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Calanoides acutus and *Calanus propinquus*, Antarctic copepods with different lipid storage modes via wax esters or triacylglycerols

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ABSTRACT: The dominant large copepods *Calanoides acutus* and *Calanus propinquus* were collected south of 65°S in the Antarctic Weddell Sea in late winter–early spring (October–November) and summer (January–February), and the lipid and fatty acid/alcohol compositions of copepodite stages V and females of these suspension feeders were analyzed. The lipids of *C. acutus* consisted mainly of wax esters. Major fatty acids in summer were 20:1($n-9$), 20:5($n-3$), 22:6($n-3$), 18:4($n-3$), 22:1($n-11$) and 16:1($n-7$). In winter the amount of 18:4($n-3$) decreased considerably in both stages, as did that of 20:5($n-3$) in females, whereas the quantity of 20:1($n-9$) showed a strong increase in females. During both seasons the fatty alcohols in the wax esters were strongly dominated by 20:1($n-9$) and 22:1($n-11$). In contrast, the bulk of the lipids of *C. propinquus* were triacylglycerols with the principal fatty acids 22:1($n-11$), 22:1($n-9$), 16:0, 20:5($n-3$) and 22:6($n-3$). Hence, an alternative to the paradigm of energy storage by means of wax esters, typical of *C. acutus* and almost all other calanoid copepods from polar and temperate oceans, was found for *C. propinquus*. The synthesis of these energy-rich triacylglycerols occurs via an unusual marine biochemical pathway, the elongation of the 20:1($n-9$) to the 22:1($n-9$) fatty acid. Our data show the existence of very different biochemical solutions to the problem of efficient energy storage for coping with the extreme seasonality in Antarctic waters, with short periods of food plenty interchanging with long phases of food scarcity.

INTRODUCTION

The copepods *Calanoides acutus* and *Calanus propinquus* have a circum-Antarctic distribution ranging from the Antarctic continent to the Antarctic convergence (Ottestad 1936, Andrews 1966). They are dominant species in the Southern Ocean, and in the Weddell Sea, for instance, copepodite stages IV to adults of these species can account for more than 20 % of the total zooplankton biomass (Boysen-Ennen et al. 1991). Both species have been described as primarily herbivorous suspension feeders (Hopkins 1985, 1987, Schnack 1985). Life history studies from sub-Antarctic regions have been published by various authors (e.g. Andrews 1966, Voronina et al. 1978, Marin 1988, Conover & Huntley 1991, Huntley & Escritor 1991). Very few investigations have been carried out in high-Antarctic waters, and especially data from the winter period are lacking (Schnack-Schiel et al. 1991). It is generally accepted that *C. acutus* overwinters in a resting stage (diapause) at depth, but recent data suggest that *C. propinquus* has developed different adaptations for surviving the austral winter, when phytoplankton biomass is extremely low (Nöthig et al. 1991, Schnack-Schiel et al. 1991, Hopkins et al. 1993, Marin & Schnack-Schiel 1993).

Although lipids play a major role as energy reserves, particularly for the herbivorous polar zooplankton, data on the lipid and fatty acid/alcohol compositions of Antarctic species are scarce. Andrews (1966) recorded seasonal changes of the oil sac size in different developmental stages of *Calanoides acutus*, but very few studies have addressed the lipid biochemistry of *C. acutus* and *Calanus propinquus* in detail (Clarke 1984,

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Reinhardt & Van Vleet 1986, Hagen 1988). These data include total lipid contents and lipid class compositions of *C. acutus*. Only Reinhardt & Van Vleet (1986) have presented data on the fatty acid compositions of individual lipid classes of this species. Even less is known about the lipid biochemistry of *C. propinquus*, and there are no lipid data available for this species apart from the study by Hagen (1988) on lipid contents and lipid class compositions.

Our investigation aimed at elucidating some of the biochemical adaptations of *Calanoides acutus* and *Calanus propinquus* to the extreme Antarctic environment, with special regard to their lipid storage modes. Both species differ remarkably in their major lipid classes. Emphasis was laid on a detailed analysis of the fatty acid and alcohol compositions in order to comprehend more clearly the different biochemical pathways which may enable these species to thrive in the polar environment.

MATERIALS AND METHODS

The copepods were collected in the southeastern Weddell Sea during 2 Antarctic expeditions with RV 'Polarstern' (ANT III/3: Jan–Feb 1985; ANT V/3: Oct–Nov 1986). The locations of the sampling stations are shown in Fig. 1. For detailed information concerning program and station data of these expeditions see Hempel (1985) and Schnack-Schiel (1987).

Specimens for the lipid analyses were sampled with a bongo net (mesh 335 µm) towed vertically from 300 m (ANT III/3) or 500 m (ANT V/3) to the surface. The catch was immediately transferred to a bucket filled with cold seawater (0 °C) and carried to a cooling container (4 °C), where only live and intact specimens were sorted under a stereomicroscope (Wild M5) according to species and stages. For a single sample of copepodite stages V (CV) or females of *Calanoides acutus* or *Calanus propinquus*, 45 to 200 specimens were pooled. Normally, the specimens of one stage were randomly sorted, but during the winter cruise (ANT V/3) we sometimes noticed pronounced differences in the size of the oil sac of specimens from the same catch. These specimens were then sorted according to the size of the oil sac, yielding samples with lipid-rich specimens and samples with lipid-poor specimens. The samples were immediately stored in glass vials at –80 °C.

After freeze-drying (48 h) and dry weight determination, total lipid content was measured gravimetrically (after Folch et al. 1957), using chloroform:methanol (2:1, v/v) for extraction, with 0.01 % butylhydroxytoluene (BHT) added as antioxidant. The lipid class composition was analyzed according to Fraser et al. (1985) by thin-layer chromatography-flame ionization

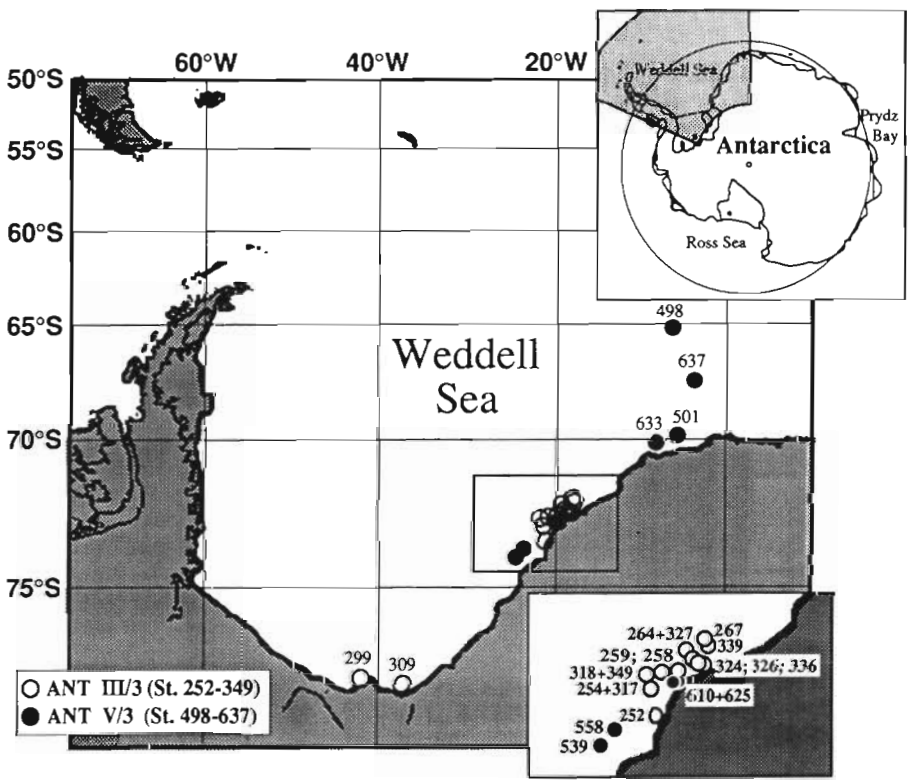

Fig. 1. Location of sampling stations during 'Polarstern' expeditions ANT III (Jan–Feb 1985) and ANT V (Oct–Nov 1986)

Table 1. *Calanoides acutus* and *Calanus propinquus*. Dry weight and lipid data (mean ± SD) for stages CV and females from summer (Jan–Feb) and late winter (Oct–Nov). Winter samples with a total lipid content <20 % DW were excluded. n: number of samples (45 to 200 specimens each); % DW: percent of dry weight; % TL: percent of total lipid; na: not analyzed

	Calanoides acutus				*Calanus propinquus*			
	CV, summer (n = 7)	CV, winter (n = 2)	Female, summer (n = 1)	Female, winter (n = 6)	CV, summer (n = 6)	CV, winter (n = 3)	Female, summer (n = 6)	Female, winter (n = 2)
Dry wt (µg ind.$^{-1}$)	625 ± 217	301 ± 153	1041	610 ± 130	1108 ± 270	884 ± 323	1762 ± 271	1264 ± 97
Lipid wt (µg ind.$^{-1}$)	281 ± 135	92 ± 76	532	220 ± 80	425 ± 254	333 ± 181	722 ± 297	377 ± 77
Total lipid (% DW)	43 ± 6	28 ± 11	51	35 ± 8	36 ± 13	35 ± 10	40 ± 11	30 ± 4
Wax esters (% TL)	92 ± 2	51 ± 3[a]	92	77 ± 3[a]	3 ± 2	na	2 ± 2	na
Triacylglycerols (% TL)	3 ± <1	na	4	na	89 ± 6	na	91 ± 6	na

[a]Calculated from fatty alcohol fraction of lipids

detection (TLC-FID) with an IATROSCAN Mark II (only summer samples were analyzed). Different standard mixtures were prepared for calibration which approximated the lipid class composition of the analyzed samples. The wax ester fraction may also include sterol esters. For details see Hagen (1988).

For gas-liquid chromatographic analysis of the fatty acid and alcohol compositions, aliquots of the extracted samples were taken. Methyl esters of fatty acids and free fatty alcohols were prepared by transesterification with 3 % concentrated sulphuric acid in methanol for 4 h at 80 °C. After their extraction with hexane the composition was analyzed with a Carlo Erba gas-liquid chromatograph (HRGC 5300) (column: 30 m × 0.249 mm; film thickness: 0.25 µm; liquid phase: DB-FFAP) using temperature programming according to the method of Kattner & Fricke (1986). Fatty acid methyl esters and alcohols were identified with standard mixtures.

RESULTS

The results of the lipid analyses from various areas of each of the 2 expeditions in the southeastern Weddell Sea (Fig. 1) were combined, but were separated into late winter and summer data sets, since the spatial variability proved to be less pronounced than the seasonal differences. The large differences in total lipid during late winter made it necessary to exclude all samples with less than 20 % lipid content as percent of dry weight (% DW). Due to their low lipid reserves, these samples did not contribute to a better understanding of energy storage mechanisms. These samples with lipid-poor specimens showed very different fatty acid and alcohol compositions, skewed towards membrane lipids.

Calanoides acutus

The dry weight of *Calanoides acutus* copepodite stages V varied between 406 and 984 µg ind.$^{-1}$; the mean dry weight was 625 ± 217 µg ind.$^{-1}$. Since females were scarce in the upper 300 m in late summer, only 1 sample (of 58 females from late January) was analyzed, with a mean dry weight of 1041 µg. The total lipid weights ranged from 146 to 504 µg ind.$^{-1}$ in CV with an average of 281 ± 135 µg ind.$^{-1}$. Females contained 532 µg of lipid ind.$^{-1}$. Total lipid contents (% DW) of CV stages in summer varied from 35 to 51 %; that of the females was 51 % (Table 1).

In late winter the 2 samples of CV had a dry weight of 192 and 409 µg ind.$^{-1}$ and a lipid weight of 38 and 145 µg ind.$^{-1}$, which corresponds to lipid contents of 20 % and 35 % DW. Dry weight of the females varied between 364 and 721 µg ind.$^{-1}$ (mean 610 ± 130 µg ind.$^{-1}$) and the lipid weight between 75 and 301 µg ind.$^{-1}$ (mean 220 ± 80 µg ind.$^{-1}$). Lipid content ranged between 21 and 42 % DW (mean 35 %) (Table 1). About half of the samples analyzed were too low in lipid content to be considered in this context.

During summer, by far the dominant lipid class in both CV stages and females was wax esters, accounting for about 90 % of total lipids. The rest were mainly phospholipids, apart from small amounts of triacylglycerols. In late winter the proportion of wax esters as calculated from the amount of fatty alcohols was lower, particularly in the CV stages (Table 1), which corresponds to the lower total lipid contents.

CV stages in summer were characterized by 6 major fatty acids: 20:1(n–9), 20:5(n–3), 22:6(n–3), 18:4(n–3), 22:1(n–11) and 16:1(n–7) (listed in order of decreasing abundance). These constituted 76 % of all fatty acids. The same major fatty acids were found in the females in summer (75 %), but with a slight change in ranks

Table 2. *Calanoides acutus* and *Calanus propinquus*. Fatty acid and alcohol compositions (% wt; mean ± SD) of total lipid in CV stages and females during summer (Jan-Feb) and late winter (Oct-Nov). -: not detected or trace amounts <0.05%

	Calanoides acutus				*Calanus propinquus*			
	CV, summer	CV, winter	Female, summer	Female, winter	CV, summer	CV, winter	Female, summer	Female, winter
Fatty acids								
14:0	2.8 ± 0.2	5.2 ± 1.5	3.6	4.9 ± 0.4	3.5 ± 0.4	3.4 ± 0.2	3.6 ± 0.3	3.6 ± 0.0
16:0	3.1 ± 0.6	4.6 ± 2.1	3.2	3.8 ± 0.7	13.0 ± 0.9	13.5 ± 0.7	12.2 ± 0.4	12.3 ± 0.3
16:1(*n*-7)	5.2 ± 1.1	8.2 ± 0.7	8.5	8.3 ± 2.3	4.2 ± 1.2	5.3 ± 0.1	3.4 ± 0.4	5.1 ± 0.2
16:1(*n*-5)	0.1 ± 0.1	0.2 ± 0.2	-	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	-
16:2(*n*-6)	0.6 ± 0.1	0.7 ± 0.2	0.7	0.6 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
16:3(*n*-3)	0.5 ± 0.2	-	0.4	-	0.3 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	-
16:4	1.8 ± 1.8	-	-	-	0.3 ± 0.1	-	0.2 ± 0.2	-
18:0	-	-	-	0.3 ± 0.3	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1
18:1(*n*-9)	4.0 ± 0.6	4.3 ± 1.0	5.0	5.9 ± 0.5	2.8 ± 0.5	2.6 ± 0.2	3.1 ± 0.6	2.6 ± 0.0
18:1(*n*-7)	1.3 ± 0.5	1.5 ± 0.2	1.0	1.4 ± 0.3	1.1 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.0
18:2(*n*-6)	1.2 ± 0.4	1.7 ± 0.5	1.7	1.9 ± 0.4	1.1 ± 0.3	1.4 ± 0.1	1.2 ± 0.3	1.3 ± 0.2
18:3(*n*-3)	0.7 ± 0.3	0.2 ± 0.3	0.8	0.7 ± 0.2	0.7 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	0.3 ± 0.0
18:4(*n*-3)	10.2 ± 5.4	1.9 ± 1.5	9.1	3.2 ± 2.3	4.1 ± 0.6	1.0 ± 0.1	4.0 ± 0.5	0.7 ± 0.1
20:1(*n*-9)	21.6 ± 1.1	23.7 ± 0.7	17.9	27.7 ± 5.3	2.7 ± 0.2	2.7 ± 0.1	2.9 ± 0.4	2.9 ± 0.1
20:1(*n*-7)	0.8 ± 0.1	0.8 ± 0.2	0.9	1.0 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
20:4	2.0 ± 0.9	1.1 ± 0.5	1.9	1.5 ± 0.5	1.2 ± 0.3	0.7 ± 0.3	1.1 ± 0.1	0.5 ± 0.0
20:5(*n*-3)	19.2 ± 3.5	19.4 ± 6.8	20.1	14.9 ± 5.7	13.9 ± 1.7	8.3 ± 0.9	12.4 ± 1.1	7.6 ± 0.4
22:1(*n*-11)	9.2 ± 0.9	10.0 ± 2.4	9.4	10.4 ± 2.2	17.7 ± 2.3	25.2 ± 1.9	19.9 ± 1.6	27.9 ± 0.3
22:1(*n*-9)	3.4 ± 0.4	3.1 ± 0.8	4.0	4.3 ± 0.6	19.2 ± 2.8	23.3 ± 0.4	20.9 ± 2.0	24.0 ± 0.7
22:5(*n*-3)	1.3 ± 0.3	0.9 ± 0.9	1.4	0.6 ± 0.3	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.8 ± 0.2
22:6(*n*-3)	11.1 ± 1.0	12.3 ± 5.6	10.1	8.2 ± 1.9	10.5 ± 1.5	7.3 ± 1.1	9.6 ± 1.7	7.3 ± 0.2
Alcohols								
14:0	6.3 ± 0.4	6.7 ± 3.0	7.5	6.3 ± 2.3	-	-	-	-
16:0	7.1 ± 1.1	8.1 ± 3.3	8.4	7.9 ± 2.4	-	-	-	-
16:1(*n*-7)	2.1 ± 0.6	2.2 ± 1.1	4.3	2.1 ± 1.3	-	-	-	-
18:1(*n*-9)	1.4 ± 0.3	1.3 ± 0.6	1.6	1.4 ± 0.5	-	-	-	-
20:1(*n*-9)	57.8 ± 1.5	55.1 ± 0.4	49.5	55.7 ± 1.8	-	-	-	-
22:1(*n*-11)	25.4 ± 2.6	26.6 ± 7.6	28.7	26.7 ± 6.2	-	-	-	-

(Table 2). In general, the variability in fatty acid composition of CV from the different areas was rather low. Especially the monounsaturated fatty acids 20:1(*n*-9) and 22:1(*n*-11) showed little variation. In contrast, 18:4(*n*-3) had a high variation coefficient of about 50 % of the mean, with lower amounts of only 6 % of all fatty acids being found at Stns 299, 309, 317 & 336 and higher amounts of about 16 % at Stns 252, 267 & 327 (Fig. 1). The fatty alcohol compositions of CV stages and females were similar and more uniform than those of the fatty acids. The principal alcohols of CV stages were 20:1(*n*-9) and 22:1(*n*-11), which made up 58 % and 25 % of total alcohols, respectively (Fig. 2). The variation in 22:1 was clearly higher than that in 20:1. Minor components were the shorter-chain alcohols 14:0 and 16:0 (Table 2).

In the late winter CV stages and females only 5 of the above 6 principal fatty acids were found in large amounts, constituting 74 % and 70 % of total fatty acids, respectively. The 18:4(*n*-3) acid was now present at only 2 to 3 %. This decrease was compensated for by a general increase in most other components, the greatest increase (from 18 % to 28 %) occurring in the 20:1(*n*-9) fatty acid in females. The variability of fatty acid composition was usually higher in winter than in summer. The alcohol composition was very similar to that of the summer specimens.

Calanus propinquus

In summer dry weights of *Calanus propinquus* CV stages ranged from 763 to 1543 µg ind.$^{-1}$. The average weight of 1108 ± 270 µg ind.$^{-1}$ was nearly 2 times higher than for *Calanoides acutus* CV stages, similar to the higher lipid weights (range 210 to 859 µg ind.$^{-1}$). From these values, a lipid content of 22 to 56 % DW was calculated. The females were also heavier than *C. acutus* females, with dry weights between 1418 and 2168 µg ind.$^{-1}$ and lipid weights of 428 and 1189 µg ind.$^{-1}$. This corresponded to a lipid content of 26 to 55 % DW. Dry weights in late winter were lower than

those in summer, ranging from 515 to 1116 µg ind.$^{-1}$ in CV and 1195 to 1332 µg ind.$^{-1}$ in females. Lipid weights were also lower, especially in the females (CV: 124 to 447 µg ind.$^{-1}$; females: 322 to 431 µg ind.$^{-1}$). Total lipid content was similar in stages CV in both seasons but lower in the females in winter (Table 1). Note that samples with low lipid contents (<20 %) were excluded from this study.

In contrast to the wax esters in *Calanoides acutus*, triacylglycerols were the dominant lipid class in *Calanus propinquus*. In summer they accounted for about 90 % in both stages. No results are available for late winter (Table 1).

The most striking difference in fatty acid composition between *Calanus propinquus* and *Calanoides acutus* was the occurrence of 22:1(n–9) as one of the 2 major fatty acids in *C. propinquus*, accounting for 19 to 24 % of the total, at the expense of 20:1(n–9) (<3 %). The proportion of 22:1(n–11) (18 to 28 %) was of the same order of magnitude as its isomer 22:1(n–9). Together with the fatty acids 16:0, 20:5(n–3) and 22:6(n–3), they made up between 74 and 80 % in both stages. Separate analysis of the fatty acid compositions of the phospholipids and triacylglycerols showed that the two 22:1 isomers were not exclusively confined to the triacylglycerol fraction – they were also a major component of the phospholipids (Kattner unpubl.). 16:1(n–7) and 18:4(n–3), which belonged to the less important of the major fatty acids in *C. acutus*, occurred only in small amounts in *C. propinquus* (1 to 5 %). The fatty acid compositions of stages CV and females from the same season were very similar (Table 2, Fig. 2). In winter specimens of *C. propinquus* the 2 isomers of the long-chain monounsaturated fatty acid 22:1 were even more abundant, together accounting for 49 % in stages CV and 52 % in females. This was associated with lower proportions of the polyunsaturated fatty acids 20:5(n–3) and 22:6(n–3) (Table 2).

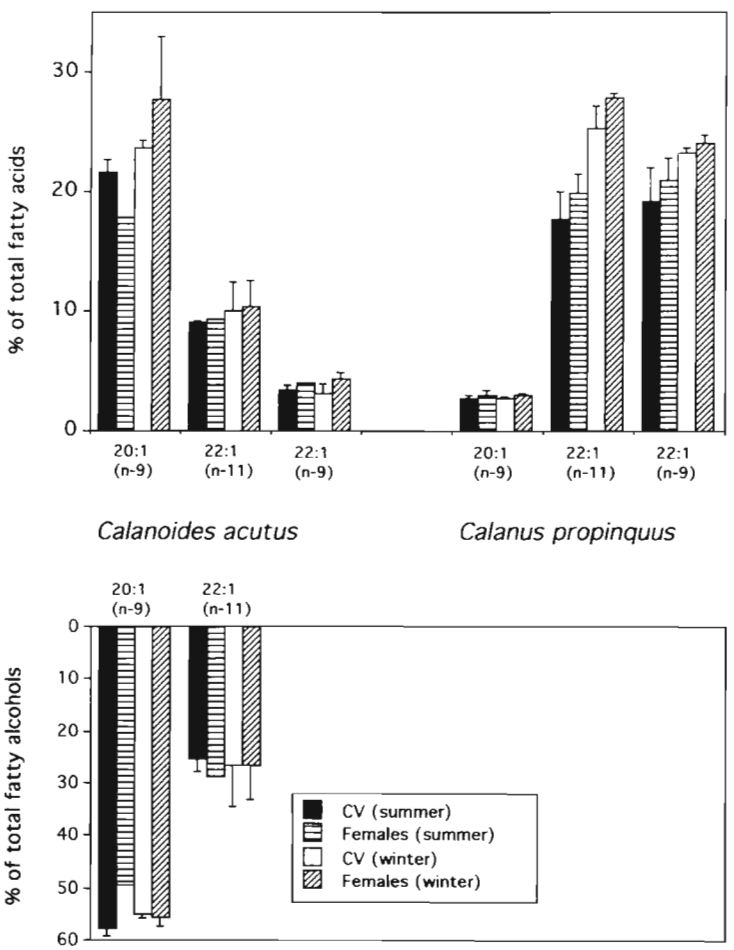

Fig. 2. *Calanoides acutus* and *Calanus propinquus*. Differences in the major end products of fatty acid and alcohol biosynthesis in stages CV and females

DISCUSSION

Calanoides acutus is a typical representative of the herbivorous calanoid copepods in the Antarctic, which store large amounts of lipids in the form of wax esters. To elucidate this phenomenon of high wax ester accumulation, investigations have been carried out in temperate and polar regions. However, most of the polar work has been done on Arctic species (see reviews by Sargent et al. 1976, Clarke 1983, Sargent & Henderson 1986). Lee (1974, 1975) reported extremely high lipid contents, almost exclusively wax esters, in the Arctic copepod *Calanus hyperboreus*. Sargent et al. (1976) suggested that wax esters are most suitable for rapid accumulation of large lipid stores, because the enzymatic regulatory mechanisms which normally inhibit lipidogenesis due to a surplus of fatty acids may be ineffective during wax ester synthesis.

Detailed lipid analyses have shown the dominance of long-chain monounsaturated fatty acids and alcohols as wax ester moieties, especially in herbivorous copepods of Arctic waters, which synthesize high amounts of 20:1(n–9) and 22:1(n–11) fatty acids and alcohols (Falk-Petersen et al. 1987, Tande & Henderson 1988, Kattner 1989, Kattner et al. 1989). This tendency is most pronounced in *Calanus hyperboreus*, and almost 60 % of its wax esters were found to be derived from combinations of these long-

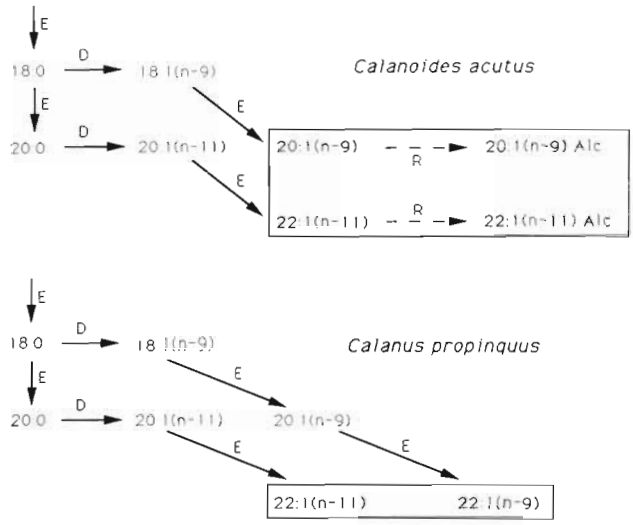

Fig. 3. *Calanoides acutus* and *Calanus propinquus*. Biochemical pathways of the major end products of fatty acid and alcohol biosynthesis. D: desaturation by delta-9 desaturase; E: elongation; R: fatty acid reduction to alcohol

chain fatty acids and alcohols (Graeve & Kattner 1992). Due to this very efficient means of energy storage, this Arctic species may be best suited for coping with the unfavourable nutritional conditions in polar oceans. The 2 smaller dominant *Calanus* species in the northern hemisphere, *C. glacialis* and *C. finmarchicus*, which inhabit Arctic and boreal Atlantic waters respectively, show a less developed tendency towards the synthesis of long-chain monounsaturated fatty acids and alcohols (Tande & Henderson 1988, Kattner et al. 1989, Hirche & Kattner in press).

The Antarctic copepod *Calanoides acutus* follows a similar pattern of synthesis of these long-chain monounsaturated fatty acids and alcohols. However, the production of the 22:1 fatty acids and alcohols is much less pronounced compared to that in Arctic species, which results in wax esters of lower calorific value. The enzymatic system of *C. acutus* may not be able - or may not need - to perform such efficient energy storage as is found in *Calanus hyperboreus*, but explanations for this phenomenon are purely speculative.

In contrast to all other polar marine herbivorous calanoids which store predominantly wax esters, only *Calanus propinquus* accumulates extensive amounts of triacylglycerols (Hagen 1988), thus deviating from the common scheme of lipid storage in herbivorous copepods from polar oceans. The existence of *C. propinquus* in high-Antarctic waters shows that energy storage via triacylglycerols is a viable alternative to wax ester accumulation. In contrast to the wax-ester synthesizing *Calanoides acutus*, *C. propinquus* compensates for the lack of wax esters by utilising an unusual pathway for fatty acid synthesis in marine zooplankton. This is achieved by an elongation of the 20:1($n-9$) to the 22:1($n-9$) fatty acid (Fig. 3). Usually this 22:1($n-9$) fatty acid is either absent or is found only in small amounts in marine planktonic organisms, whereas in *C. propinquus* it is one of the major components. This 22:1($n-9$) fatty acid occurred not only in the triacylglycerol fraction but also in the phospholipids (Kattner unpubl.), a clear indication of the general importance of this pathway in fatty acid biosynthesis. The addition of 2 carbon atoms results in a higher calorific content and thus provides an energetic advantage during the mobilization of these reserve lipids.

This benefit - although possibly less significant than that provided by wax ester storage - may be sufficient to compensate for the energetic requirements arising out of the different life history of *Calanus propinquus*: Nöthig et al. (1991) as well as Hopkins et al. (1993) found active *C. propinquus* in maximum concentrations in Weddell Sea surface waters during winter, and Schnack-Schiel et al. (1991) recorded high respiration rates and swimming activities for *C. propinquus* in the southeastern Weddell Sea during late winter, while *Calanoides acutus* was still inactive. Hence, the overwintering strategies of *C. acutus* and *C. propinquus* are fundamentally different: the purely herbivorous *C. acutus* goes into diapause at depth during winter and saves energy through a severely reduced metabolism, whereas *C. propinquus* stays active in winter and may switch from a summer phytoplankton diet to a more opportunistic feeding mode in winter, possibly even benefiting from the rich life in and under the sea-ice during this period (see also Marin & Schnack-Schiel 1993). The storage of triacylglycerols supports these findings that *C. propinquus* occupies a different ecological niche which may include year-round feeding, whereas the wax ester accumulation in *C. acutus* is more indicative of seasonal feeding [see Sargent et al. (1981), who discuss this aspect for wax-ester- and triacylglycerol-storing polar euphausiids].

Apart from these overwintering strategies, the incorporation of food seems to be different as well. Often, typical phytoplankton fatty acids such as 16:1 and 18:4 are incorporated without modification by the herbivorous copepods, which makes these compounds good indicators of the major diet ingested (Lee et al. 1971, Sargent & Falk-Petersen 1981, Kattner et al. 1989, Graeve 1992). These trophic markers of a phytoplankton diet occur only in small amounts in *Calanus propinquus*. These data support our earlier suggestion that *C. propinquus* is not a pure herbivore, but rather displays a more opportunistic feeding mode (Hopkins 1987, Hopkins & Torres 1989, Hopkins et al. 1993). We detected no clear seasonal variation in the 16:1 fatty acid in *C. propinquus* or *C. acutus*, but there is a

decrease of the 18:4 fatty acid during winter, although more pronounced in *Calanoides acutus* than in *C. propinquus*. This may indicate that 18:4 is ingested with phytoplankton by *C. acutus* during summer and later utilized or converted into other fatty acids.

It is known from studies on starvation effects (e.g. during overwintering) that wax-ester-storing calanoids first make use of fatty acids with lower calorific content, such as dietary fatty acids, which thus increases the proportion of long-chain fatty acids and alcohols (Lee 1974, Kattner et al. 1989, Hirche & Kattner in press). However, a comparison of the fatty acid compositions during the different seasons shows only a slight tendency towards the longer-chain 22:1 fatty acids in late winter for *Calanoides acutus*, which may be evidence for minimal utilization of lipid reserves during diapause. In contrast, and although it has a different lipid storage mode, our data for *Calanus propinquus* clearly reflect this tendency towards long-chain 22:1 fatty acids. Hence, *C. propinquus* is also able to apply this strategy for using its energy reserves most efficiently.

In summary, both species have developed alternative strategies for surviving the pronounced seasonality and strongly pulsed primary production in the Antarctic ocean. They both store large amounts of lipids in oil sacs, which are accumulated during periods of plentiful food, but what is most striking is that these 2 species store biochemically quite different compounds. Previously, it was assumed that herbivorous calanoid copepods could only survive in polar oceans due to their ability to synthesize wax esters, and *Calanoides acutus* was a prime example of this survival strategy in the Antarctic. However, our study verifies that within marine calanoid copepods there exists a viable alternative to this paradigm: the accumulation of triacylglycerols with long-chain fatty acid moieties in *Calanus propinquus*.

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LITERATURE CITED

Andrews, J. H. (1966). The distribution and life-history of *Calanoides acutus* (Giesbrecht). 'Discovery' Rep. 34: 117–162

Boysen-Ennen, E., Hagen, W., Hubold, G., Piatkowski, U. (1991). Zooplankton biomass in the ice-covered Weddell Sea, Antarctica. Mar. Biol. 111: 227–235

Clarke, A. (1983). Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr. mar. Biol. A. Rev. 21: 341–453

Clarke, A. (1984). The lipid content and composition of some Antarctic macrozooplankton. Brit. Antarct. Surv. Bull. 63: 57–70

Conover, R. J., Huntley, M. E. (1991). Copepods in ice-covered seas – distribution, adaptations to seasonally limited food, metabolism, growth patterns and life cycle strategies in polar seas. J. mar. Syst. 2: 1–40

Falk-Petersen, S., Sargent, J. R., Tande, K. (1987). Lipid composition of zooplankton in relation to the sub-Arctic food web. Polar Biol. 8: 115–120

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

Fraser, A. J., Tocher, D. R., Sargent, J. R. (1985). Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J. exp. mar. Biol. Ecol. 88: 91–100

Graeve (1992). Umsatz und Verteilung von Lipiden in arktischen marinen Organismen. Ph.D. thesis, Bremen Univ.

Graeve, M., Kattner, G. (1992). Species-specific differences in intact wax esters of *Calanus hyperboreus* and *C. finmarchicus* from Fram Strait–Greenland Sea. Mar. Chem. 39: 269–281

Hagen, W. (1988). On the significance of lipids in Antarctic zooplankton. Ber. Polarforsch. 49: 1–129 (in German) [English version (1989): Canadian Transl. Fish. Aquat. Sci. 5458: 1–149]

Hempel, G. (ed.) (1985). Die Expedition ANTARKTIS III mit FS 'Polarstern' 1984/85. Ber. Polarforsch. 25: 1–209

Hirche, H.-J., Kattner, G. (1993). Egg production and lipid content of starved and fed *Calanus glacialis*: indication of food-dependent and food-independent reproductive mode. Mar. Biol. (in press)

Hopkins, T. L. (1985). Food web of an Antarctic midwater ecosystem. Mar. Biol. 89: 197–212

Hopkins, T. L. (1987). Midwater food web in McMurdo Sound, Ross Sea, Antarctica. Mar. Biol. 96: 93–106

Hopkins, T L., Lancraft, T. M., Torres, J. J., Donnelly, J. (1993). Community structure and trophic ecology of zooplankton in the Scotia Sea Marginal Ice Zone in winter (1988). Deep Sea Res. 40(1): 81–105

Hopkins, T. L., Torres, J. J. (1989). Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep Sea Res. 36(4): 543–560

Huntley, M. E., Escritor, F. (1991). Dynamics of *Calanoides acutus* (Copepoda: Calanoida) in Antarctic coastal waters. Deep Sea Res. 38: 1145–1167

Kattner, G. (1989). Lipid composition of *Calanus finmarchicus* from the North Sea and the Arctic. A comparative study. Comp. Biochem. Physiol. 94B: 185–188

Kattner, G., Fricke, H. S. G. (1986). Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromat. 361: 263–268

Kattner, G., Hirche, H.-J., Krause, M. (1989). Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar. Biol. 102: 473–480

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

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

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

Marin, V. (1988). Qualitative models of the life cycles of *Calanoides acutus*, *Calanus propinquus*, and *Rhincalanus gigas*. Polar Biol. 8: 439–446

Marin, V., Schnack-Schiel, S. B. (1993). On the occurrence of *Rhincalanus gigas*, *Calanoides acutus* and *Calanus propinquus* (Copepoda: Calanoida) in late May in the area of the Antarctic Peninsula, Antarctica. Polar Biol. 13: 35–40

Nöthig, E.-M., Bathmann, U., Jennings , J. C. Jr, Fahrbach, E., Gradinger, R., Gordon, L. I., Makarov, R. (1991). Regional relationships between biological and hydrographical properties in the Weddell Gyre in late austral winter 1989. Mar. Chem. 35: 325–336

Ottestad, P. (1936). On Antarctic copepods from the 'Norvegia' expedition 1930–31. Scient. Results Norw. Antarctic Exped. 1927–1928 et SQQ 15: 5–44

Reinhardt, S. B., Van Vleet, E. S. (1986). Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Mar. Biol. 91: 149–159

Sargent, J. R., Falk-Petersen, S. (1981). Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis* during midwinter. Mar. Biol. 62: 131–137

Sargent, J. R., Gatten, R. R., Henderson, R. J. (1981). Lipid biochemistry of zooplankton from high latitudes. Oceanis 7: 623–632

This article was submitted to the editor

Sargent, J. R., Henderson, R. J. (1986). Lipids. In: Corner, E. D. S., O'Hara, S. (eds.) Biological chemistry of marine copepods. Univ. Press, Oxford, p. 59–108

Sargent, J. R., Lee, R. F., Nevenzel, J. C. (1976). Marine waxes. In: Kolattukudy, P. E. (ed.) Chemistry and biochemistry of natural waxes. Elsevier, Amsterdam, p. 50–91

Schnack, S. B. (1985). Feeding by *Euphausia superba* and copepod species in response to varying concentrations of phytoplankton. In: Siegfried, W. R., Condy, P. R., Laws, R. M. (eds.) Antarctic nutrient cycles and food webs. Springer, Berlin, p. 311–323

Schnack-Schiel, S. (ed.) (1987). The winter expedition of RV 'Polarstern' to the Antarctic (ANT V/1–3). Ber. Polarforsch. 39: 1–259

Schnack-Schiel, S. B., Hagen, W., Mizdalski, E. (1991). Seasonal comparison of *Calanoides acutus* and *Calanus propinquus* (Copepoda: Calanoida) in the southeastern Weddell Sea, Antarctica. Mar. Ecol. Prog. Ser. 70: 17–27

Tande, K. S., Henderson, R. J. (1988). Lipid composition of copepodite stages and adult females of *Calanus glacialis* in Arctic waters of the Barents Sea. Polar Biol. 8: 333–339

Voronina, N. M., Vladimirskaya, Ye. V., Zmiyevskaya, M. I. (1978). Seasonal variations in the age composition and vertical distribution of common zooplankton species in the Southern Ocean. Oceanology 18: 335–339

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