Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate

W. C. M. Klein Breteler1,*, M. Koski1, 2, S. Rampen1

1 Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB, Den Burg, The Netherlands
2 Present address: Danish Institute for Fisheries Research, Kavalergården 6, 2920 Charlottenlund, Denmark

ABSTRACT: The ciliate Strombidium sulcatum was used to feed and grow young stages of the copepods Temora longicornis (Müller) and Pseudocalanus elongatus (Boeck). The ciliate was cultured in the laboratory using either bacteria or the green alga Dunaliella sp. as a food source. Young copepodites of both copepod species consumed S. sulcatum at significant rates, but after 3 d, weight-specific ingestion decreased more than 2-fold. Larvae and copepodites feeding on S. sulcatum developed at suboptimal rates, survived poorly and showed abnormal morphology in comparison to control individuals fed a good-quality Rhodomonas sp. diet. The specific mass of fatty acids in S. sulcatum was much lower than in the Dunaliella sp. diet. However, the fatty acid composition of the protozoan more or less resembled that of the food, lacking long-chain highly unsaturated fatty acids (HUFAs). Sterols only occurred in Dunaliella sp., although in low abundance of useless ∆7 sterols. Obviously, S. sulcatum did not biochemically enhance bacterial or algal food for subsequent use at higher trophic levels, and only transferred fatty acids without further conversion. The results indicate a deficiency in the ciliate of HUFAs and sterols which are essential nutrients for copepod growth. Apart from energy, ciliates seem to contribute little nutritive value to the diet of higher trophic levels, and this may limit secondary production during periods of low algal abundance.

KEY WORDS: Copepods · Ciliates · Grazing · Food quality · Fatty acids · Sterols

INTRODUCTION

The biochemical composition of marine microorganisms is well recognised as influencing the productivity of aquatic systems. In particular, the lipids contained in algae and other organisms at the base of the food chain seem to determine the quality of food for the higher trophic levels. In aquatic environments ω3 polyunsaturated fatty acids (PUFAs) are an essential requirement for most metazoans, but they cannot be synthesised by these animals and must be obtained from algal food. A subset of PUFAs, the long-chain, highly-unsaturated fatty acids (HUFAs) eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA) are ‘semi-essential’ compounds that animals can produce only slowly; hence, HUFAs can enhance zooplankton growth rates when supplied with the food (Brett & Müller-Navarra 1997 and references therein). Laboratory observations show that copepods also require sterols as an essential component (Ederington et al. 1995, Klein Breteler et al. 1999), which they, like all crustaceans, cannot produce themselves (Dewey 1967, Goad 1981).

For zooplankton, plant material is the major source of HUFAs and sterols (Sargent et al. 1987, Brown et al. 1997). However, copepods also feed on micro- and nanoozooplankton, of which the lipid compositions are poorly known (Wood 1988) and the synthesis or bioconversion of HUFAs and sterols is uncertain. Recently, the heterotrophic flagellate Bodo sp. was found to produce EPA and DHA at high rates (Zhukova & Kharlamenko 1999), and among heterotrophic dinofla-
gellates a few species are known to produce DHA (Harrington & Holz 1968, Barclay et al. 1994, Klein Breteler et al. 1999). Sterols are synthesised by some species of amoebae and trypanosomatid protozoans (Volkman 2003), and by the heterotrophic dinoflagellate Oxyrrhis marina (Klein Breteler et al. 1999). Apparently, heterotrophic dinoflagellates have maintained the capability of their autotrophic conspecifics to produce HUFAs and sterols. Since protozoans feed on detritus and pico- and nanoplancton, they may both channel energy and enhance biochemical quality from the microbial food web towards higher trophic levels. Such trophic upgrading has been shown for O. marina. This heterotrophic dinoflagellate transfers the unsuitable Dunaliella sp., containing no HUFAs and hardly any sterols, into high-quality food rich in both essential lipids, which allows fast development of copepods that feed on O. marina (Klein Breteler et al. 1999). Recently, the intermediary dinoflagellate Gyrodinium dominans growing on Phaeocystis globosa has been shown to improve the nutritional quality of P. globosa for Acartia tonsa, as indicated by the much higher egg production rate of A. tonsa feeding on the dinoflagellate than of those feeding on P. globosa directly (Tang et al. 2001).

In marine ciliates, synthesis of sterols has not been detected, except for cholesterol in the hypotrich Euplotes sp. (class Nassophorea) (Harvey & McManus 1991). However, Tetrahymena spp. and several scuticociliates (both belonging to the class Oligohymenophora) produce tetrathymanol and hopanoids, which functionally replace sterols when algal sterols are not available (Harvey & McManus 1991, Harvey et al. 1997). The fatty acid composition of ciliates tends to resemble that of the food they have consumed. Since PUFAs generally do not occur in bacteria (Phillips 1984), they also do not occur in bacterivorous ciliates, except in the rare case when PUFAs are present in the bacteria consumed (Harvey et al. 1997). Ciliates may convert lipids obtained from their food to ω-6-PUFAs (Kaneshiro et al. 1979, Sul & Erwin 1997). There are also a few indications of ω3-PUFA and -HUFA production by ciliates, but only in marine species. Thus, linolenic acid, 18:3ω3, has been found in the bacterivorous marine scuticociliate Pleuronema sp. (Ederington et al. 1995). The marine scuticociliate Parauronema acutum produces EPA and DHA at low rates, when shorter-chain ω3-fatty acids (linolenic acid), among others, occur in the medium (Sul & Erwin 1997); Euplotes crassus does the same, but from bacteria containing only up to 18C mono-unsaturated fatty acids (Zhukova & Kharlamenko 1999). Obviously, our present knowledge of the biochemical capability of ciliates is not complete. Due to their significance in the food-chain (Sanders & Wickham 1993), upgrading of food quality by marine ciliates would stimulate secondary production, particularly under oligotrophic conditions when algae are not abundant.

In this study we used the marine bacteri-/herbivorous oligotrich ciliate Strombidium sulcatum (class Polyhymenophora), to test the hypothesis that lipid compounds essential for copepods can be produced by the ciliate. We fed S. sulcatum with bacteria, probably containing neither PUFAs nor sterols, and with the chlorophyte Dunaliella sp., which does contain the essential 18:3ω3 PUFA, but no HUFAs or useful Δ5 sterols. Both bacteria and Dunaliella sp. are known not to support copepod growth alone. To assess any improved food value after trophic transfer to the protozoan, we compared concentrations of fatty acids and sterols in S. sulcatum and in the algal food, and we measured the rate of development and grazing of copepods feeding on S. sulcatum.

**MATERIALS AND METHODS**

Using continuous cultures, the ciliate Strombidium sulcatum was grown using either bacteria or the chlorophycean alga Dunaliella sp. as the food source. The ciliate was fed to young stages of the copepods Temora longicornis (Müller) and Pseudocalanus elongatus (Boeck). Development, mortality and grazing rates of the copepods were monitored. The cryptophycean Rhodomonas sp. was used as a control food, allowing good growth and low mortality of the copepods. The fatty acid and sterol composition of algal and protozoan food were measured as described below.

**Cultures.** Copepods were obtained from brood stocks of Temora longicornis and Pseudocalanus elongatus, which were continuously cultured in the laboratory at 15°C with a surplus of food (>300 μg C l⁻¹). The food consisted of the cryptophyte Rhodomonas sp., the haptophyte Isochrysis galbana and the heterotrophic dinoflagellate Oxyrrhis marina. The algae were cultured in 3 l chemostats, using a dilution rate of 0.16 d⁻¹ of 1/2 medium (Guillard 1975), a constant air supply, a light intensity of ca. 150 μE m⁻² s⁻¹, a 16:8 h light-dark regime and with the temperature held at 15°C. All algal cultures were unialgal, but contained low concentrations of bacteria. *O. marina* was cultured using *Rhodomonas* sp. as food. Details of the culture conditions have been described by Klein Breteler & Gonzalez (1986, 1988) and Klein Breteler & Laan (1993). Strombidium sulcatum (33 μm equivalent spherical diameter, ESD, 2240 pg C cell⁻¹), obtained from X. Mari (Station Zoologique, Villefranche sur Mer, France), was grown in batch culture on natural bacteria developing on wheat grains. From this culture, we inoculated 2 different 3 l continuous cultures. In one, a bacteria medium was supplied to promote the growth
of bacteria as food for *S. sulcatum*. The bacteria medium consisted of autoclaved filtered seawater containing 60 mg l⁻¹ bactopeptone and 60 mg l⁻¹ yeast extract (both from Difco) supplied at a rate of 0.25 d⁻¹. The other culture was part of a 2-stage chemostat, in which *Dunaliella* sp. (6.7 µm ESD, 29 pg C cell⁻¹) was continuously supplied to *S. sulcatum* at a dilution rate of 0.08 d⁻¹. *Dunaliella* sp. was cultured in conditions similar to those of the other algae, except for a bottle volume of 0.5 l and a dilution rate of 0.5 d⁻¹. The cultures of *S. sulcatum* were aerated at a low rate of about 1 bubble per 2 s using compressed air, and stirred for 2 s every 80 s using a magnetic stirrer with an electronic time-control unit.

Concentrations and cell volumes of algae and protozoans were measured in samples from the continuous cultures using an Elzone electronic particle counter (Particle Data). For determination of carbon and lipid content, samples were filtered onto combusted Whatman GF/F filters and stored under N₂ at –50°C. The carbon content was measured using a Carlo Elba CHN analyser. Since no separate cultures of the bacteria were available, the lipid composition of the bacteria could not be assessed under the present continuous culture conditions.

**Lipid analysis.** Samples were saponified by reflux (1 h) with 6 ml 1 N KOH/methanol (MeOH) solution. After acidifying the solution to pH 3 with 2 N HCl in water/MeOH (1:1, v/v), the solution was added in a separatory funnel with double-distilled water. Thereafter, the filters were extracted twice with double-distilled water and subsequently with double-distilled water/MeOH (1:1, v/v), and 3 times with dichloromethane (DCM). All fractions were collected in the separation funnel. After separation of the DCM fraction, the remaining water/MeOH fraction was washed with DCM 2 more times, separating the DCM after each washing. Samples were dried on a rotavap, transferred into a sample jar and weighed. As an internal standard, 6,6-dideutero-3-methylheneicosane was added to quantify the amount of fatty acids. Extracts were methylated with diazomethane in DCM and eluted over a silica column with ethyl acetate.

For sterol analysis, an aliquot of the extract was dried under N₂ and separated over Al₂O₃ using hexane/DCM (1:1 v/v) and DCM/methanol (1:1 v/v) to remove the methylated fatty acids from the remaining sample with sterols. Immediately before gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analysis, samples were treated with bis(trimethylsilyl) trifluoracetamide to convert compounds containing free hydroxyl groups to their trimethylsilyl-ether (TMS) derivatives. GC analyses were performed using a Hewlett-Packard 5890 gas chromatograph with an on-column injector and a flame-ionisation detector. Samples were injected onto a fused silica capillary column (50 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 µm). Helium was used as the carrier gas. Starting at 70°C, the temperature was raised to 130°C at 20°C min⁻¹ and then at 4°C min⁻¹ to 320°C. This temperature was maintained for 25 min.

GC/MS analyses were performed using a Hewlett-Packard 6890 gas chromatograph with on-column injector, interfaced to a Hewlett-Packard 5973 mass spectrometer operated at 70 eV with a mass range of m/z 50–800 and a measuring rate of 2 scans s⁻¹. Samples were injected onto a fused silica capillary column (50 m × 0.25 mm) coated with CP Sil-8 (film thickness 0.25 µm). Helium was used as the carrier gas. Starting at 100°C, the temperature was raised to 200°C at 15°C min⁻¹ and then at 6°C min⁻¹ to 320°C. This temperature was maintained for 25 min.

Double-bond positions of fatty acids were determined by comparing retention times of the extracted compound with those of PUFA No. 1 and 2 standard mixtures (Matreya). For sterols, no standards were used. Since sterols with double bonds at Positions 7 and 8 give similar mass spectra, our identification of these sterols was tentative.

**Experiments.** The development times of the copepods were measured using an experimental set-up with 1.2 l glass bottles, which were rotated at 1 rpm using a rolling-table in a temperature-controlled room held at 15°C under dim light conditions. Young larvae and copepodites (250 to 490 specimens) of *Temora longicornis* or *Pseudocalanus elongatus* were taken from the broodstock, rinsed with double-filtered (2 µm) seawater and incubated in 2 µm-filtered seawater with food. The stages of the larvae mainly varied from Nauplius IV to Copepodite II. Younger stages were not included, since they were considered unable to consume the large ciliates used as prey in our experiments (Berggreen et al. 1988). Incubation continued until most copepods were mature or dead. Food was supplied from the continuous cultures of *Rhodomonas* sp. or *Strombidium sulcatum* at a concentration of ≥300 µg C l⁻¹. The experiments with *S. sulcatum* were carried out 3 times, using either bacteria or *Dunaliella* sp. as food for the ciliate. Every 1 or 2 d, 90% of the food medium was removed, using reverse flow filtration through a 50 µm mesh, and replaced by new medium with food. Twice a week, the food concentration was measured using the Elzone particle counter. Sampling of copepods began 1 d after the start of the incubation, to allow for adaptation to the new food. The sampling was done 3 times per week to determine stage distribution and mortality, at the same time diluting the culture to keep the copepod biomass below a value equivalent to about 40 adult copepods l⁻¹. Usually,
30–50 copepods were collected per sampling time. Stage duration was calculated from the median development time (MDT) of successive stages. MDT is defined as the time when 50% of the population has not yet passed a certain stage. The statistical package S-plus was used to estimate MDT from gamma distribution functions, which adequately describe the cumulative stage distribution, as explained by Klein Breteler et al. (1994). Occasionally, when there was premature extermination of a population, MDT of the last stage was estimated by extrapolation of the gamma function. Extrapolation was applied only when at least 20% of the population had entered this stage, and these results are specifically indicated.

Instantaneous rates of mortality \( (Z \text{ d}^{-1}) \) were calculated according to \( N_t = N_0 e^{-zt} \), where \( N_0 \) is the number of individuals \( l^{-1} \) at Time 0 and \( N_t \) is the number of individuals \( l^{-1} \) at Time t. \( N_t \) was corrected for sampling mortality by multiplying the observed number of individuals by \( V^{n-1}/[(V-v_1)·(V-v_2)·...·(V-v_{n-1})] \), where \( V \) is the volume of the experimental bottle, \( v \) is the volume of the sample and \( n \) is the rank number of the sample.

Grazing rates were measured using individuals collected from the above experiments after 1 and 3 d of incubation. The 1 d collections contained 24 to 72 copepodites, mainly CI and CII, and the 3 d collections had 12 to 34 copepodites, mainly CII and C III (see Table 2). They were collected on a sieve and resuspended in 3 replicate 135 ml bottles with seawater containing \( Strombidium sulphatum \) at an initial concentration of 220 to 290 \( \mu \text{g C l}^{-1} \); 6 bottles without copepodites served as a control. The bottles were closed with a PTFE-lined screw cap, avoiding air bubbles, and were incubated on the rolling table under the same conditions as described for the development experiments. The concentrations of \( S. sulphatum \) were determined using the Elzone particle counter. In 3 control bottles, the abundance was determined immediately after preparation; the remaining 3 bottles together with the 3 bottles containing copepods were analysed after 24 h of incubation. At that time, the copepods were collected and enumerated according to their developmental stage. Grazing rates were calculated according to Frost (1972) and tested for differences using the ‘bootstrap’ method (Manly 1997).

The ash-free dry weight of the copepods was approximated after Klein Breteler et al. (1982) and Klein Breteler & Gonzalez (1988), with correction for slight differences in size between the adults from the present and the former cultures. Carbon weight was calculated assuming 41% carbon content of the ash-free dry weight (Williams & Robins 1982).

**RESULTS**

**Development**

There was a strong effect of food quality on the rate of copepod development, as reflected by the duration of the individual stages (Table 1) and the cumulative development time (Fig. 1). Differences among experiments were partly due to differences in age of the copepods at the start of the incubations (cf. levels of curves in Fig. 1). However, fast development occurred with the control food \( Rhodomonas sp. \), with \( Pseudocalanus elongatus \) and \( Temora longicornis \) developing through the copepodite stages in about 9 d (Fig. 1).
contrast, when using Strombidium sulcatum as food, the rate of development was strongly reduced and most nauplii or young copepodes did not pass more than 2 successive stages. The reduced development seen with S. sulcatum did not seem to depend on the kind of food (viz. Dunaliella sp. or bacteria) fed to the ciliate, as shown in the experiment with T. longicornis (Fig.1).

Mortality

When fed Rhodomonas sp., the instantaneous rates of mortality of Temora longicornis and Pseudocalanus elongatus were low (5–12% d


contrast, when using Strombidium sulcatum as food, the rate of development was strongly reduced and most nauplii or young copepodes did not pass more than 2 successive stages. The reduced development seen with S. sulcatum did not seem to depend on the kind of food (viz. Dunaliella sp. or bacteria) fed to the ciliate, as shown in the experiment with T. longicornis (Fig.1).

Mortality

When fed Rhodomonas sp., the instantaneous rates of mortality of Temora longicornis and Pseudocalanus elongatus were low (5–12% d


In Table 1, the duration (d) of developmental stages using Rhodomonas sp. (Rho) or the ciliate Strombidium sulcatum (Strom) as food source. S. sulcatum was grown using either bacteria (b) or Dunaliella sp. (D; 1 and 2 indicate separate experiments). Numbers in italics: estimates obtained by extrapolation of gamma distribution functions. Stages 5 and 6: Naupliar Stages 5 and 6; Stages 7–11: Copepodite Stages 1–5.

Grazing

After 1 d of adaptation to Strombidium sulcatum, young copepodes (mainly CI and II) filtered ca. 1.5 ml ind.\(^{-1}\) d\(^{-1}\) (Table 2), equivalent to an average ingestion of 34 and 58% of the body carbon content in Pseudocalanus elongatus and Temora longicornis, respectively (Fig. 3). After another 2 d of feeding on S. sulcatum, the copepodes, albeit having developed to 1 higher stage on average (Table 2), cleared less water than initially, resulting in an average ingestion of only about 18% of their body carbon content (Fig. 3). The results from the T. longicornis experiments were to some extent rather variable, probably due to insuffi-
cient mixing of the fast swimming ciliates during preparation of the grazing bottles. The reduced grazing after 3 d of exposure to *S. sulcatum* was significant (*p* < 0.05) in *P. elongatus* only.

**Behaviour and morphology**

After 5 d of feeding on *Strombidium sulcatum*, a significant number of young copepodites of both *Temora longicornis* and *Pseudocalanus elongatus* became inactive, with some of them showing abnormal morphology. In those individuals, the distal segments of the swimming feet were strongly swollen, along with the caudal rami of the urosome in a few of them.

**Lipid content and composition**

The total fatty acid content of *Strombidium sulcatum* was 7 times lower than that of *Dunaliella* sp. (Table 3). Their fatty acid compositions were more or less similar, particularly with regard to HUFAs, which were absent from both of them (Table 3). An accurate comparison is complicated by a high contribution (19% on the basis of C) of *Dunaliella* sp. in the ciliate culture, while contamination was intensified by the high specific fatty acid content of the alga. After correction for *Dunaliella* sp. in the ciliate culture, the proportions of fatty acids in *S. sulcatum* were not reliable and often not significantly different from zero. The dominant fatty acid in *Dunaliella* sp. was 18:3ω3, whereas in the ciliate C18:1ω7 was most abundant. Of sterols, only low amounts of 24-methylcholesterol-7-en-3β-ol (Δ7C28:1) could be detected in *Dunaliella* sp., as well as traces of 3 other Δ7 sterols. In the samples of *S. sulcatum*, only traces of 2 of these Δ7 sterols were detectable, and these were probably due to *Dunaliella* sp. present in the samples. Most probably, therefore, the ciliate did not contain any sterol at all. Tetrahymanol or other sterol surrogates were also not detected in the samples from the ciliate.

Table 3. *Dunaliella* sp. and *Strombidium sulcatum*. Composition (%) and content (µg mg⁻¹ C) of fatty acids and sterols. Fatty acid values of *S. sulcatum* corrected for presence of *Dunaliella* sp., based on cell counts. tr: traces of sterols; (tr): traces include contamination due to *Dunaliella* sp.; SD: standard deviation within samples (*n* = 3) due to analytical errors; x: impossible; ?: uncertain identification of double-bond positions from PUFA standard mixtures (among others due to different GC-column used); nd: not detectable; nq: not quantifiable due to coelution with hydroxy fatty acids (both insignificant amounts); ns: not significantly different from zero. Detection limit of sterols was ca. 0.3 µg mg⁻¹ C

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Dunaliella sp. Mean</th>
<th>Dunaliella sp. SD</th>
<th>S. sulcatum Mean</th>
<th>S. sulcatum SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.3</td>
<td>0.1</td>
<td>8.7</td>
<td>0.4</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.7</td>
<td>0.2</td>
<td>4.9</td>
<td>0.5</td>
</tr>
<tr>
<td>C16:4</td>
<td>12.8</td>
<td>2.2</td>
<td>−10.4ns</td>
<td>7.7</td>
</tr>
<tr>
<td>C16:3ω1</td>
<td>0.6</td>
<td>0.1</td>
<td>nq</td>
<td>nq</td>
</tr>
<tr>
<td>C16:3ω2</td>
<td>3.2</td>
<td>0.3</td>
<td>1.7ns</td>
<td>1.6</td>
</tr>
<tr>
<td>C16:1ω1</td>
<td>0.2</td>
<td>0.0</td>
<td>0.6ns</td>
<td>0.6</td>
</tr>
<tr>
<td>C16:1ω2</td>
<td>0.1</td>
<td>0.0</td>
<td>3.7</td>
<td>0.6</td>
</tr>
<tr>
<td>C16:1ω3</td>
<td>4.1</td>
<td>0.6</td>
<td>−4.4</td>
<td>0.3</td>
</tr>
<tr>
<td>C16:1ω4</td>
<td>1.3</td>
<td>0.3</td>
<td>10.1</td>
<td>3.3</td>
</tr>
<tr>
<td>C16:1ω7</td>
<td>0.6</td>
<td>0.1</td>
<td>4.5</td>
<td>0.6</td>
</tr>
<tr>
<td>C16:0</td>
<td>23.8</td>
<td>4.4</td>
<td>13.8ns</td>
<td>8.3</td>
</tr>
<tr>
<td>C18:3ω3</td>
<td>1.0</td>
<td>0.2</td>
<td>3.7ns</td>
<td>3.2</td>
</tr>
<tr>
<td>C18:3(ω6ω7)</td>
<td>3.5</td>
<td>0.5</td>
<td>2.8ns</td>
<td>2.5</td>
</tr>
<tr>
<td>C18:3(ω3ω7)</td>
<td>35.0</td>
<td>4.6</td>
<td>15.6ns</td>
<td>22.3</td>
</tr>
<tr>
<td>C18:2ωx</td>
<td>1.4</td>
<td>0.1</td>
<td>5.4</td>
<td>0.8</td>
</tr>
<tr>
<td>C18:2ω6</td>
<td>3.1</td>
<td>0.6</td>
<td>5.1ns</td>
<td>2.4</td>
</tr>
<tr>
<td>C18:1ω9</td>
<td>5.7</td>
<td>1.0</td>
<td>−0.4ns</td>
<td>0.8</td>
</tr>
<tr>
<td>C18:1ω7</td>
<td>2.2</td>
<td>0.3</td>
<td>31.4</td>
<td>0.8</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.5</td>
<td>0.1</td>
<td>3.1</td>
<td>0.9</td>
</tr>
<tr>
<td>C20:5</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C22:6</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

### Total fatty acids

<table>
<thead>
<tr>
<th>μg mg⁻¹ C</th>
<th>μg mg⁻¹ C</th>
</tr>
</thead>
<tbody>
<tr>
<td>151.6</td>
<td>21.6</td>
</tr>
</tbody>
</table>

### Sterols

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Dunaliella sp. Mean</th>
<th>Dunaliella sp. SD</th>
<th>S. sulcatum Mean</th>
<th>S. sulcatum SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ7C28:1</td>
<td>0.7</td>
<td>(tr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ7,24(28)C28:2</td>
<td>tr</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ7,24(28)C29:2</td>
<td>tr</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ7C29:1</td>
<td>tr</td>
<td>(tr)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

In this study, the ciliate Strombidium sulcatum was grown using low quality food deficient in HUFAs and Δ5 sterols, which are ‘semi-essential’ and essential compounds, respectively, for copepods. When feeding on S. sulcatum, the 2 species of copepods could not develop through more than a few stages. Their rate of development was low, they died at high rates, and reduced feeding was evident within a few days. Reduced motility and abnormal morphology unequivocally reflected the unsuitability of S. sulcatum as a food source. Probably this ciliate was not toxic for the copepods (see below). Biochemical analyses indicated that S. sulcatum did not upgrade the nutritional quality of food available to the copepods, in contrast to the heterotrophic dinoflagellate Oxyrrhis marina (Klein Breteler et al. 1999).

Sterols occurred in Dunaliella sp. at low concentration. They were not Δ5 sterols, but they were tentatively identified as Δ7 sterols (Table 3). Although Δ7 sterols are common to most Chlorophyceae (Volkman 1986, 2003), evidence for their digestion by copepods is contradictory (Prah et al. 1984, Harvey et al. 1987). Lack of growth (Koski et al. 1998, Klein Breteler et al. 1999 and this study) and reduced egg production (Ederington et al. 1995) of copepods feeding on organisms with only Δ7 sterols also support the idea that Δ7 sterols are not efficiently used by copepods. We found no evidence for the presence of sterols in Strombidium sulcatum, an observation which has been made previously in 2 other species of bacterivorous ciliates by Ederington et al. (1995) and Harvey et al. (1997). Even the small amounts of Δ7 sterols detected in the Dunaliella sp. food were hardly or not found in S. sulcatum. This indicates that the ciliate neither produced nor transferred sterols to higher trophic levels. Since copepods have an essential requirement for sterols, the lack of sterols was likely to be the primary cause of the reduced performance of the copepods feeding on the ciliates. In future studies, a new technique developed recently to supplement sterols to food algae (VonElert et al. 2003), may make it possible to prove sterol limitation due to ciliate food.

Most animals, including marine calanoid copepods, have an absolute requirement for ω3-PUFAs in their food (Brett & Müller-Navarra 1997). Dunaliella sp. is a rich source of linolenic acid (18:3ω3), which could cover the ω3-PUFA requirement of copepods. In our experiments, from the biochemical composition of Strombidium sulcatum it seems that this ciliate transferred ω3-PUFAs from the alga to the copepods. In copepods, further chain elongation and desaturation of linolenic acid into HUFAs have been shown in harpacticoids (Norsker & Støttestrup 1994, Nanton & Castell 1999) as well as in the marine calanoid Paracen-
whereas the other authors used cereal grasses (De-Biasi et al. 1990) or hay infusions (Abdullahi 1992) to grow bacteria together with the ciliates. Other potential sources of sterols include agar, latex, filters, yeasts and fungi (J. K. Volkman pers. comm.). Unfortunately, no biochemical analyses were performed in the above studies, which makes it impossible to exclude transfer of essential lipids from the media.

Juvenile copepods feeding on Strombidium sulcatum showed abnormal morphology, which we have never observed before in studies on food availability (Klein Breteler et al. 1982, 1995, Klein Breteler & González 1986, 1988, Klein Breteler & Schogt 1994). Therefore, shortage of food does not seem to explain the swollen appendages observed after feeding on S. sulcatum. In Temora stylifera, morphological aberrations due to toxic hydrocarbons present in diatom food have been reported (Carotenuto et al. 2002). Swollen extremities seem to indicate a lack of rigidity of the exoskeleton, such as that observed in insects due to microviridine, and in cladocerans due to an unknown bioactive compound with a molecular weight corresponding to microviridine that occurred in a cyanobacterial diet (Kaebernick et al. 2001). However, strong evidence against the possibility of toxic or bioactive compounds in S. sulcatum was provided by a test using a mixture of Rhodomonas sp. and S. sulcatum as food for copepods. In this mixture, Nauplii IV and V of T. longicornis significantly grazed on both the algae and the ciliates (M. Koski unpubl.). At the same time, they developed well at a rate comparable to the rate observed with the Rhodomonas sp. diet alone (Fig. 1). Since this rules out toxicity as an explanation, the lack of sterols in the ciliate diet remains as the primary cause for a reduced rigidity of the exoskeleton. Sterols are required for cellular architecture by reinforcing cell walls. They are precursors of steroid hormones and, therefore, play a role in the moulting process. Moreover, there is evidence that sterol–protein complexes are involved in tanning and hardening of crustacean cuticles (Dennell 1960). Therefore, a disturbed moulting and cuticle physiology due to a sterol deficiency is probably the cause of the morphological abnormalities we observed.

Studies on biochemical synthesis and transfer of lipids by ciliates would profit from the availability of algal species deficient in either sterols or HUFAs, but not in both, as was the case in our study with Dunaliella sp. To date, we have also attempted to grow Strombidium sulcatum on Synechococcus sp., Isochrysis galbana and Rhodomonas sp., of which the latter 2 species contain sterols as well as HUFAs and are known for their good quality as food for copepods. Unexpectedly, Strombidium sulcatum did not grow on the cyanobacterium nor on the 2 algal species, although the algae are also naked flagellates of similar motility and of a size smaller than or similar to Dunaliella sp. It is possible that S. sulcatum did not feed on these algae, which would account for the low growth rates. S. sulcatum also did not consume the slightly larger Hymenomonas elongata and Thalassiosira weissflogii (Bonnet & Carlotti 2001). Recently, S. sulcatum has been grown using the flagellate Marsupiononas pelliculata as food (Broglio et al. 2003). Another, larger species, Strombidium sp., only grew on Pavlova (Monochrysis) lutheri, but not on Isochrysis sp. and, surprisingly, also not on Dunaliella sp. (Scott 1985). Apart from selective food ingestion, differences in the nutritional demands of Strombidium species may underlie their specific growth responses to different diets.

Species of freshwater ciliates are known for their different requirements of lipids for growth (Dewey 1967, Anderson 1988, Sleigh 1989). The present study suggests that Strombidium sulcatum does not require HUFAs or sterols to prosper. Like most ciliates (Ederington et al. 1995, Harvey et al. 1997), S. sulcatum neither needs these compounds in its food, nor does it have a biochemical pathway to synthesise them de novo or by conversion from other lipids. Exceptions to this are the scuticociliate Parauronema acutum (Sul & Erwin 1997) and the hypotrich Euplotes crassus (Zhu-kova & Kharlamenko 1999), which synthesise HUFAs, although at a low rate. Euplotes sp. is also the only ciliate known to produce a sterol (Harvey & McManus 1991). However, sterols are indispensable in maintaining cell membrane fluidity (Ourisson et al. 1987), and, hence they occur in virtually all higher organisms. In bacteria they are functionally replaced by hopanoids. In ciliates, different surrogates of sterols have been found. Scuticociliates and Tetrahymena pyriformis (both in the class Oligohymenophora) contain tetrahymanol and hopanoids as sterol surrogates, but in Euplotes sp. (class Nassophorea) and in the heterotrich Fabrea salina, tetrahymanol does not occur (Harvey & McManus 1991, Harvey et al. 1997). We also did not detect tetrahymanol in the oligotrich S. sulcatum (class Polyhymenophora). Possibly this ciliate uses hopanoids as a sterol surrogate which, however, may have remained undetected due to their low solubility and difficulty to extract (Ourisson & Rohmer 1992).

In summary, our study supports that marine ciliates are generally incapable of synthesising sterols and HUFAs. Deficiency of sterols seems to make bacterivorous ciliates unsuitable as a single food for copepods. The food selection habits and/or the nutritional demands of ciliates seem to be highly specific, and both constitute gaps in our knowledge that need to be filled to understand their biochemical capabilities and intermediary role between the microbial capabilities and higher trophic levels in the food chain.

Klein Breteler WCM, Gonzalez SR (1986) Culture and development of Temora longicornis (Copepoda, Calanoida) at different conditions of temperature and food. Sylleucus 38:71–84


Acknowledgements. We are grateful to Dr. J. van der Meer for calculation of the Gamma distribution functions. Thanks are due to Dr. G. J. Herndl for critically reading the manuscript. An anonymous reviewer is thanked for detailed improvements of the text.

LITERATURE CITED


Klein Breteler WCM, Gonzalez SR (1986) Culture and development of Temora longicornis (Copepoda, Calanoida) at different conditions of temperature and food. Sylleucus 38:71–84


Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: May 5, 2003; Accepted: February 24, 2004
Proofs received from author(s): June 9, 2004