the 3 species. Both of these fatty acids are abundant in diatoms (Sargent et al. 1987, Graeve et al. 1994), consistent with diatoms providing the main dietary precursors of the wax esters in all 3 species studied. This is supported by C16 PUFA characteristic of diatoms being present in minor but significant % in all 3 species. The flagellate markers 18:4n-3 and 22:6n-3 (Sargent et al. 1987, Graeve et al. 1994) were present in small and minor %, respectively, in all 3 species. The % of both 18:4n-3 and 22:6n-3 were higher in *Calanus hyperboreus* than in the other 2 species but the difference was significant only for 22:6n-3. Higher % of flagellate markers in the wax esters of *C. finmarchicus* and *C. glacialis* have previously been reported (Kattner & Krause 1987, Kattner et al. 1989, Albers et al. 1996), but % in wax esters in *C. glacialis* have generally been low (Tande & Henderson 1988, Albers et al. 1996). One factor underlying such apparent species differences is that *Calanus* copepods may feed selectively on diatoms or flagellates including *Phaeocystis* (Mullin 1965, Meyer-Harms et al. 1999). However, the abundance of individual algae, i.e. the species composition of the phytoplankton, which depends on the timing and stage of development of the bloom, which in turn differs in open Arctic waters from waters adjacent to ice, is probably the major factor determining which algae the copepods ingest. Although the fatty acid compositions in Table 2 are very similar between the 3 species, the standard deviations indicate that considerable variation existed between individual samples for all the species. The Stage V and female copepods studied here had accumulated their abundant wax ester reserves at times, varying from a few weeks to many months during preceding summer(s) and spring(s), before they were sampled. Consequently, the similarity of the fatty acid compositions of the wax esters in the 3 species indicates that, overall, their historical diets were similar, irrespective of when and in what location these diets were assimilated. This is not to say, however, that their individual diets do not vary significantly in time and space.

Although diatom biomarkers dominated the fatty acids of the copepods' wax esters, the flagellate biomarker 22:6n-3 consistently dominated the fatty acids in their polar lipids, i.e. this fatty acid is preferentially directed by the copepods to phospholipids. It is possible that dietary 20:5n-3 derived from diatoms is converted to 22:6n-3 by the copepods, but this is unlikely because flagellates, including dinoflagellates and prymnesiophytes, commonly contain around 4 times as much 22:6n-3 as 18:4n-3 (Sargent et al. 1995a). The small % of flagellate-derived 18:4n-3 in the copepods' wax esters fatty acids indicates that the dietary input of flagellate-derived 22:6n-3 is likely to be sufficiently large to account for its presence in phospholipids. Because diatom-derived fatty acids

dominate the copepods' wax esters, the contribution of flagellates to the copepods' diet is underestimated by considering the wax esters alone. Nonetheless, polar lipid is a minor albeit a very important constituent of the copepods' total lipid relative to wax esters (accounting for ca. 14% of the total lipid) so that, overall, diatoms contribute substantially more fatty acids to the copepods' lipids than do flagellates. Very high % of 22:6n-3 and the correspondingly high ratio of 22:6n-3/20:5n-3 (2:1) in the copepods' polar lipids have been recorded previously (Lee 1974, Tande & Henderson 1988, Albers et al. 1996). This is in contrast to the situation in other polar zooplankton, e.g. gammarids (Scott et al. 1999) and euphausiids (Falk-Petersen et al. 2000), where 20:5n-3 is the dominant fatty acid in polar lipid and the ratio of 22:6n-3/20:5n-3 is generally ca. 1:2.

Clearly the % of 20:5n-3 and 22:6n-3 in marine invertebrate phospholipids are regulated much more tightly than those in neutral lipids, even though the PUFA in question are derived, often in variable amounts, from the copepods' diets. Such tight regulation is presumably determined genetically and is essential to maintain specific cellular function(s). One such function is the critical role 22:6n-3 has in neural tissues of vertebrates including fish, where it is concentrated in nerve synaptosomal junctions (Sargent et al. 1993). Some copepods have recently been shown, unusually for invertebrates, to have myelinated nerves (Davis et al. 1999) which facilitate their fast strike and escape responses. Interestingly, Lee (1974) reported that the phospholipids of *Calanus hyperboreus* contained high levels (15 to 19%) of sphingomyelin, a major lipid in myelin. However, whether there are specialisations in nerve junctions in copepods involving 22:6n-3 remains to be investigated. Shulman & Yakovleva (1983) noted that the levels of 22:6n-3 in marine and freshwater fish are correlated closely with their level of mobility. Yuneva et al. (1992) reported that mobile, predatory euphausiids that make lengthy migrations have higher levels of 22:6n-3 than less active, non-predatory forms. The biological function(s) of 22:6n-3 in organisms remains elusive, but Rabinovich & Ripatti (1990) demonstrated that the molecule is conformationally stable over a wide range of temperatures and pressures ensuring that critical tissue functions, e.g. neural functions, can operate efficiently over a wide range of temperatures and pressures. This view of 22:6n-3 differs from the traditional concept that it is principally concerned with ensuring cell membrane fluidity at low ambient temperature. The latter concept has often been questioned, not least on the grounds of the phase transition points of individual PUFA and that the content of 22:6n-3 in marine animal phospholipids does not readily correlate with ambient temperature (e.g. Sargent et al. 1995b). The possibility that 22:6n-3 has special properties in copepods relating to their mobility and migrations rather than to adaptation to low temperatures is worthy of future research. This is but one aspect of the fascinating interplay between environment (including diet) and genetics in high-latitude zooplankton.

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