Marine Biology 62, 131–137 (1981)

MARINE BIOLOGY

Ecological Investigations on the Zooplankton Community in Balsfjorden, Northern Norway: Lipids and Fatty Acids in *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* During Mid-Winter

J. R. Sargent[1] and S. Falk-Petersen[2]

[1] Natural Environment Research Council, Institute of Marine Biochemistry; St. Fittick's Road, Aberdeen AB1 3RA, Scotland
[2] Aquatic Biology Group, Institute of Biology and Geology, University of Tromsø; P.O. Box 790, N-9001 Tromsø, Norway

Abstract

Total lipid of *Meganyctiphanes norvegica* (M. Sars) contained 53% triacylglycerols and traces of wax esters, that of *Thysanoessa raschi* (M. Sars) contained 44% triacylglycerols and 10% wax esters and that of *T. inermis* (Krøyer) contained 28% triacylglycerols and 40% wax esters. The triacylglycerols of *M. norvegica* were relatively rich in 20:1 and 22:1 fatty acids and its traces of wax esters resembled those of calanoid copepods. The triacylglycerols of both *Thysanoessa* species were deficient in 20:1 and 22:1 fatty acids but were richer in 16:1 ($n-7$) and 18:1 ($n-7$) acids than those of *M. norvegica*. The wax esters of *T. raschi* contained phytol as almost the only fatty alcohol and were rich in 16:0 and 18:1 ($n-9$) fatty acids. The wax esters of *T. inermis* contained mainly 16:0 and 14:0 fatty alcohols with lesser amounts of phytol and their dominant fatty acid was 18:1, especially the ($n-9$) isomer. The triacylglycerols of *T. inermis* had 18:4 ($n-3$) as the major polyunsaturated fatty acid. From these and other aspects of fatty acid and fatty alcohol analyses it is concluded that a major foodstuff of *M. norvegica* in Balsfjorden is wax ester-rich calanoid copepods. *T. raschi* and especially *T. inermis* are concluded to have much more preference for phytoplanktonic food. Results are discussed in terms of current knowledge of the lipid chemistry of krill in the northern and southern hemispheres.

Introduction

Lee and Hirota (1973) proposed that wax esters were elaborated in large amounts by marine animals, especially zooplanktonic species, present in environments where short periods of food plenty were followed by long periods of food shortage. This situation is likely to occur especially in polar regions where primary production, although intense, occurs over a short period. Arctic herbivorous calanoid copepods such as *Calanus hyperboreus* have high levels of wax esters that are rich in 20:1 and especially 22:1 fatty alcohols (Lee, 1974; reviewed by Sargent *et al.*, 1978).

The euphausiids (krill) form a large part of the polar zooplanktonic biomass (Mauchline and Fisher, 1969) and in recent years attention has focussed on the possible commercial exploitation of krill, especially in the Antarctic (Eddie, 1977). The major Antarctic krill, *Euphausia superba*, contains at most traces of wax esters but is instead rich in triacylglycerols (Raymont *et al.*, 1971; Bottino, 1975; Clarke, 1980). Its more southerly neighbour, *E. crystallorophias*, which is commonly found close to or under the ice edge, is rich in wax esters that contain 14:0 and 16:0 as their major fatty alcohols (Bottino, 1975).

Krill are found in abundance in the Barents Sea and in fjords in Northern Norway (Hopkins *et al.*, 1978). Balsfjorden, near Tromsø in Northern Norway, contains three krill species, *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis*, that have been subjected to detailed biological analyses in recent years (Hopkins *et al.*, 1978; Falk-Petersen, 1981; Falk-Petersen and Hopkins, 1981; Falk-Petersen *et al.*, 1981). The present study was undertaken to characterise the lipids of these krill in detail to further our understanding of the role of lipids and fatty acids in the marine food-chain at high latitudes.

Materials and Methods

Euphausiids

Individuals of *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa raschi* (M. Sars) and *T. inermis* (Krøyer) were caught in Balsfjorden, near Tromsø, during November and December of 1976 and 1977 by methods already detailed (Falk-Petersen, 1981). The present

0025-3162/81/0062/0131/$01.40

Table 1. *Meganyctiphanes norvegica, Thysanoessa raschi* and *T. inermis*. Lipid class composition during mid-winter

	M. norvegica	*T. raschi*	*T. inermis*
Wet wt (mg)	533.3 ± 92.1(8)	76.4 ± 5.9(12)	98.3 ± 11.3(5)
Dry wt (mg)	136.9 ± 23.7	19.5 ± 1.6	30.5 ± 4.3
Total lipid (% dry wt)	41.9 ± 2.8	40.2 ± 1.7	53.9 ± 1.7
Lipid class composition (%)			
phospholipids	11.4 ± 0.4	19.8 ± 1.5	14.0 ± 1.3
monoacylglycerols	6.0 ± 0.3	8.0 ± 0.3	6.8 ± 0.6
sterols	6.7 ± 0.6	6.8 ± 0.7	3.3 ± 0.8
free fatty acids	19.1 ± 1.4	11.5 ± 1.3	8.3 ± 1.1
triacylglycerols	53.3 ± 3.4	43.6 ± 2.6	27.5 ± 1.3
unknown	3.5 ± 1.5	0.0	0.0
wax esters	0.0	10.3 ± 1.5	40.1 ± 2.7

work was based on 5 male + 3 female *M. norvegica*, 6 male + 6 female *T. raschi* and 4 male + 1 female *T. inermis*. The wet weights, dry weights and percentage total lipid present in the dry weight of these euphausiids have already been published (Falk-Petersen, 1981). Mean values are shown in Table 1.

Lipid Analyses

Individual euphausiids were dried in desiccators over silica gel at ambient temperature until constant weight was reached. Total lipid was extracted from each specimen by the method of Folch *et al.* (1957) and made to a concentration of 10 mg ml^{-1} in chloroform:methanol (2:1 by volume). Samples were chromatographed on thin layers of silicic acid (250 μm thick) using hexane: diethyl ether:acetic acid (90:10:1 by volume) as solvent, stained with the copper acetate-phosphoric acid reagent of Fewster *et al.* (1969) and analysed by quantitiative densitometry as detailed by Sargent *et al.* (1977). Samples of total lipid from all individuals in a given species were then pooled and subjected to preparative chromatography under the same conditions as above. Plates were stained lightly with dichlorofluorescein and individual lipid classes detected by reference to known standards. Wax ester, triacylglycerol and free fatty acid zones were eluted with diethyl ether; the origin zone which was equated with phospholipid was eluted with chloroform:methanol (2:1 by volume). Eluates were extracted with 2% $KHCO_3$ twice to remove dichlorofluorescein, twice with water and dried under a stream of nitrogen.

Wax esters were saponified using potassium *t*-butoxide (Lee *et al.*, 1971), the reaction mixture acidified with HCl, extracted with hexane and the hexane phase subjected to preparative thin-layer chromatography using hexane:diethyl ether:acetic acid (70:30:1 by volume. Light staining with dichlorofluorescein revealed the presence of free fatty acids and free fatty alcohols with traces of wax esters; sterols were not detected. Free fatty acid and free fatty alcohol zones were recovered as previously, using diethyl ether as eluting agent.

Methyl esters of free fatty acids, phospholipids and triacylglycerols were prepared using H_2SO_4-catalysed methylation in methanol (Christie, 1973). Fatty alcohol acetates were prepared by reacting with acetic anhydride in pyridine (Farquhar, 1962). Trimethylsilyl (Me_3Si) derivatives of fatty alcohols were also prepared by reacting small quantities of alcohols with 20 μl of bis (trimethylsilyl)-trifluoroacetamide (Applied Science) for 20 min at room temperature and removing excess reagent under a stream of nitrogen. Methyl esters of fatty acids and fatty alcohol acetate or Me_3Si derivatives were analysed by gas-liquid chromatography using a Packard 429 gas chromatograph (Packard Instruments Ltd) equipped with open glass capillary tubing 25 m in length and 0.25 mm internal diameter and coated with the liquid-phase silar-5CP. The injection port and flame ionisation detector were operated at 250 °C and the oven was programmed to rise from 160° to 200 °C over a 45 min period. Helium was used as carrier gas. Analyses were also carried out under similar conditions using a CP-Wax 51 fused silica capillary column (25 m × 0.22 mm) (Chrompack Nederland, B.V.). Individual fatty acids and fatty alcohols were identified by reference to known standards and to the data of Ackman and Eaton (1978). Derivatives of fatty alcohols were also analysed by combined gas-liquid capillary chromatography-mass spectrometry using a Finnigan 4000 quadropole mass spectrometer coupled to a Finnigan 9610 capillary gas chromatograph as detailed by Volkman *et al.* (1980).

Results

Lipid class analyses were carried out on individual specimens of *Meganyctiphanes norvegica, Thysanoessa raschi* and *T. inermis*. No marked differences were noted between sexes nor were there obvious differences between individuals within a given species. For these reasons data in Table 1 are presented as means ± SE.

More than one half of the total lipid in *Meganyctiphanes norvegica* occurs as triacylglycerols (Table 1). One fifth is free fatty acids. Sterols and "monoacylglycerols", the latter identified only by mobility on

Table 2. *Meganyctiphanes norvegica*. Fatty acid and fatty alcohol analyses of lipids. Data are expressed as wt %. Lipid classes were isolated and derivatives prepared for GLC analyses as detailed in "Materials and Methods". Dash indicates < 0.5%; PUFA: polyunsaturated fatty acids

Constituent	Free fatty acid	Phospholipid	Triacylglycerol	Wax esters	
				Acid	Alcohol
14:0	6.8	7.1	8.0	1.0	1.2
16:0	26.8	22.6	19.2	31.3	16.4
16:1(*n* −7)	4.3	2.7	4.5	3.3	–
16 PUFA	1.4	–	2.1	–	–
Phytanate	–	–	0.7	–	–
18:0	1.8	1.3	1.3	13.0	–
18:1(*n* −9)	11.4	18.0	14.2	28.4	2.2
18:1(*n* −7)	7.6	2.2	7.4	2.0	–
18:2(*n* −6)	2.2	1.7	1.2	1.9	–
18:3(*n* −3)	1.7	–	0.8	–	–
18:4(*n* −3)	0.6	–	1.8	–	–
20:1(*n* −9)	6.3	6.0	11.7	9.1	28.7
20:5(*n* −3)	8.7	11.1	6.6	4.1	–
20 PUFA	4.1	0.7	3.0	–	1.3
22:1(*n* −11)	2.9	–	6.3	4.8	49.7
22:6(*n* −3)	7.3	22.9	6.4	–	–
22 PUFA	1.9	1.8	0.5	–	–

chromatograms, are minor constituents. The origin zone, which was well separated from the adjacent zone of "monoacylglycerols", is presumably mainly phospholipids and accounts for about one tenth of the total lipid. About 3% of the total lipid occurred as an unknown zone, which was present in 4 out of 8 individuals analysed in the range 4 to 11% of their total lipid. This zone migrated on thin layers with a mobility similar to that of alkyldiacylglycerols or phthalate esters. It was not investigated further, nor was it present in the other species analysed. Traces of wax esters could just be seen in all individuals of *M. norvegica* but these were below the limits of detection of the analytical method used.

The major lipid in *Thysanoessa raschi* is triacylglycerols. Small but very significant amounts of wax esters are also present. The wax esters account for the same percentage of total lipid as free fatty acids, whereas phospholipids constitute some 20% of total lipid. A different situation holds for *T. inermis* where, although substantial amounts of triacylglycerols are present, the major lipid is now wax esters. Polar lipids and free fatty acids account for smaller percentages of the total lipid in *T. inermis* than in *T. raschi*.

Since large differences were not observed between the individual analyses in Table 1, the respective lipid classes in a given species were isolated from pooled samples of total lipid prior to fatty acid and fatty alcohol analyses. Sufficient amounts of the traces of wax esters in *Meganyctiphanes norvegica* could be isolated by heavily loading several preparative plates and combining the wax ester zones.

Table 2 shows the acid and alcohol analyses of the lipid classes of *Meganyctiphanes norvegica*. The major fatty acids in the triacylglycerols are 16:0 and 18:1. Of the latter the (*n* −9) isomer, oleic acid, is present in almost twice the amount of the (*n* −7) isomer, *cis*-vaccenic acid. Small amounts of 16:1 are also present. 20:1 and 22:1 fatty acids are present in the ratio 2:1 approximately. The major polyunsaturated fatty acids in the triacylglycerols are 20:5 (*n* −3) and 22:6 (*n* −3), present in equal amounts, and quite significant amounts of C_{18} polyunsaturates including 18:4 (*n* −3) are also present. Small amounts of phytanic acid were detected. In general, the percentage composition of the free fatty acids recovered from *M. norvegica* is similar to that of triacylglycerols.

The phospholipid fraction of *Meganyctiphanes norvegica* is characterised by large amounts of 20:5(*n* −3) and 22:6(*n* −3) with the latter predominating. The major saturated and unsaturated fatty acids are 16:0 and 18:1(*n* −9). Small amounts of 20:1 and lesser amounts of 18:1(*n* −7), are present but 22:1 was not detected.

The traces of wax esters in *Meganyctiphanes norvegica* contain 16:0, 20:1 and 22:1 as major fatty alcohols. The fatty acids of the wax esters are rich in 16:0, 18:0, 18:1 (*n* −9), 20:1 and 22:1 moieties. The latter two are present in the ratio approximately 2:1. The major polyunsaturate is 20:5 (*n* − 3), and 22:6 (*n* − 3) was not detected.

Table 3 shows the triacylglycerols of *Thysanoessa raschi* to have 14:0 and 16:0 as major saturated fatty acids, with lesser amounts of 18:0. The major monounsaturated fatty acids are 16:1, 18:1(*n* −9) and 18:1 (*n* −7), present in approximately equal amounts. Only traces of 20:1 and 22:1 fatty acids are present. The triacylglycerols of *T. raschi* are not particularly rich in polyunsaturates; the major component is 20:5(*n* −3), with only small amounts of 22:6 (*n* −3). Small amounts of phytanic acid were detected. The free fatty acids of

Table 3. *Thysanoessa raschi.* Fatty acid and fatty alcohol analyses of lipids. Data are expressed as wt %. Lipid classes were isolated and derivatives prepared for GLC analyses as detailed in "Materials and Methods". Dash indicates < 0.5%; PUFA: polyunsaturated fatty acids

Constituent	Free fatty acid	Phospholipid	Triacylglycerol	Wax esters	
				Acid	Alcohol
14:0	8.3	7.8	14.1	4.8	1.7
16:0	30.6	27.1	31.2	34.6	2.3
16:1(n −7)	3.8	6.2	11.9	7.8	–
16 PUFA	–	–	2.4	–	–
Phytanate	–	–	1.7	1.2	–
18:0	3.8	2.9	2.4	6.2	–
18:1(n −9)	8.2	8.2	11.6	9.1	–
18:1(n −7)	10.9	11.1	10.8	11.4	–
18:2(n −6)	3.8	2.0	1.6	1.2	–
18:3 (n −3)	–	0.5	0.6	–	–
18:4(n −3)	0.9	1.6	1.8	0.9	–
20:1(n −9)	–	–	0.7	3.9	–
20:5(n −3)	19.8	19.3	3.6	7.1	–
20 PUFA	0.9	0.6	2.4	2.5	–
22:1(n −11)	–	–	0.6	3.2	–
22:6(n −3)	6.6	10.5	0.6	–	–
22 PUFA	–	–	0.7	2.5	–
Phytol	–	–	–	–	93.0

Table 4. *Thysanoessa inermis.* Fatty acid and fatty alcohol analyses of lipids. Data are expressed as wt %. Lipid classes were isolated and derivatives prepared for GLC analyses as detailed in "Materials and Methods". Dash indicates < 0.5%; PUFA: polyunsaturated fatty acids

Constituent	Free fatty acid	Phospholipid	Triacylglycerol	Wax esters	
				Acid	Alcohol
14:0	5.5	4.2	8.5	1.6	28.8
16:0	25.8	22.5	33.7	5.6	52.0
16:1(n −7)	5.6	4.6	15.3	6.1	5.8
16 PUFA	–	3.5	1.5	–	–
Phytanate	–	–	2.3	–	–
18:0	3.7	2.6	2.8	1.5	–
18:1(n −9)	22.9	10.6	6.8	53.8	–
18:1(n −7)	15.0	10.0	12.2	22.5	–
18:2(n −6)	3.5	1.9	2.0	2.4	–
18:3(n −3)	–	–	0.8	–	–
18:4(n −3)	2.1	0.7	7.3	0.8	–
20:1(n −9)	–	5.3	0.9	–	–
20:5(n −3)	15:1	20.6	4.8	4.7	–
20 PUFA	–	2.0	–	–	–
22:1(n −11)	–	–	–	–	–
22:6(n −3)	–	9.2	–	–	–
22 PUFA	–	–	–	–	–
Phytol	–	–	–	–	10.3

T. raschi are quite different from those of the triacylglycerols, being much more similar in composition to those of the phospholipids. Both free fatty acids and phospholipids contain 16:0, 18:(n −7) and 20:5 (n −3) as major saturated, monounsaturated and polyunsaturated fatty acids, respectively. Both classes contain additionally small amounts of 14:0, 16:1 and 18:0 moieties.

A wide range of fatty acids is present in the small amounts of wax esters present in *Thysanoessa raschi*, with 16:0 and 18:1 moieties predominating. The (n −9) and (n −7) isomers present in the latter occur in very similar amounts. Small amounts of 20:1 and 22:1 fatty acids are also present, with the former predominating. The major polyunsaturate in *T. raschi* wax esters is 20:5 (n −3); small amounts of C_{18} polyunsaturates including 18:4(n −3) were detected but 22:6(n −3) was not.

A quite unexpected finding in Table 3 is that the wax esters of *Thysanoessa raschi* contain phytol as essentially their only fatty alcohol. Phytol present in the wax esters

had the same retention time as authentic phytol when analysed by capillary gas-liquid chromatography on silar-5CP as both the acetate and Me_3Si derivatives. Confirmation of phytol was obtained by combined capillary gas-liquid chromatography-mass spectrometry carried out through the courtesy of Dr. J. Volkman, Organic Geochemistry Unit, University of Bristol, England.

The triacylglycerols of *Thysanoessa inermis* have 16:0 followed by 14:0 and 18:0 as major saturated fatty acids (Table 4). The major monounsaturated fatty acid is 16:1, followed by 18:1 whose isomers (n −9) and (n −7) are present in a ratio of approximately 1:2. Only traces of 20:1 and no 22:1 fatty acids were detected in the triacylglycerols of *T. inermis*, but small amounts of phytanic acid were present. The major polyunsaturated acid was 18:4(n −3); lesser amounts of 20:5 (n −3) occurred, but 22:6 (n −3) was not detected. As is the case for *T. raschi* the free fatty acids present in *T. inermis* are quite similar to those present in phospholipids. Both classes have 16:0 and 18:1 in abundance, the (n −9) and (n −7) isomers of the latter being present in approximately equal amounts. 20:5(n −3) is the major polyunsaturated acid in both cases, although 22:6 (n −3) is well represented in the phospholipids but not in the free fatty acids. A further difference is the presence of 20:1 in phospholipids but not in free fatty acids. Neither class contained significant amounts of 22:1.

The wax esters of *Thysanoessa inermis* present a relatively simple picture (Table 4). The fatty acids are dominated by 18:1, with the (n −9) isomer present in twice the amount of the (n −7) isomer. Smaller amounts of 16:0 and 16:1 acids are also present. The acids are relatively deficient in polyunsaturates, with small amounts of 20:5 (n −3) and C_{18} moieties being present. The major alcohols present in *T. inermis* wax esters are 16:0, followed by 14:0 and lesser amounts of 16:1. Approximately 10% of the total fatty alcohols are accounted for by phytol, identified by the same methods as for *T. raschi* wax esters.

Discussion

Triacylglycerols but not wax esters were present in *Meganyctiphanes norvegica* recovered from the stomachs of whales caught in the North Atlantic Ocean, and this held also for *Thysanoessa inermis* from the same source (Ackman *et al.*, 1970). *T. raschi* caught in Saanich Inlet, British Columbia, Canada, contained triacylglycerols but not wax esters (Sargent and Lee, 1975). These earlier results could indicate that not all individuals of the species of *T. raschi* and *T. inermis* contain wax esters but rather that the presence of these lipids depends on the individual's exact location. Certainly the *Thysanoessa* species analysed in the present study were caught at relatively high latitudes. While the individuals analysed here were caught in mid-winter, it is known that *T. raschi* and *T. inermis* contain wax esters throughout the year although highest levels are found in both species, especially the former, in mid-winter (Falk-Petersen *et al.*, 1981). In a parallel study of the seasonal lipid composition of krill in Balsfjorden (Falk-Petersen *et al.*, 1981), we have considered that the lipid class composition of the three species is consistent with *M. norvegica* being mainly carnivorous whereas both *Thysanoessa* species are omnivorous, tending to consume phytoplankton when these are available during spring-summer, but consuming smaller zooplankton when phytoplankton are absent from the water later in the year. We have also commented on the fact that the levels of wax esters accumulated by the two polar euphausiids, *Euphausia crystallorophias* (studied by Bottino, 1975) and *T. inermis* (examined by Falk-Petersen *et al.*, 1981; and the present results) are substantially less than those found by Lee (1974) to be accumulated by polar calanoid copepods such as *Calanus hyperboreus*. This may be correlated with the generally omnivorous and herbivorous feeding habits of euphausiids and calanoids, respectively.

The above considerations may be amplified by considering the detailed fatty acid and alcohol composition of the various krill species. As a preliminary, however, we may consider general aspects of our present understanding of fatty acids in the marine food chain (Ackman, 1980; Sargent and Whittle, 1981). Phytoplankton are rich in 16:1(n −7) fatty acid that is formed by a Δ9 desaturase operating on 16:0 from the carboxyl end of the molecule. Oleic acid, 18:1(n −9), is a major fatty acid of most marine animal lipids, formed by a Δ9 desaturase operating on 18:0. The 20:1(n −9) present in large quantities in calanoid copepods is probably formed by carboxyl chain elongation of 18:1(n −9), whereas the 22:1(n −11) present in very large quantities in calanoids is probably formed by a Δ9 desaturase operating on 20:0 to yield 20:1(n −11), which is immediately elongated to 22:1(n −11) (Pascal and Ackman, 1976). Should the desaturase in marine animals operate only with a Δ9 specificity, then the 18:1 (n − 7) acid frequently found in large quantities in marine animal oils (Ackman, 1980) can only derive from the elongation of 16:1(n −7) that is likely to derive to a substantial extent ultimately from phytoplankton. That is, 16:1(n −7) and 18:1(n −7) in animal lipid will tend to reflect a phytoplanktonic dietary input, whereas 18:1(n −9), 20:1 (n −9) and 22:1(n −11) will reflect an animal dietary input. The latter two fatty acids are together present in very large amounts in calanoid copepods (as fatty alcohols), although they have also been found in Arctic amphipods and decapods (Lee, 1975). 22:1 fatty acid is not present in significant amounts in phytoplanktonic material. C_{16}, C_{18}, C_{20} and C_{22} polyunsaturated fatty acids are all well represented in phytoplankton, although marine animals tend to deposit C_{20} and C_{22} polyunsaturates in their phospholipids, presumably removing C_{16} and C_{18} polyunsaturates either through chain elongation to higher derivatives or through catabolism (reviewed by Sargent and Whittle, 1981). That is, the shorter chain C_{16} and C_{18} polyunsaturates tend to be more characteristic of phytoplankton. Finally, it is to be emphasised

that these are guiding principles only, that can not be regarded as definite rules of general application.

On the basis of the foregoing considerations, it can be concluded that *Meganyctiphanes norvegica* in the present study is a carnivore consuming large amounts of wax ester-rich calanoid copepods. Thus, its major lipid, triacylglycerols, is relatively deficient in 16:1(n −7), and 18:1(n −7) is a minor fraction of total 18:1, both of which indicate a relatively small input of phytoplanktonic lipid. 20:1 and 22:1 fatty acids in the ratio 2:1 are prominent in its triacyglycerols, consistent with a large dietary input of 20:1 and 22:1 fatty alcohols from calanoid copepod wax esters. It is notable that the ratio of 20:1/22:1 fatty alcohols in calanoid wax esters is commonly less than unity, whereas the corresponding ratio in the 20:1 and 22:1 fatty acids found in large quantities in zooplanktonivorous fish, e.g. capelin, is commonly greater than unity, indicating selective catabolism of 22:1 with respect to 20:1 (Jangaard, 1974). The lipids in *M. norvegica* are generally deficient in C_{16} and C_{18} polyunsaturated fatty acids, but the phospholipids have 22:6(n −3) as their major polyunsaturate. These results are consistent in the main with an input of animal rather than phytoplanktonic polyunsaturates into *M. norvegica*. Finally, the composition of the traces of wax esters present in *M. norvegica* is very characteristic of calanoid wax esters, particularly in terms of the fatty alcohol composition. It is probable that such wax esters are present in those stomach contents of the euphausiid stemming directly from dietary calanoids. We conclude that a calanoid copepod rich in wax esters, probably *Calanus finmarchicus*, is a major item in the diet of *M. norvegica* in Balsfjorden.

With respect to *Thysanoessa raschi* we note initially that 20:1 and 22:1 fatty acids are minor components of its lipids (Table 3). Wax ester-rich calanoids such as *Calanus finmarchicus* cannot be major components of its diet, although non wax-ester containing calanoids are not excluded. *T. raschi* has substantial amounts of 16:1 in its triacylglycerols, and 18:1(n −7) accounts for about one half of the total 18:1. This can be interpreted as being consistent with a very significant dietary input of phytoplankton. Finally, the major polyunsaturated fatty acid in *T. raschi* is 20:5(n −3), with 22:6(n −3) being present in much smaller amounts. This is consistent with *T. raschi* being closer trophically to phytoplankton than is *Meganyctiphanes norvegica*.

The relatively small quantities of wax esters present in *Thysanoessa raschi* contain essentially only phytol as fatty alcohol. The latter is a true phytoplanktonic marker, that can only be derived from chlorophyll, phaeophytin or some other phytoplanktonic material such as the phytyl esters present in the eye spot of a dinoflagellate (Withers and Nevenzel, 1977). Parallel analyses have shown that the wax esters in *T. raschi* are maximal during mid-winter when living phytoplankton is essentially absent from Balsfjorden (Falk-Petersen *et al.*, 1981). A dietary input of phytol could stem from detrital feeding on bottom sediments, or the relatively abundant non-living particulate material suspended in the water column during mid-winter (Falk-Petersen *et al.*, 1981).

Considerations of fatty acid and fatty alcohol composition in *Thysanoessa inermis* (Table 4) suggest that its diet is even more phytoplanktonic than that of *T. raschi*. Thus 16:1 is present in substantially greater amounts and the ratio 18:1 (n −7)/18:1 (n −9) is higher in *T. inermis* than in *T. raschi*. Minor and major amounts of 22:6(n −3) and 20:5(n −3), respectively, occur in *T. inermis*, with 18:4(n −3) being the major polyunsaturated fatty acid in its triacylglycerols. Previous analyses of *Calanus finmarchicus* from Norwegian fjords have shown that 18:4(n −3) is a major component of the fatty acids of its wax esters (Volkman *et al.*, 1980). It is probable that 18:4(n −3) derives directly from the phytoplankton. Major phytoplankters in Balsfjorden include *Phaeocystis pouchetti*, diatoms and dinoflagellates (Eilertsen, 1979). Preliminary analyses have established that a *P. pouchetti*-rich sample of mixed phytoplankton from Balsfjorden contains 21% of its total fatty acids as 18:4(n −3) (Sargent, unpublished data).

Phytol accounts for only 10% of the total fatty alcohols of the wax esters of *Thysanoessa inermis*, although this species has both a higher proportion of its total lipid as wax esters and a higher lipid content than *T. raschi*. Nonetheless, the absolute levels of phytol per individual *T. raschi* are approximately twice that per individual *T. inermis*, indicating that the detrital feeding habit discussed earlier is less well developed in *T. inermis*.

The results of the present fatty acid and fatty alcohol analyses of the krill in Balsfjorden are, therefore, consistent with the conclusion that *Meganyctiphanes norvegica* is mainly carnivorous, consuming wax ester-rich copepods, whereas *Thysanoessa raschi* – and especially *T. inermis* – are mainly herbivorous. This conclusion supports the contention that it is the high-latitude herbivores that accumulate the highest levels of wax esters.

It is interesting finally to compare all euphausiid species so far analysed in both northern and southern hemispheres. Both the present results and those of Ackman *et al.* (1970) indicate that *Meganyctiphanes norvegica* in the northern hemisphere consumes large quantities of wax ester-rich calanoids; this holds for none of the other euphausiids so far examined. Triacylglycerols have not yet been analysed in detail in the Antarctic *Euphausia crystallorophias*, although both wax ester content and the composition of the wax esters of this species are very similar to those of *Thysanoessa inermis* in the present study (Bottino, 1975). *E. crystallorophias* has 14:0 followed by 16:0 as its major fatty alcohols (Bottino, 1975), whereas the reverse is true for *T. inermis* (Table 4). The wax esters of *E. crystallorophias* do not contain phytol (Bottino, 1975), but it is still reasonable to conclude that this species like *T. inermis* has marked herbivorous tendencies. Certainly, the lipids of *E. crystallorophias*, like those of both *T. inermis* and *E. superba*, do not contain significant amounts of 20:1 and 22:1 acids (Bottino, 1975; Clarke, 1980). *E. superba* has only traces of wax esters, and the composition of its major lipid, triacylglycerols (described

by Clarke, 1980) is not too dissimilar from that of *T. raschi*. On these bases, *E. superba* may be categorised mainly as a herbivore. It is perhaps remarkable that the present biochemical classification based on lipid analyses should group *E. superba* with *T. raschi* and *E. crystallorophias* with *T. inermis*, leaving *M. norvegica* in isolation. The placing of other euphausiids in such a classification is awaited with interest.

Literature Cited

Ackman, R. G.: Fish lipids. *In*: Advances in fish science and technology, pp 86–103. Ed. by J. J. Connell. Farnham, Surrey, England. Fishing News Books Ltd. 1980

Ackman, R. G. and C. A. Eaton: Some contemporary applications of open-tubular gas liquid chromatography in analyses of methyl esters of longer-chain fatty acids. Fette Seifen Anstr-Mittel *80*, 21–37 (1978)

Ackman, R. G., C. A. Eaton, J. C. Sipos, S. N. Hooper and J. D. Castell: Lipids and fatty acids of two species of North Atlantic krill (*Meganyctiphanes norvegica* and *Thysanoessa inermis*) and their role in the aquatic food web. J. Fish. Res. Bd Can. *27*, 513–533 (1970)

Bottino, N. R.: Lipid composition of two species of Antarctic krill: *Euphausia superba* and *E. crystallorophias*. Comp. Biochem. Physiol. *50B*, 479–484 (1975)

Christie, W. W.: Lipid analysis, 338 pp. Oxford: Pergamon Press 1973

Clarke, A.: The biochemical composition of *Euphausia superba* Dana from South Georgia. J. exp. mar. Biol. Ecol. *43*, 221–236 (1980)

Eddie, G. O.: The harvesting of krill, 76 pp. Rome: FAO 1977. (United Nations Development Programme, Southern Oceans Fisheries Survey Programme No. GLO/SO/77/2)

Eilertsen, H. Chr.: Planteplankton, minimumskaftorer og primaer produksjon i Balsfjorden, 1977, 130 pp. Candidat real thesis in marine biology, University of Trømso 1979

Falk-Petersen, S.: Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: seasonal changes in body weight and the main biochemical composition of *Thysanoessa inermis* (Krøyer), *T. raschii* (M. Sars) and *Meganyctiphanes norvegica* (M. Sars) in relation to environmental parameters. J. exp. mar. Biol. Ecol. *49*, 103–120 (1981)

Falk-Petersen, S., R. R. Gatten, J. R. S. Sargent and C. C. E. Hopkins: Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: seasonal changes in the lipid class composition of *Meganyctiphanes norvegica, Thysanoessa raschii* and *Thysanoessa inermis*. J. exp. mar. Biol. Ecol., (In press) (1981)

Falk-Petersen, S. and C. C. E. Hopkins: Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: population dynamics of the euphausiids *Thysanoessa inermis* (Krøyer), *Thysanoessa raschii* (M. Sars) and *Meganyctiphanes norvegica* (M. Sars) in 1976 and 1977. J. Plankton Res. (In press). (1981)

Farquhar, J. W.: Identification and gas-liquid chromatographic behaviour of plasmalogen aldehydes and their acetal, alcohol and acetylated alcohol derivatives. J. Lipid Res. *3*, 21–30 (1962)

Fewster, M. E., B. J. Burns and J. F. Mead: Quantitative densitometric thin-layer chromatography of lipids using copper acetate reagent. J. Chromat. *43*, 120–126 (1969)

Folch, J., M. Lees and G. H. Sloane-Stanley: A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. *226*, 497–509 (1957)

Hopkins, C. C. E., S. Falk-Petersen, K. Tande and H. C. Eilertsen: A preliminary study of zooplankton sound scattering layers in Balsfjorden: structure, energetics and migration. Sarsia *63*, 255–264 (1978)

Jangaard, P. M.: The capelin (*Mallotus villosus*). Biology, distribution, exploitation, utilization and composition. Bull. Fish. Res. Bd Can. *176*, 1–70 (1974)

Lee, R. F.: Lipid composition of the copepod *Calanus hyperboreus* from the Arctic Ocean. Changes with depth and season. Mar. Biol. *26*, 313–318 (1974)

Lee, R. F.: Lipids of Arctic zooplankton. Comp. Biochem. Physiol. *51B*, 263–266 (1975)

Lee, R. F. and J. Hirota: Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. Limnol. Oceanogr. *18*, 227–239 (1973)

Lee, R. F., J. C. Nevenzel and G. A. Paffenhöfer: Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar. Biol. *9*, 99–108 (1971)

Mauchline, J. and L. R. Fisher: The biology of euphausiids. Adv. mar. Biol. *7*, 1–454 (1969)

Pascal, J. C. and R. G. Ackman: Long chain monoethylenic alcohol and acid isomers in lipids of copepods and capelin. Chemy Phys. Lipids *16*, 219–223 (1976)

Raymont, J. E. G., R. T. Srinivasagam and J. K. B. Raymont: Biochemical studies on marine zooplankton, IX. The biochemical composition of *Euphausia superba*. J. mar. biol. Ass. U.K. *51*, 581–588 (1971)

Sargent, J. R., R. R. Gatten, E. D. S. Corner and C. C. Kilvington: On the nutrition and metabolism of zooplankton. XL. Lipids in *Calanus helgolandicus* grazing *Biddulphia sinensis*. J. mar. biol. Ass. U.K. *57*, 525–533 (1977)

Sargent, J. R. and R. F. Lee: Biosynthesis of lipids in zooplankton from Saanich Inlet, British Columbia, Canada. Mar. Biol. *31*, 15–23 (1975)

Sargent, J. R., R. F. Lee and J. C. Nevenzel: Marine waxes. *In*: Chemistry and biochemistry of natural waxes, pp 50–91. Ed. by P. Kolattukudy. Amsterdam: Elsevier 1978

Sargent, J. R. and K. J. Whittle: Lipids and hydrocarbons in the marine food web. *In*: Analysis of marine ecosystems, Ed. by A. R. Longhurst. London: Academic Press (In press) (1981)

Volkman, J. K., R. R. Gatten and J. R. Sargent: Composition and origin of milky water in the North Sea. J. mar. biol. Ass. U.K. *60*, 626–632 (1980)

Withers, N. W. and J. C. Nevenzel: Phytyl esters in a marine dinoflagellate. Lipids *12*, 989–993 (1977)

Date of final manuscript acceptance: January 7, 1981.
Communicated by J. Mauchline, Oban