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Lipid Composition of Some Typical North Sea Copepods

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ABSTRACT: Copepods of the southern North Sea and Wadden Sea were investigated with special respect to the main lipid groups as well as to their fatty-acid pattern. The seasonal variation of lipids of the small copepods *Temora longicornis, Acartia clausi* and *Centropages hamatus* was determined; in addition some zooplankters, which could only be sampled sporadically, were analysed. The seasonal values for total lipids from the small copepods varied between 0,3 and 1 μg ind.$^{-1}$, with maximal concentrations during spring and autumn. In addition to fatty-acid pattern, the proportions of triglycerides, phospholipids and free fatty acids were determined. The triglycerides are fixed as depot lipid with high seasonal variation in their concentrations (the main lipid component). Phospholipids exhibited less variation, which is to be expected, since they are incorporated as structural lipids in membranes. A comparison of the feeding behavior between omnivorous copepods and copepods characterized by selective feeding shows that the fatty-acid pattern seems to depend on the type of food ingested. In selective feeders the proportion of polyunsaturated fatty acids is greater than in omnivorous copepods.

INTRODUCTION

Many factors may affect the lipid composition and fatty-acid pattern, e. g. depth, temperature, salinity, geographical location, season, food availability and starvation (Lee et al., 1970; Gardner and Riley, 1972; Lee, 1974a; Mayzaud, 1976; Sargent et al., 1977). Until now, lipid composition of zooplankters has only been determined for large copepods or for mixed zooplankton (e. g. Ackman et al., 1974; Lee, 1974b; Sargent and Lee, 1975). The distribution of wax esters, the main lipid component of the copepods *Calanus* and *Pseudocalanus*, has been well reviewed (e. g. Nevenzel, 1970; Lee et al., 1971, 1972; Sargent and McIntosh, 1974; Sargent and Lee, 1975).

In this paper the lipid composition of small copepods is presented and discussed, as well as the fatty-acid pattern which indicates the types of fatty acids present at a given time. The seasonal variation of fatty acids was determined for *Temora longicornis, Acartia clausi* and *Centropages hamatus*, the dominant copepod species in the southern North Sea and the Wadden Sea. In addition, fatty-acid patterns were analyzed for copepods and other zooplankters, which could be sampled only sporadically.

METHODS

In 1977 and 1978 plankton samples were collected at about monthly intervals. The sampling site 'Lister Tief' is located near the German North Sea coast outside the Wadden Sea of Sylt between the islands of Sylt and Rømø. Samples were taken by two neustonic nets (300 μm mesh, 0 and 30 cm depth). After sampling, the living copepods were immediately identified and sorted. Only adults and copepodite stage V were used for lipid analyses. For each analysis about 100 individuals of the small copepods and 50 of the larger zooplankters were used. The copepods were stored in chloroform in glass tubes (Sovirel) at –20 °C until lipid analyses were carried out.

The copepods were crushed in a mortar and extracted with chloroform/methanol (2 : 1, v/v) using the modified methods of Folch et al. (1957) and Bligh and Dyer (1959). The lipid extracts were dried under nitrogen. One part of the extract was fractionated by means of thinlayer chromatography using silica gel HR 60 (Merck, Darmstadt). The plates were developed in the solvent system hexane/diethylether/concentrated acetic acid (80 : 20 : 1, v/v/v) (Skipski et al., 1965). They were then sprayed with half concentrated H_2SO_4

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and ashed for 30 min at 250 °C. The densitometer measurements (Spectrodensitomenter 3000, Schoeffel) were made with a slit width of 0.5 mm and at a wavelength of 500 nm. Methods for determining fatty-acid composition using gas chromatography with column materials EGSS-X and Silar 5 CP have been described previously. Fatty acids and lipid classes were quantified using internal standards (Brockmann et al., 1976; Kattner and Brockmann, 1978).

RESULTS

Lipid Content

The lipid determinations were carried out during 1977 and 1978. The neritic copepods *Temora longicornis* and *Centropages hamatus,* as well as *Acartia clausi,* were studied in particular because they were sampled during all seasons. Only in plankton samples collected during 1977 *Pseudocalanus elongatus* was present in numbers necessary for lipid analyses; the same applied to *Calanus finmarchicus,* in autumn and winter near Sylt. During summer 1977 and 1978 only a few lipid analyses of the carnivorous copepod *Anomalocera patersoni* could be performed. This neustonic copepod was found only in the surface net.

Figure 1 shows the amount of total fatty acids (FA), expressed as μg ind.$^{-1}$; it represents nearly the total lipid content. The values of these 4 main species were nearly the same and ranged between 0.3 and 1.0 μg ind.$^{-1}$. The mean values were 0.55 μg ind.$^{-1}$ for *Temora longicornis,* 0.66 for *Centropages hamatus,* 0.50 for *Acartia clausi,* and 0.62 for *Pseudocalanus elongatus.* Maximum values occurred during May and in autumn. The amount of lipids during May was highest, decreasing to minimum values during June, then rising slightly until autumn and finally decreasing again during winter. There is a significant positive linear correlation (99 % level) between the annual lipid distributions in *T. longicornis* and *A. clausi;* however between *A. clausi* and *C. hamatus* only a significant rank correlation (according to Spearman) was found.

Main Lipid Classes

Figure 2 shows the lipid components of *Temora longicornis, Acartia clausi* and *Centropages hamatus.* Phospholipids, free fatty acids and triglycerides (TG) were determined. Wax esters and diglycerides, also including similar less polar substances, occurred only in amounts less than 5 %. The absolute amount of the lipid components was calculated by means of the sum of FA, in order to obtain comparable values. In the

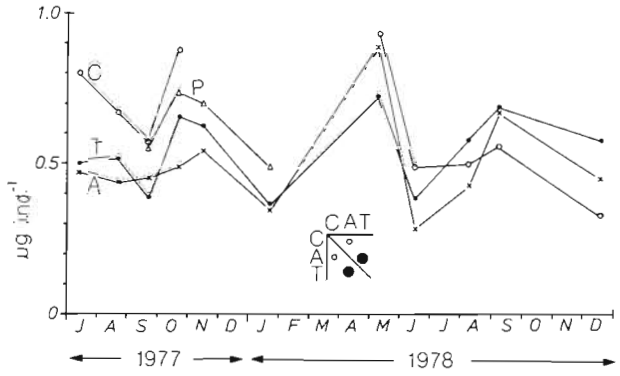

Fig. 1. Monthly concentrations of total fatty acids of *Centropages hamatus* (C); *Acartia clausi* (A), *Temora longicornis* (T), and *Pseudocalanus elongatus* (P). Inset: linear positive significant correlation • 99.0 to 99.9 %; positive significant rank-correlation ○

development of TG of the 3 main species there was a significant positive correlation (95 to 99 % level), but for *T. longicornis/A. clausi* and *A. clausi/C. hamatus* there was a highly significant correlation (99 to 99.9 % level).

During May the increase of the lipid concentration was mainly caused by the increase of TG, which rose again during autumn, with lowest concentrations in winter and during June and August. The free FA increased only slightly during May, parallel to the TG with a second maximum in autumn. A significant correlation of free FA existed only between *Temora longicornis* and *Centropages hamatus.* The phospholipids showed only a slight variation in distribution over the

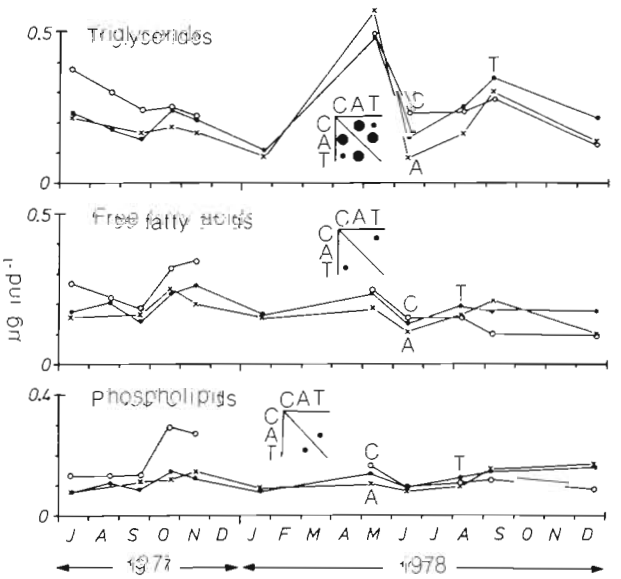

Fig. 2. Monthly concentrations of lipid composition of *Centropages hamatus* (C), *Acartia clausi* (A), and *Temora longicornis* (T). Inset: linear positive significant correlation ● 99.0 to 99.9 %, • 95.0 to 99.0 %

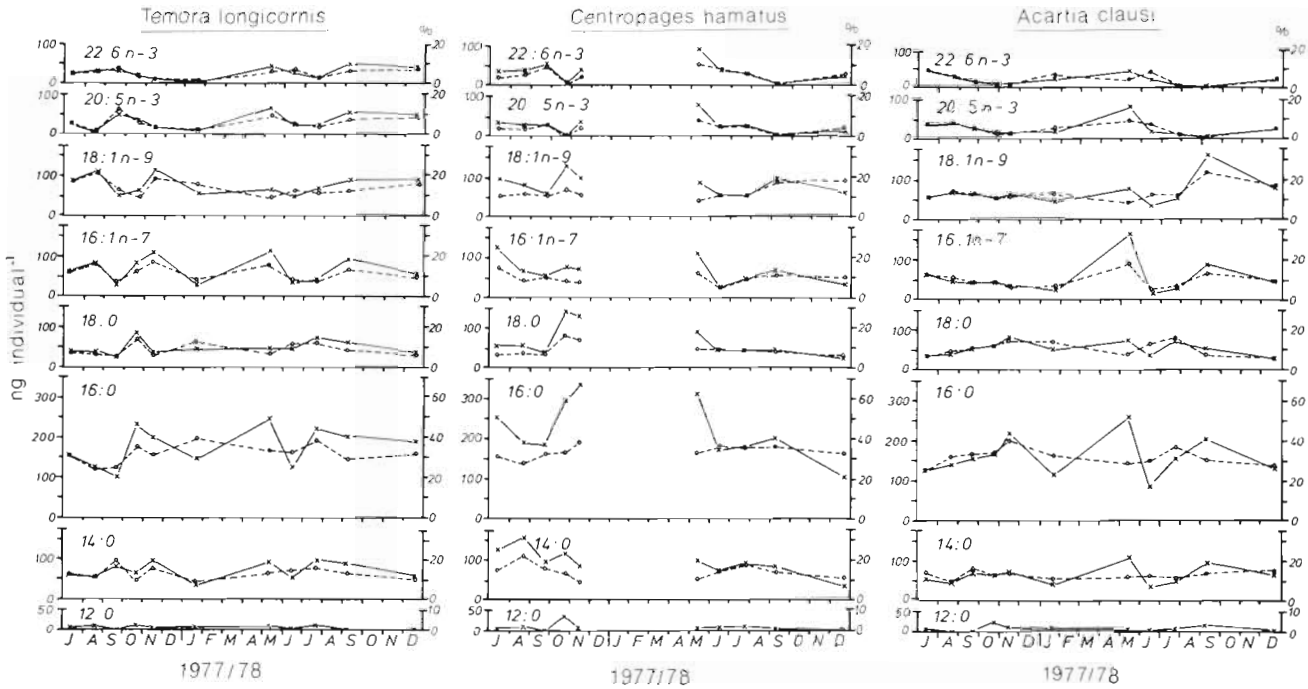

Fig. 3. Monthly concentrations of main components of fatty-acid pattern. Crosses: absolute amounts; circles: percentage composition

year. Only the phospholipid concentration of *C. hamatus* revealed a large increase in winter of 1977. A significant correlation existed between *Acartia clausi* and *T. longicornis*.

The TG were the main part of the lipid fraction. Free FA and phospholipids showed similar concentrations. During the lipid maximum in May the ratio of TG to phospholipids was 3 : 1 for *Centropages hamatus*, 5.1 : 1 for *Acartia clausi* and 3.4 : 1 for *Temora longicornis*. During winter the ratio changed to 0.8 : 1 for *C. hamatus*, 1.1 : 1 for *A. clausi*, and 1.6 : 1 for *T. longicornis*.

Fatty Acid Composition and Variation

Figures 3 and 4 show the main components of total FA for *Temora longicornis*, *Acartia clausi* and *Centropages hamatus*. In addition to the absolute amount of

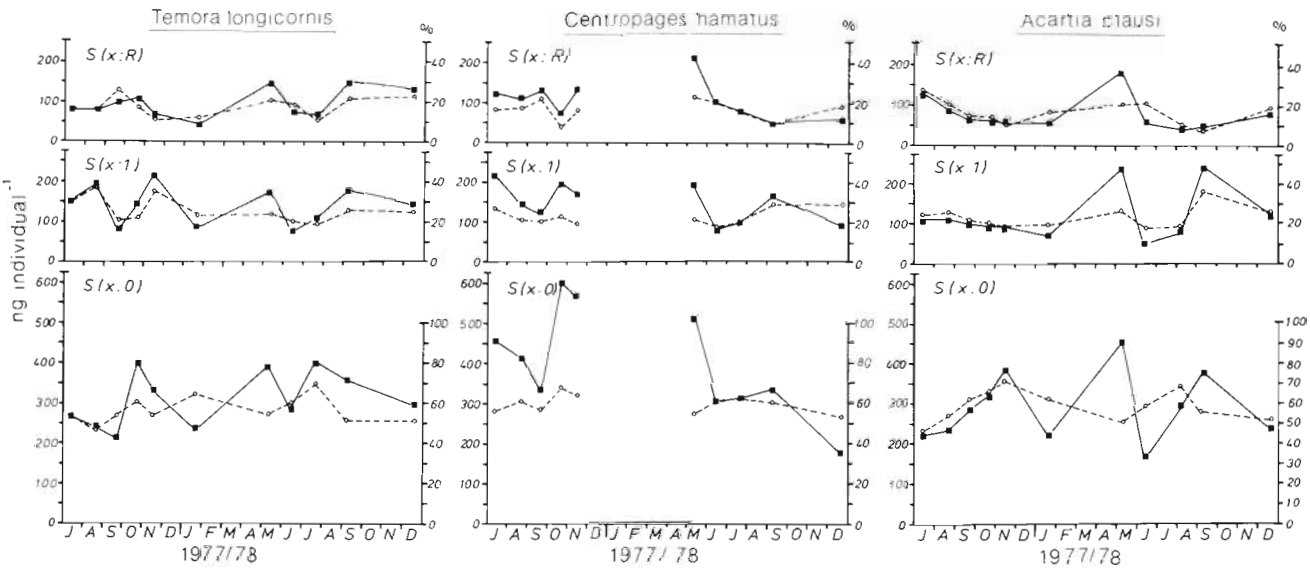

Fig. 4. Monthly concentrations of saturated FA (x:o), monosaturated FA (x:1), and polyunsaturated FA (x:R). Squares: absolute amounts; circles: percentage composition

Table 1. Relative quantities of fatty acids (%)

Fatty acid	*Temora longicornis* n = 11 m sd	*Acartia clausi* n = 11 m sd	*Centropages hamatus* n = 10 m sd	*Pseudocalanus elongatus* n = 5 m sd	*Calanus finmarchicus* n = 2 m sd	*Anomalocera patersoni* n = 3 m sd	*Sagitta elegans* n = 1 m	*Crangon crangon* (zoeae) n = 2 m sd	Cumaceae n = 2 m sd
12 : 0	1.1±0.7	1.5±1.3	1.2±1.1	0.9±0.4	0.5±0.1	0.2±0.2	0.3	0.4±0.0	0.9±0.8
14 : 0	13.4±3.2	13.4±1.6	14.7±3.9	13.3±3.5	22.9±4.0	11.1±1.3	6.2	8.1±0.9	10.3±0.4
16 : 0	32.2±4.7	32.3±4.1	34.0±2.9	30.3±2.7	24.1±1.6	37.1±5.5	30.8	31.8±1.1	28.1±0.0
18 : 0	8.8±2.9	11.0±3.4	9.7±3.3	8.7±3.7	4.7±1.9	8.6±2.3	9.5	12.5±0.9	11.0±3.0
19 : 0	0.3±0.3	0.3±0.2	0.1±0.2	0.3±0.2					
20 : 0	0.6±0.2	0.8±0.4	0.7±0.3	0.7±0.4	0.2±0.0	0.7±0.7	1.1	1.3±1.1	0.7±0.1
S (x . 0)	56.3±6.6	59.1±7.8	60.3±4.8	54.1±6.4	52.2±7.6	57.7±7.8	47.9	54.3±4.2	51.0±4.2
16 : 1 n–7	11.6±3.6	10.1±3.8	10.1±2.7	14.3±3.7	11.1±2.8	6.8±1.7	15.8	7.6±1.1	15.1±4.6
18 : 1 n–9	14.3±3.7	13.8±4.0	12.8±3.1	16.7±4.1	8.0±0.4	11.2±5.2	12.2	25.3±3.0	20.4±5.7
S (x · 1)	25.9±6.0	23.9±5.5	23.0±4.2	31.0±7.4	19.1±2.5	18.0±3.6	28.0	32.8±2.0	35.5±1.1
16 : 2 n–6	1.2±0.3	1.0±0.4	0.9±0.3	1.4±0.3	1.2±0.1	0.6±0.1	0.5	1.0±0.4	1.7±1.1
18 : 2 n–9	0.7±0.3	1.0±0.3	1.0±0.3	0.9±0.3	1.3±0.6	0.8±0.2	0.4	1.5±1.0	0.5±0.1
18 : 3 n–9 / 20 : 1 n–3	0.9±0.4	0.9±0.5	0.8±0.5	0.7±0.3	4.3±1.0	0.7±0.6	2.4	0.9±0.1	1.0±0.3
18 : 4 n–6	0.7±0.3	1.0±0.7	0.7±0.4	0.7±0.2	0.6±0.5	1.1±0.9		0.3±0.1	0.9±0.2
20 : 2 n–6	0.2±0.2	0.1±0.1	0.1±0.1	0.1±0.1		0.1±0.2	0.2		
20 : 3 n–9	0.7±0.9	0.6±0.6	0.5±0.4	0.5±0.3	0.1±0.0	0.4±0.4	0.8	0.3±0.3	0.6±0.1
20 : 5 n–3	5.9±3.3	5.2±2.6	4.6±2.4	4.7±1.6	4.8±2.1	6.1±3.2	5.4	5.4±2.6	6.0±0.5
22 : 3 n–6 / 22 : 4 n–9	0.6±0.4	0.9±1.0	0.7±0.5	0.7±0.4	0.1±0.0	0.3±0.1	1.2	0.3±0.2	0.4±0.1
22 : 3 n–3	1.3±0.6	1.1±0.4	1.2±0.8	1.3±0.7	3.1±0.1	4.0±1.0	5.2	0.1±0.0	
22 : 6 n–3	5.1±2.2	4.5±2.8	5.7±3.1	3.3±2.5	5.1±1.5	9.1±4.7	5.5	2.6±0.7	1.7±1.0
n.i.		0.7±0.8	0.6±0.5	0.5±0.6	8.3±4.7	1.1±1.1	2.4		0.9±0.6
S (x : R)	17.8±5.0	17.0±6.2	16.7±4.8	14.8±4.9	28.7±10.1	24.4±7.4	24.0	12.9±2.1	13.6±3.2

m = mean; sd = standard deviation; for further explanations consult text

FA, the percentage composition of individual FA is shown. The saturated FA contributed 50–60 % of the total. The main component was palmitic acid (16 : 0), followed by myristic acid (14 : 0) and stearic acid (18 : 0). The monounsaturated FA accounted for 20 to 35 % of the total. They consisted, in equal amounts, of palmitoleic acid (16 : 1 n-7)* and oleic acid (18 : 1 n-9). The remainder were polyunsaturated FA, including FA typical for marine plankton such as eicosapentaenoic acid (20 : 5 n-3) and docosahexaenoic acid (22 . 6 n-3). The percentage distribution of FA exhibited smaller variations than the absolute amounts of FA. During the May maximum a slight increase of palmitoleic acid could be observed for all 3 species. The increase of monounsaturated FA after the increase of saturated FA is notable. Individual FA, as well as FA grouped according to the number of double bonds per molecule, show the same distribution as the sum of all individual FA. In particular, FA of *T. longicornis* and *A. clausi* were significantly correlated, but no correlation existed between *T. longicornis* and *C. hamatus*.

In view of the small variation in the percentage distribution of FA, Table 1 lists the mean values of individual FA for all samples of one species. In addition to *Temora longicornis, Acartia clausi* and *Centropages hamatus,* included in the table are also other species, which could not be sampled regularly in quantities necessary for lipid analyses. In comparison with the 3 main species, the proportion of polyunsaturated FA is larger in the carnivorous species *Anomalocera patersoni* and *Sagitta elegans*. In *Crangon* larvae and Cumaceae the proportion of monounsaturated FA was higher. In the lipid fraction of *Calanus finmarchicus* wax esters accounted for up to 60 %. In the fraction of *Pseudocalanus elongatus* wax esters also formed a large proportion (up to 30 %). In the other species wax esters could not be detected or were only present in very small amounts.

DISCUSSION

The small copepods *Temora longicornis, Acartia clausi, Centropages hamatus* and *Pseudocalanus elongatus* are dominant zooplankters of the German Bight.

* x : y n-z; where x = number of carbon atoms in the FA; y = number of double bonds; z = terminal C atoms of the first double bond

Chemical determinations of the composition of these relatively small copepods were thus far, scarce (Mayzaud, 1976). These small copepods are omnivorous, their feeding behavior depending on the food available (Marshall and Orr, 1966; Petipa, 1978). An adaptation to the respective ecosystem and the variation in carbon content of the organisms concerned during the year was described by Boucher et al. (1976). Regarding the complexity of the lipid variations, it seems probable that the main factor affecting the lipid composition is the natural assemblage of phytoplankton species with their different fatty acid patterns (Morris, 1971). Following the phytoplankton blooms in spring and autumn, maximum lipid concentrations were observed in copepods. Especially after the spring bloom lipid concentrations were high. The maxima were due to an increase in TG (Sargent et al., 1977) and a small increase in free FA and phospholipids. The copepods produce TG as depot lipids if the food concentration is high. In case of low food availability the TG are reduced before other lipid components are used. The increase in TG may be directly related to the lipid composition of the particulate matter available as food. An increasing build-up of lipids was observed during the stationary growth phase of phytoplankton (Collyer and Fogg, 1955; Kattner et al., 1980). It appears possible that TG of phytoplankton were directly taken over by copepods as depot lipids.

Further support for this assumption is provided by the fact that, at maximal lipid concentrations in copepods the percentage proportion of unsaturated FA increased, then decreased during summer and finally rose again during autumn. The unsaturated FA, especially the polyunsaturated FA, are characteristic of marine phytoplankton (Kates and Volcani, 1966; Ackman et al., 1968; Chuecas and Riley, 1969; Harrington et al., 1970). Selective utilization of these compounds as reserve material under conditions of low food availability seems possible (Gardner and Riley, 1972). In contrast to the TG, the concentrations of phospholipids, a nondepot lipid fraction, show only small variation. As structure lipids they are incorporated as membrane elements in cells. In the literature on lipid investigations, the free fatty-acid fraction is hardly described (Lee et al., 1970; Takahashi and Yamada, 1976). The free FA may make up a large part of the total lipids, especially lipids of small copepods, which build up no wax esters as depot lipids. Takahashi and Yamada recorded a high proportion of polyunsaturated FA in the free FA fraction. It seems that copepods may have an intracellular FA pool.

The percentage composition of FA of *Temora longicornis, Acartia clausi* and *Centropages hamatus* shows only slight differences (Tab. 1). The food of these species consists of phytoplankton, as well as detritus and younger stages of copepods. Thus, it seems appropriate to compare the FA pattern of omnivorous copepods with the FA pattern of particulate matter which has been determined near Sylt (Kattner and Brockmann, 1978). In particulate matter, the proportion of polyunsaturated FA is smaller than comparable data for individual phytoplankton species. This is due to the differential resistance of the various FA determined: saturated FA are more resistant to photolytic, biological and chemical degradation than their polyunsaturated counterparts; hence saturated FA will be concentrated in detritus. This may explain the relatively low percentage proportion of polyunsaturated FA in omnivorous small copepods. The same was found in *Crangon crangon* (zoea stages) and Cumaceae (Table 1).

In contrast to the omnivorous copepods, the proportion of polyunsaturated FA in *Calanus finmarchicus* is quite high, possibly because *C. finmarchicus* is a herbivorous copepod which grazes on phytoplankton employing a selective feeding behavior (Gamble, 1978). Typical for *C. finmarchicus* and *Pseudocalanus elongatus* is the formation of oil drops serving as reserve material. The drops consist, in particular, of wax esters. The same high porportion of polyunsaturated FA could be observed in the carnivorous zooplankters *Anomalocera patersoni* and *Sagitta elegans*. They are also selective feeders. The presence of *Anomalocera patersoni* is limited to the summer; it is found only in waters with temperatures above 15 °C (Champalbert, 1969).

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