Marine Biology (2006) 148: 779–788
DOI 10.1007/s00227-005-0123-1

RESEARCH ARTICLE

Adriana J. Veloza · Fu-Lin E. Chu · Kam W. Tang

Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*

Received: 25 May 2005 / Accepted: 2 September 2005 / Published online: 18 November 2005
© Springer-Verlag 2005

Abstract To test whether heterotrophic protists modify precursors of long chain *n*–3 polyunsaturated fatty acids (LC*n*−3PUFAs) present in the algae they eat, two algae with different fatty acid contents (*Rhodomonas salina* and *Dunaliella tertiolecta*) were fed to the heterotrophic protists *Oxyrrhis marina* Dujardin and *Gyrodinium dominans* Hulbert. These experiments were conducted in August 2004. Both predators and prey were analyzed for fatty acid composition. To further test the effects of trophic upgrading, the calanoid copepod *Acartia tonsa* Dana was fed *R. salina*, *D. tertiolecta*, or *O. marina* that had been growing on *D. tertiolecta* (OM-DT) in March 2005. Our results show that trophic upgrading was species-specific. The presence of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the heterotrophic protists despite the lack of these fatty acids in the algal prey suggests that protists have the ability to elongate and desaturate 18:3 (*n*–3), a precursor of LC*n*−3PUFAs, to EPA and/or DHA. A lower content of these fatty acids was detected in protists that were fed good-quality algae. Feeding experiments with *A. tonsa* showed that copepods fed *D. tertiolecta* had a significantly lower content of EPA and DHA than those fed OM-DT. The concentration of EPA was low on both diets, while DHA content was highest in *A. tonsa* fed *R. salina* and OM-DT. These results suggest that *O. marina* was able to trophically upgrade the nutritional quality of the poor-quality alga, and efficiently supplied DHA to the next trophic level. The low amount of EPA in *A. tonsa* suggests EPA may be catabolized by the copepod.

Communicated by J. P. Grassle, New Brunswick

A. J. Veloza · F.-L. E. Chu (✉) · K. W. Tang
Virginia Institute of Marine Science, 1208 Greate Road,
Gloucester Point, VA 23062, USA
E-mail: chu@vims.edu
Tel.: +1-804-6847349
Fax: +1-804-6847186

Introduction

The availability of long-chain *n*–3 polyunsaturated fatty acids (LC*n*–3PUFAs) in seston is an important nutritional factor for zooplankters (Muller-Navarra et al. 2004). The amount of LC*n*–3PUFAs such as eicosapentaenoic acid [EPA; 20:5 (*n*–3)] and docosahexaenoic acid [DHA; 22:6 (*n*−3)] in diets is correlated with growth and development in copepods (Koski et al. 1998; Klein Breteler et al. 1999; Tang et al. 2001). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are usually synthesized de novo by algae, while higher trophic organisms obtain these important molecules through bioaccumulation or by converting LC*n*−3PUFA precursors such as α-linolenic acid [18:3 (*n*−3)], 18:4 (*n*−3), and 18:5 (*n*−3), to DHA and EPA via elongation and desaturation. However, this bioconversion is slow and usually does not meet the physiological demands of the organisms (Sargent and Whittle 1981).

The level of these (*n*−3) essential fatty acids (*n*−3 EFAs) in algae can be highly variable (Morris et al. 1983). The LC*n*−3PUFA content is highest during periods of rapid cell growth or bloom episodes (Fraser and Sargent 1989). The quantity of DHA and EPA in algae also varies significantly among major taxa (Sargent and Whittle 1981). Calanoid copepods that were fed *Dunaliella tertiolecta* and *Phaeocystis globosa*, which are deficient in EPA and DHA, exhibited reproductive failure and high mortality (Koski et al. 1998; Lacoste et al. 2001; Tang et al. 2001). On the contrary, copepods feeding on the cryptophyte *Rhodomonas salina*, which is rich in LCn−3PUFAs, exhibited high egg production efficiency and naupliar growth rate (Tang et al. 2001).

Besides planktonic algae, heterotrophic protists are also a significant component in the copepods' diet (Gifford and Dagg 1991; Atkinson 1994; Levinson et al. 2000). The nutritional quality of heterotrophic protists can be different from that of their algal food, and as prey, they can in turn support higher growth in zooplankters. For example, Klein Breteler et al. (1999)

reported that the heterotrophic dinoflagellate *Oxyrrhis marina* grown on the EFA deficient alga *D. tertiolecta* supported the rapid growth of the copepods *Temora longicornis* and *Pseudocalanus elongatus* from naupliar stages to adulthood. Tang et al. (2001) and Broglio et al. (2003) also showed that heterotrophic protists as a trophic link between poor nutritional quality algae and copepods resulted in higher egg production and egg hatching success. This improvement of poor algal quality for subsequent use by higher trophic organisms has been dubbed "trophic upgrading" (Klein Breteler et al. 1999), and the underlying mechanisms are largely unknown. It has been suggested that heterotrophic protists may be able to synthesize or accumulate essential fatty acids (Kleppel et al. 1998), or convert precursor fatty acids to EPA and DHA (Broglio et al. 2003; Park et al. 2003).

Although there is evidence that the upgrading of biochemical components occurs in heterotrophic protists, the process appears to be species-specific and to affect the reproductive parameters of copepods differently. For example, growth in juvenile copepods was not supported by feeding on the ciliate *Strombidium sulcatum* grown on *D. tertiolecta* (Klein Breteler et al. 2004). Biochemical analysis showed that the ciliate incorporated the fatty acids from the algae without any modification. Kleppel et al. (1998) and Broglio et al. (2003) fed bacteria and algae of different nutritional quality to heterotrophic protists and produced protists with different fatty acid contents. Broglio et al. (2003) noted that in *Acartia tonsa* egg viability, but not egg production, was correlated with the ingestion of EFAs found in the heterotrophic prey. In addition, Tang and Taal (2005) also reported differences in egg production efficiencies when copepods were fed heterotrophic protists grown under different algal treatments.

Several questions about trophic upgrading remain unanswered: are good quality algae upgraded as well? Do different heterotrophic protists upgrade food to the same extent? How does trophic upgrading affect the EFA content of zooplankton, hence its subsequent transfer to higher trophic levels? A study by Graeve et al. (1994) suggested that copepod biochemical composition resembles that of their diet. Kattner et al. (1981) compared the fatty acid composition of omnivorous and herbivorous copepods to their prey and concluded that the fatty acid composition appeared to depend on the type of food consumed. Zooplankters, especially calanoid copepods, are important prey items for larval fish. Information on the effects of diet on copepod biochemical composition is necessary to better understand fish recruitment and yield. The objectives of the present study were (1) to investigate the ability of heterotrophic protists to modify ingested EFAs from high and low-quality planktonic algae, and (2) to investigate the potential trophic upgrading effects of heterotrophic protists on the LCn−3 PUFA content of the copepod *Acartia tonsa*.

Materials and methods

Algae, protist, and copepod cultures

The chlorophyte *Dunaliella tertiolecta* (CCMP 1320) and the cryptophyte *Rhodomonas salina* (CCMP 1319) were obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and cultured in 1-l round-bottom flasks in f/2 culture medium as recommended by CCMP. *Dunaliella tertiolecta* and *R. salina* were chosen based on their EFA content as low- and high-quality algae, respectively. Algae were maintained in the log phase for biochemical analysis and feeding experiments by diluting the culture with new f/2 medium every few days. Cultures were kept in a walk-in environmental room at 19°C in a 12 h light:12 h dark cycle.

The heterotrophic dinoflagellates *Oxyrrhis marina* Dujardin and *Gyrodinium dominans* Hulbert were obtained from the Shannon Point Marine Center and cultured in f/2 medium and fed monocultures of *Dunaliella tertiolecta* or *Rhodomonas salina*. Cultures were started in 250-ml Pyrex bottles with approximately 40 cells ml^{-1}. Every 2–3 days half of the culture was transferred to a 500-ml culture bottle and fed ad libitum for approximately 2 weeks. The protist cultures were maintained for more than five cell divisions on the experimental diets to assure equilibrium between the protist cell chemical contents and its algal food. This also allowed the protists to reach a high density (3–5x10^3 cells ml^{-1}) and to maintain a constant supply of organisms for the biochemical analysis. The bottles were placed on a rotating plankton wheel in a walk-in environmental room at 19°C in the dark.

The calanoid copepod *Acartia tonsa* Dana was collected from the York River, Virginia, and maintained in 0.2 µm filtered artificial seawater (ASW) of 20 S under a 12 h:12 h dark cycle. The copepods were fed *Rhodomonas salina* and *Thalassiossira weissflogii* for 3–4 days until used in experiments.

Feeding experiments

Cell carbon contents of *Dunaliella tertiolecta* (31.1 pg C $cell^{-1}$), *Rhodomonas salina* (29.8 pg C $cell^{-1}$), *Oxyrrhis marina* (516.3 pg C $cell^{-1}$), and *Gyrodinium dominans* (270.2 pg C $cell^{-1}$) were estimated based on size-to-carbon conversion (Strathmann 1967; Menden-Deuer and Lessard 2000). The carbon content of *A. tonsa* was previously measured to be 4.6 µg C $copepod^{-1}$ (Tang et al. 1999).

Algae and heterotrophic protists used for lipid analysis were collected when cultures were in the active growth phase during August 2004. Three sub-samples from each planktonic alga and protist culture were taken and concentrated on GF/F filters. Protists were concentrated on the filter via gravitational filtration.

Aliquots of all cultures were taken and preserved in 2% acid Lugol's iodine for cell counts. Cell counts showed that remnant algal food in the protists' cultures at the time of harvest was negligible. The filters were stored in −80°C until lipid analysis.

Copepod feeding experiments were conducted in a walk-in environmental room at 19°C with a 12 h light:12 h dark photoperiod during March 2005. Three replicate of approximately 100 live *Acartia tonsa* were collected for measuring initial fatty acid content prior to the experiments. The copepods were then fed, in triplicates, monocultures of *Dunaliella tertiolecta* (DT), *Rhodomonas salina* (RS), or *Oxyrrhis marina* grown on *D. tertiolecta* (OM-DT). To maximize ingestion rates, all diet treatments were standardized to at least 300 μg C l^{-1}. Experiments were conducted in 3-l containers with 40 copepods ml^{-1}. Two thirds of the water was replaced daily to prevent ammonia build-up and food was also replenished daily. Mortality of copepods was similar among all treatment groups (about 8% day^{-1}). After 5 days of feeding, the experiment was terminated and 121–158 live copepods were collected from each replicate treatment. All samples were stored at −80°C until biochemical analysis.

Lipid analyses

Total lipids were extracted by homogenizing the samples in a mixture of chloroform:methanol:water (2:2:1 by volume) according to Folch et al. (1957) and Bligh and Dyer (1959). A two-phase system was created with the upper phase (aqueous) containing the non-lipid impurities. The lower phase (chloroform containing lipids) was removed and evaporated to dryness with nitrogen. Lipids were then re-suspended in 1:1 chloroform:methanol and stored at −20°C until fatty acid analysis. For fatty acid analysis, each lipid sample was transesterified with boron trifluoride (BF_3) and hexane, and heated for 15 min at 100°C (Metcalfe and Schmitz 1961). Samples were then extracted with carbon disulfide, and the organic phase was evaporated under nitrogen and re-suspended in hexane (Marty et al. 1992). The fatty acid methyl esters (FAMEs) were analyzed according to Chu and Ozkizilcik (1995) using gas/liquid chromatography and identified by comparing their retention times with known standards (Sigma, Supelco, Bellefonte, USA) and confirmed with GC/MS. Differences in fatty acid profiles among treatments were tested with one-way ANOVA followed by a Tukey test.

Results

Lipid content and fatty acid composition of algae and heterotrophic protists

Rhodomonas salina had a higher total lipid content than *Dunaliella tertiolecta* (mean ± SD hereafter; 246.76 ± 34.74 and 88.00 ± 7.65 μg mg^{-1} C, respectively). *Dunaliella tertiolecta* had a fatty acid composition typical of the class Chlorophyceae, which is characterized, with a few exceptions, by high proportions of the fatty acids 16:0 and 16:4 ($n-3$) and very little, or no C20 and C22 LC$n-3$PUFAs (Ackman et al. 1968; Sargent 1976). The main fatty acids detected in our *D. tertiolecta* culture included 16:0, 16:4 ($n-3$) and 18:3 ($n-3$) (13.31 ± 1.00, 14.52 ± 1.37, 29.51 ± 2.73 μg mg^{-1} C, respectively, Table 1). DHA and EPA were not detected in *D. tertiolecta* (Table 1). In the cryptophyte *Rhodomonas salina*, the principal fatty acids observed included 14:0, 16:0, 18:3 ($n-3$), and 18:4 ($n-3$) (22.87 ± 3.52, 48.12 ± 7.61, 45.36 ± 7.42, 42.30 ± 7.06 μg mg^{-1} C, respectively, Table 2). EPA (13.07 ± 2.05 μg mg^{-1} C) and DHA (13.85 ± 4.37 μg mg^{-1} C) were also found in *R. salina* (Table 2).

The total lipid content of *Oxyrrhis marina* feeding on *Dunaliella tertiolecta* or *Rhodomonas salina* was higher (42.27 ± 11.61 and 83.24 ± 4.77 μg mg^{-1} C, respectively) than the total lipid content of *Gyrodinium dominans* grown on either diet (22.92 ± 10.14 and 22.00 ± 1.21 μg mg^{-1} C, respectively). *Oxyrrhis marina* fed *R. salina* had the highest lipid content.

A significant amount of DHA was detected in both *Oxyrrhis marina* (15.17 ± 3.40 μg mg^{-1} C) and *Gyrodinium dominans* (4.47 ± 1.61 μg mg^{-1} C) (Table 1) when fed on *Dunaliella tertiolecta*, however, the DHA content in *O. marina* was significantly higher than in *G. dominans* on that diet ($P = 0.002$, Fig. 1). In contrast, *G. dominans* had a significantly higher concentration of EPA (1.55 ± 0.61 μg mg^{-1} C) than *O. marina* (< 2% total fatty acids) in this treatment ($P < 0.006$; Fig. 1). Except for the presence of EPA and DHA in the protists feeding on *D. tertiolecta*, the fatty acid profile of the algae and protists was similar, albeit the amounts of fatty acids differed.

When fed *Rhodomonas salina*, the DHA contents of *Oxyrrhis marina* (24.46 ± 9.56 μg mg^{-1} C) and *G. dominans* (6.78 ± 1.46 μg mg^{-1} C) (Table 2) were not significantly different from their algal diet, but were significantly different from each other ($P = 0.027$, Fig. 1). The EPA content of *R. salina* (13.07 ± 2.05 μg mg^{-1} C) was significantly higher than that of *O. marina* (2.55 ± 0.94 μg mg^{-1} C) and *G. dominans* (1.95 ± 0.24 μg mg^{-1} C) ($P < 0.001$). There were no significant differences in EPA concentration between the two heterotrophic dinoflagellates (Fig. 1).

Some differences were observed in protists fed different algae. For example, *Oxyrrhis marina* fed *Dunaliella tertiolecta* (OM-DT) had detectable concentrations of 18:1(n−9), while only a trace amount of EPA (< 2% of total fatty acids) was detected. On the other hand, only trace amounts of 18:1(n−9) were detected in *O. marina* fed *Rhodomonas salina* (OM-RS), but the protist contained 2.55 ± 0.94 μg mg^{-1} C of EPA. Contrarily, *Gyrodinium dominans* grown on both algae had similar profiles and concentrations of fatty acids (Tables 1 and 2).

Table 1 *Oxyrrhis marina* and *Gyrodinium dominans* Fatty acid profile (μg mg^{-1} C) of heterotrophic dinoflagellates fed on *Dunaliella tertiolecta*. Values are mean ± SD ($n=3$), tr trace amounts (< 2% total fatty acid), — = not detected, RT retention time of unknown (min)

	Dunaliella tertiolecta (DT)	*Oxyrrhis marina* (OM-DT)	*Gyrodinium dominans* (GD-DT)
Saturated fatty acids			
14:0	Tr	0.94 ± 0.83	1.40 ± 0.67
15:0	—	—	Tr
16:0	13.31 ± 1.00	13.21 ± 3.45	4.51 ± 1.60
17:0	—	—	Tr
18:0	—	—	Tr
iso17:0dma	—	—	Tr
Monounsaturated fatty acids			
16:1(*n*−7)	—	—	Tr
16:1(*n*−13)t	2.56 ± 0.21	—	—
18:1(*n*−9)	3.32 ± 0.20	2.79 ± 0.68	0.99 ± 0.40
18:1(*n*−7	Tr	1.68 ± 0.38	1.57 ± 0.76
17:1(*n*−7)	3.36 ± 0.32	—	Tr
24:1(*n*−9)	—	—	Tr
Polyunsaturated fatty acids			
16:2(*n*−6)	Tr	—	—
16:2(*n*−4)	—	—	Tr
16:3(*n*−6)	Tr	—	—
16:4(*n*−3)	14.52 ± 1.37	Tr	Tr
16:4(*n*−1)	—	—	Tr
18:2(*n*−6)	5.23 ± 0.42	1.83 ± 0.44	Tr
18:2(*n*−4)	—	—	Tr
18:3(*n*−6)	2.74 ± 0.24	Tr	—
18:3(*n*−3)	29.51 ± 2.73	4.88 ± 1.10	Tr
18:4(*n*−3)	Tr	—	Tr
20:3(*n*−3)	—	—	Tr
20:4(*n*−6)	—	—	Tr
20:5(*n*−3)	—	Tr	1.55 ± 0.61
22:5(*n*−6)	—	—	Tr
22:6(*n*−3)	—	15.17 ± 3.40	4.47 ± 1.61
Unknown			
	—	—	Tr
RT = 10.79	2.36 ± 0.27	—	—
Total	88.00 ± 7.65	42.27 ± 11.61	22.92 ± 10.14

Fatty acids of bacterial origin (branched and odd chains) were detected in small concentrations (Tables 1 and 2) in algal and protist cultures because cultures were not axenic. Although it is possible that the protists may have consumed some of the ambient bacteria present in the seawater, it is likely that these fatty acids came from free-living bacteria which were retained on the filters during filtration.

Lipid content and fatty acid composition of *Acartia tonsa*

Prior to the feeding experiments, *Acartia tonsa*, which were fed a mixture of *Rhodomonas salina* and *Thalassiossira weissflogii*, had a lipid content comparable to *A. tonsa* fed the treatment diets (91.69 ± 14.13 μg mg^{-1} C; Table 3). Fatty acids such as 12:0 and 16:0 were the most abundant saturated fatty acids, while the monounsaturated fatty acid, 16:1(n−7) and the polyunsaturated fatty acids 18:3 (*n*−3), 20:5 (*n*−3), and 22:6 (*n*−3) were also present but in lower amounts (Table 3).

Acartia tonsa in the *Rhodomonas salina* treatment (RS) had a higher total lipid content (143.47 ± 16.24 μg mg^{-1} C) than *A. tonsa* in the *Dunaliella tertiolecta* treatment (DT; 92.16 ± 11.61 μg mg^{-1} C) and the *Oxyrrhis marina* treatment (OM-DT; 96.74 ± 9.17 μg mg^{-1} C). All copepods in the experiments were > 200 μm (total length) and of comparable size. Saturated fatty acids such as 14:0, 16:0 and 18:0, which are typical of calanoid copepods, were present in high concentrations in the RS treatment. Copepods in the DT and OM-DT treatments had lower amounts of 14:0 (Table 3).

Differences in the fatty acid profile of *Acartia tonsa* among the diet treatments were also observed. Copepods in the OM-DT treatment had a low amount of 18:3 (*n*−3) (5.28 ± 0.43 μg mg^{-1} C), while those in the DT treatment contained 34.50 ± 22.21 μg mg^{-1} C (Table 3). Only a trace amount of 18:4 (*n*−3) was detected in copepods in the DT and OM-DT treatments. Copepods fed *Rhodomonas salina* (RS) had the highest concentrations of both 18:3 (*n*−3) and 18:4 (*n*−3) (Table 3). The fatty acid 16:4 (*n*−3), which was relatively high in *Dunaliella tertiolecta*, was found only in trace amounts in *A. tonsa* fed this alga. Fatty acids of bacterial origin were also detected in trace amounts in the copepod cultures.

The highest concentration of EPA was found in copepods fed *Rhodomonas salina* (21.94 ± 4.02 μg mg^{-1} C; Table 3) and the content was significantly higher than

Table 2 *Oxyrrhis marina* and *Gyrodinium dominans*

Fatty acid profile (μg mg^{-1} C) of heterotrophic dinoflagellates fed on *Rhodomonas salina*. Values are mean ± SD ($n=3$), tr trace amounts (< 2% total fatty acid), — = not detected, RT retention time of unknown (min)

	Rhodomonas salina (RS)	*Oxyrrhis marina* (OM-RS)	*Gyrodinium dominans* (GD-RS)
Saturated fatty acids			
12:0	—	Tr	—
13:0	—	Tr	—
14:0	22.87 ± 3.52	3.15 ± 1.12	2.06 ± 0.24
15:0	Tr	—	Tr
16:0	48.12 ± 7.61	16.99 ± 6.16	4.73 ± 0.76
17:0	Tr	Tr	Tr
18:0	Tr	Tr	Tr
17:0dma	Tr	Tr	—
iso17:0dma	Tr	—	Tr
22:0	—	Tr	—
24:0	—	—	Tr
Monounsaturated fatty acids			
14:1(*n*−7)		Tr	—
16:1(*n*−13)t	Tr	Tr	—
16:1(*n*−9)	Tr	—	—
16:1(*n*−7)	Tr	Tr	Tr
16:1(*n*−5)	—	Tr	—
18:1(*n*−11)	Tr	Tr	—
18:1(*n*−9)	12.84 ± 1.95	Tr	0.91 ± 0.29
18:1(*n*−7)	Tr	4.22 ± 1.60	1.49 ± 0.39
20:1dma	—	Tr	—
20:1(*n*−9)	—	Tr	—
Polyunsaturated fatty acids			
16:3(*n*−6)	—	Tr	—
16:3(*n*−3)	—	Tr	—
16:4(*n*−3)	—	Tr	—
16:4(*n*−1)	—	Tr	—
18:2(*n*−6)	17.60 ± 2.65	2.99 ± 1.27	Tr
18:3(*n*−6)	Tr	Tr	—
18:3(*n*−3)	45.36 ± 7.42	3.80 ± 1.63	Tr
18:4(*n*−3)	42.30 ± 7.06	Tr	Tr
20:3(*n*−3)	—	Tr	—
20:4(*n*−6)	Tr	Tr	—
20:5(*n*−3)	13.07 ± 2.05	2.55 ± 0.94	1.95 ± 0.24
22:5(*n*−6)	—	Tr	Tr
22:5(*n*−3)	Tr	Tr	—
22:6(*n*−3)	13.85 ± 4.37	24.46 ± 9.56	6.78 ± 1.46
24:1(*n*−9)	—	Tr	—
Unknown	—	—	—
RT = 12.33	—	—	Tr
Total	246.76 ± 34.74	83.24 ± 4.77	22.00 ± 1.21

those fed the other two diets ($P < 0.001$; Fig. 2). EPA concentrations in the other two treatments were 6.53 ± 4.09 and 7.54 ± 0.63 μg mg^{-1} C (Table 3). The DHA concentration in *Acartia tonsa* in the DT treatment (8.30 ± 5.27 μg mg^{-1} C) was significantly lower than that in the RS treatment (27.72 ± 3.08 μg mg^{-1} C) and the OM-DT treatment (36.00 ± 5.25 μg mg^{-1} C) ($P < 0.001$; Fig. 2).

Comparison of the fatty acid profiles between initial copepod samples and copepods in the three diet treatments shows that the EPA content of *Acartia tonsa* decreased significantly when fed *Dunaliella tertiolecta* ($P = 0.014$; Fig. 2) and *Oxyrrhis marina* ($P = 0.001$; Fig. 2). The turnover rates of EPA on these diets were −2.08 and −1.88 μg mg^{-1} C d^{-1}, respectively (Table 4). In contrast, DHA content of the copepod increased significantly when fed *Rhodomonas salina* (2.63 μg mg^{-1} C d^{-1}) and *O. marina* (4.23 μg mg^{-1} C d^{-1}; Table 4) ($P = 0.002$; Fig. 2). The mean DHA content in copepods fed *D. tertiolecta* was lower but not significantly different from the initial copepods (Fig. 2).

Discussion

The present study suggests that trophic upgrading is specific to the species of algae and heterotrophic protists involved. The alga *Dunaliella tertiolecta* lacks both EPA and DHA, but, significant amounts of both were detected in the two heterotrophic dinoflagellates, *Oxyrrhis marina* and *Gyrodinium dominans*, that fed on *D. tertiolecta*. These results are consistent with those of Klein Breteler et al. (1999), who also detected high levels of DHA in *O. marina* fed *D. tertiolecta*. Our results and previous studies suggest that the heterotrophic protists modify and/or convert long chain (*n*−3)

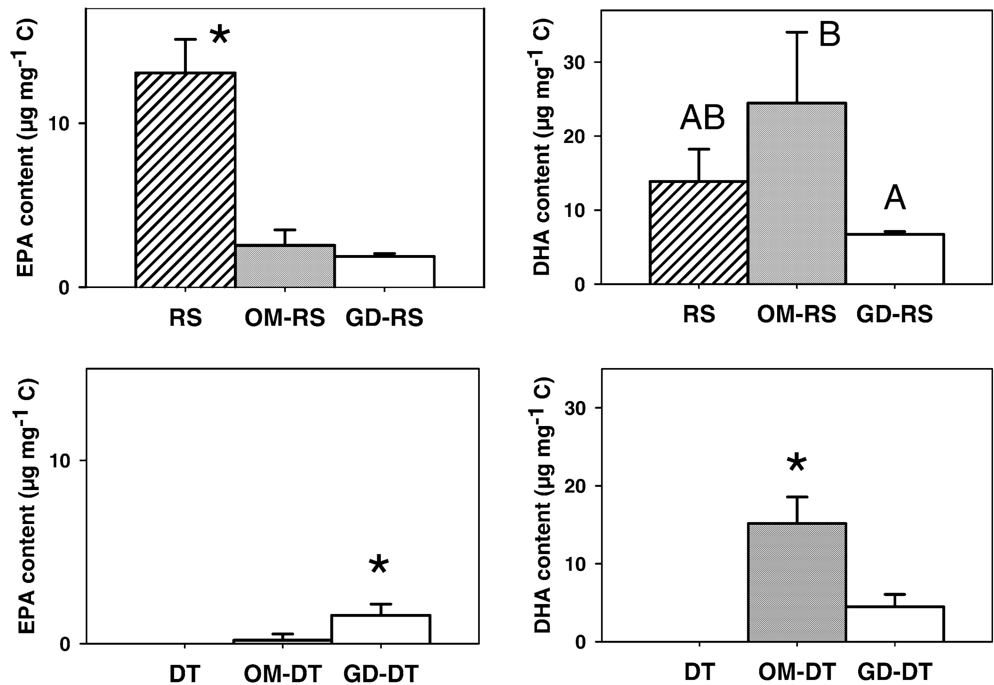

Fig. 1 *Oxyrrhis marina* and *Gyrodinium dominans*. EPA and DHA concentrations (mean ± SD) in heterotrophic dinoflagellates fed on *Dunaliella tertiolecta* (DT) or *Rhodomonas salina* (RS). OM *O. marina*, GD *G. dominans*. EPA and DHA were not detectable in *D. tertiolecta*. Asterisks and letters above bars indicate significance ($P < 0.05$; 1-ANOVA), with different letters denoting significant difference between treatments

polyunsaturated fatty acid precursors in *D. tertiolecta* to EPA and DHA, thereby providing better nutrition for higher trophic organisms. For example, *D. tertiolecta* is readily ingested by copepods, but it does not support growth in many copepod species (Støttrup and Jensen 1990; Koski et al. 1998). Tang and Taal (2005) reported that *Acartia tonsa* fed *O. marina* that grew on *D. tertiolecta* had a higher egg production rate and egg hatching success than *A. tonsa* that fed on *D. tertiolecta* directly, despite the higher ingestion rates observed with the alga alone. Similarly, *O. marina* that was fed *D. tertiolecta* supported rapid growth and development of two other copepod species, *Temora longicornis* and *Pseudocalanus elongatus* (Klein Breteler et al. 1999).

In addition to EPA and DHA, Klein Breteler et al. (1999) also reported the presence of cholesterol, brassicasterol, and other sterols in *Oxyrrhis marina* fed *Dunaliella tertiolecta*. No sterol was detected in *D. tertiolecta* (Klein Breteler et al. 1999), although various phytosterols are present in algae (Kayama et al. 1989; Soudant et al. 1996, 1998). Sterols are necessary for cell structure and functioning, reproduction, and growth (Ederington et al. 1995). Cholesterol is the principal sterol in crustaceans (Goad 1981). Because copepods are incapable of synthesizing sterols de novo, they must rely on their diets to obtain these compounds. Thus, the absence of sterols in *D. tertiolecta* could also be responsible for the low egg production observed in copepods fed this alga. The ability of *O. marina* to synthesize or modify sterols further emphasizes the important role of heterotrophic protists in trophic upgrading.

Since *Dunaliella tertiolecta* lacks EPA and DHA, the DHA and/or EPA detected in the two heterotrophic dinoflagellates apparently did not come from bioaccumulation, although this is typically the mechanism by which most organisms in higher trophic levels obtain their EFAs. Heterotrophs are generally believed to be incapable of synthesizing EFAs. So far only the heterotrophic dinoflagellate *Crypthecodinium cohnii* (Barclay et al. 1994) and the marine zooflagellate *Bodo* sp. (Zhukova and Kharlamenko 1999) are known to synthesize DHA. Kleppel and Burkart (1995) and Kleppel et al. (1998) did not detect any EFAs in *O. marina* grown on yeast, which lacks EPA, DHA, and other PUFAs. Thus it is likely that *O. marina* acquired precursors such as α-linolenic acid and 18:4 ($n-3$) from *D. tertiolecta*, and synthesized DHA via elongation and desaturation rather than by de novo synthesis of DHA. While *D. tertiolecta* had a relatively high level of α-linolenic acid (29.51 ± 2.73 µg mg^{-1} C), *Gyrodinium dominans* fed with this alga had only a trace amount of this fatty acid. These results lead us to hypothesize that α-linolenic acid, perhaps along with other shorter chain $n-3$ fatty acids [16:4($n-3$)] obtained from the algal food, were used by the protists to produce EPA and DHA. This pathway is typical among marine algae and mosses (Gurret al. 2002). Nevertheless, it is unlikely that all the 18:3($n-3$) and other short chain $n-3$ fatty acids present in *D. tertiolecta* were utilized to produce DHA and EPA. Some of them may have been consumed for energy. The capability to synthesize EFAs from precursor fatty acids has also been demonstrated in a protozoan *Parauronema acutum* (Sul and Erwin 1997), another copepod *Paracalanus parvus* (Nanton and Castell 1998), and the oyster *Crassostrea virginica* (Chu and Greaves 1991); however, this bioconversion appears inefficient in maintaining optimal growth rates for the studied invertebrates.

Table 3 *Acartia tonsa*

Fatty acid profile (μg mg^{-1} C) of copepods fed on algae and heterotrophic dinoflagellate. Values are mean ± SD ($n=3$), tr trace amounts (< 2% total fatty acid), — = not detected, RT retention time of unknown (min)

	Initial	*Dunaliella tertiolecta* (DT)	*Rhodomonas salina* (RS)	*Oxyrrhis marina* (OM-DT)
Saturated fatty acids				
12:0	55.62 ± 3.36	18.66 ± 9.71	26.78 ± 6.56	25.71 ± 1.47
14:0	9.75 ± 1.42	4.82 ± 0.89	10.81 ± 2.95	4.03 ± 0.37
16:0	42.90 ± 3.02	49.35 ± 8.15	42.32 ± 5.97	41.34 ± 2.73
18:0	9.36 ± 0.65	11.25 ± 0.60	14.87 ± 0.87	9.56 ± 1.77
22:0	Tr	Tr	Tr	Tr
24:0	Tr	—	Tr	Tr
Monounsaturated fatty acids				
16:1(*n*−7)	23.96 ± 2.88	7.95 ± 3.96	9.26 ± 1.91	5.34 ± 0.33
16:1(*n*−5)	Tr	Tr	Tr	Tr
16:1(*n*−13)t	Tr	Tr	Tr	Tr
18:1(*n*−11)	Tr	Tr	Tr	Tr
18:1(*n*−9)	Tr	4.46 ± 1.83	Tr	Tr
18:1(*n*−7)	6.33 ± 0.40	Tr	8.44 ± 1.34	4.37 ± 0.50
20:1(*n*−9)	Tr	Tr	Tr	Tr
20:1(*n*−7)	—	Tr	—	—
22:1(*n*−11)	Tr	Tr	Tr	Tr
Polyunsaturated fatty acids				
16:2(*n*−4)	—	Tr	—	—
16:3(*n*−6)	Tr	Tr	Tr	3.90 ± 0.76
16:3(*n*−4)	—	Tr	Tr	Tr
16:4(*n*−3)	—	Tr	Tr	Tr
16:4(*n*−1)	Tr	—	Tr	Tr
18:2(*n*−6)	Tr	Tr	6.57 ± 1.23	Tr
18:2(*n*−6)t	—	Tr	Tr	—
18:3(*n*−6)	Tr	Tr	Tr	Tr
18:3(*n*−3)	15.62 ± 1.31	34.50 ± 22.2	30.88 ± 7.62	5.28 ± 0.43
18:4(*n*−3)	13.73 ± 1.55	Tr	31.24 ± 9.05	Tr
20:4(*n*−6)	Tr	Tr	Tr	Tr
20:3(*n*−3)	—	Tr	Tr	Tr
20:5(*n*−3)	16.92 ± 1.36	6.53 ± 4.09	21.94 ± 4.02	7.54 ± 0.63
22:5(*n*−6)	—	Tr	Tr	—
22:5(*n*−3)	Tr	Tr	Tr	Tr
22:6(*n*−3)	14.59 ± 1.15	8.30 ± 5.27	27.72 ± 3.08	36.00 ± 5.25
Unknown				
RT = 18.20	5.25 ± 0.81			
Total	91.69 ± 14.13	92.16 ± 11.61	143.47 ± 16.24	96.74 ± 9.17

In the high food quality treatment, *Rhodomonas salina* had a significantly higher concentration of EPA than *Oxyrrhis marina* (OM-RS) and *Gyrodinium dominans* (GD-RS) fed with this alga, while DHA concentration was not significantly different. Concentrations of other fatty acids were also lower in the two heterotrophic protists compared to the alga. It is likely that most of the DHA and EPA found in both protists was assimilated from the algal food rather than synthesized from precursors such as 18:3(*n*−3) and 18:4(*n*−3), which are abundant in *R. salina*. It also appears that when heterotrophic protists feed on phytoplankton rich in EPA and DHA, they preferentially accumulate DHA over EPA. Dietary EPA may be metabolized by the protists for energy or other physiological demands. Tang and Taal (2005) reported lower egg production efficiency in *Acartia tonsa* when *O. marina* (RS) was the prey, versus *A. tonsa* fed on the alga directly. The lower content of EPA detected in the protist on this diet could be responsible for the lower egg production in *A. tonsa*.

Regardless of the diets both protists had less EPA than DHA, and Klein Breteler et al. (1999) and Broglio et al. (2003) reported that heterotrophic dinoflagellates tend to be richer in DHA than in EPA. The difference in the concentration of the fatty acids suggests that (1) there may be a higher physiological demand for DHA in heterotrophic protists or (2) EPA is further elongated and desaturated to produce DHA, or (3) EPA is utilized as an energy source.

There were also differences in DHA concentration between the two heterotrophic dinoflagellates. *Oxyrrhis marina* was significantly richer in DHA than *Gyrodinium dominans* when they fed on the same algal food (*Dunaliella tertiolecta*). It is not known whether copepods require a high ratio of DHA to EPA in their diets for growth and development. A high ratio of DHA to EPA in the diet is critical for the growth and development of larval and juvenile fish (Watanabe 1993; Rainuzzo et al. 1997). Although both *O. marina* and *G. dominans* upgraded their food, if the demand for DHA is higher than EPA for effective reproduction, then *O. marina* (DT) may have a higher nutritional quality than *G. dominans* (DT) for *Acartia tonsa*. Tang and Taal (2005) reported that the egg production efficiency of *A. tonsa* was sig-

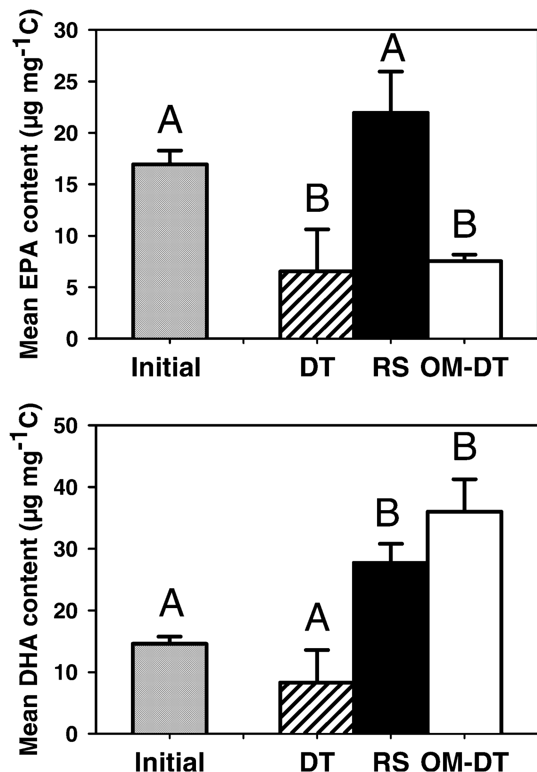

Fig. 2 *Acartia tonsa*. Initial and final EPA and DHA concentrations (mean ± SD) on three treatment diets. (DT *Dunaliella tertiolecta*, RS *Rhodomonas salina*, OM-DT *Oxyrrhis marina* grown on *Dunaliella tertiolecta*). *(n = 3)*. Letters above bars indicate significance ($P < 0.05$; 1-ANOVA), with different letters denoting significant difference between treatments

nificantly higher with *O. marina* fed *D. tertiolecta* than with *G. dominans* fed *D. tertiolecta*. The DHA concentration was also lower in *G. dominans* (GD-RS) than in *O. marina* (OM-RS). The lower concentration of DHA in *G. dominans* suggests that EFA enrichment capabilities may vary among protist species.

It is well documented that long chain ($n-3$) PUFAs are important for growth and development in marine calanoid copepods (Jónasdóttir 1994; Jónasdóttir and Kiørboe 1996), but few studies have examined the potential trophic upgrading effects on the biochemical composition of copepods. In the present study, we compared the effects of heterotrophic and autotrophic diets on the EFA content of *Acartia tonsa*. Copepods that fed on *Dunaliella tertiolecta* had a significantly lower content of EPA and DHA. In contrast, *A. tonsa* feeding on *Oxyrrhis marina* grown on this alga had the highest concentration of DHA, suggesting that *O. marina* had trophically upgraded the nutritional value of the poor-quality alga, and efficiently provided DHA to the next trophic level. Based on the carbon content and fatty acid content per cell of *Rhodomonas salina* (EPA = $9.7\text{x}10^{-7}$ µg cell^{-1}; DHA = $1.03\text{x}10^{-6}$ µg cell^{-1}) and *Oxyrrhis marina* (EPA = $2.5\text{x}10^{-7}$ µg cell^{-1}; DHA = $1.96\text{x}10^{-5}$ µg cell^{-1}), we calculated that the observed EPA and DHA content of *A. tonsa* was equivalent to 3.10–71.60 µg C of the food. Tang and Taal (2005) reported ingestion rates of *A. tonsa*, with the same diets and food concentrations as in the present study, ranging from 2.2 to 5.4 µg C ind^{-1} day^{-1}. Assuming a gut passage time of 60 min for *A. tonsa* at 17°–19°C (Arashkevich 1977), the copepods would have accumulated at most 0.09–0.23 µg C ind^{-1} in their gut at the time of sampling. This analysis shows that the detected EPA and DHA in the copepods were not due to remnant food in the guts but to fatty acids assimilated into the copepod body tissues.

Although *Dunaliella tertiolecta* lacks EPA and DHA, it is a rich source of α-linolenic acid, which could fulfill the demand for LC$n-3$PUFAs in the copepods. However, studies show that copepods continuously feeding on *D. tertiolecta* suffer from female sterility, oocyte deterioration (Lacoste et al. 2001), poor growth and development (Koski et al. 1998; Tang et al. 2001). EPA and DHA were detected in *A. tonsa* that fed on *D. tertiolecta*, but at low concentrations. The EPA and DHA detected in *A. tonsa* in the *D. tertiolecta* treatment is likely to have originated from the two algae, *Rhodomonas salina* and *Thalassiossira weissflogii*, that were used to feed the copepods prior to the feeding experiments since both DHA and EPA declined in *A. tonsa* after feeding with *D. tertiolecta* for 5 days. Moreover, there was an abundance of 18:3($n-3$) in *A. tonsa* fed with this alga and *Rhodomonas salina*. As *R. salina*, *Thalassiossira weissflogii* contains both EPA and DHA (Soudant and Chu, unpublished data). Nevertheless, the ability to convert α-linolenic acid into EPA and DHA has been demonstrated in the calanoid copepod *Paracalanus parvus* (Moreno et al. 1979) and in some harpacticoid copepod species (Norsker and Støttrup 1994; Nanton and Castell 1998). Desvilletes et al. (1997) also suggested that the cyclopoid *Eucyclops serrulatus* may convert α-linolenic acid into DHA. However, Brett and Müller-Navarra (1997) argued that this bioconversion is too slow to support growth and development. The lack or low availability of dietary EPA and DHA might have

Table 4 *Acartia tonsa*

	Turnover rate	
	(µg mg C^{-1} day^{-1})	(% day^{-1})
EPA		
DT	–2.08	–12.28
RS	1.00	5.93
OM-DT	–1.88	–11.09
DHA		
DT	–1.26	–8.62
RS	2.63	18.00
OM-DT	4.23	29.35

Turnover rates of EPA and DHA in the copepod in the three diet treatments in terms of net change (µg mg C^{-1} day^{-1}) and percent change (% day^{-1}) calculated based on the initial EPA and DHA contents of *A. tonsa* (Table 3). Negative values indicate net loss; positive values indicate net gain. DT *Dunaliella tertiolecta*, RS *Rhodomonas salina*, OM-DT *Oxyrrhis marina* grown on *D. tertiolecta*

contributed to the poor reproductive and growth capabilities often observed in marine calanoid copepods, although EPA and DHA may not be the sole factors essential for growth and reproduction.

Rhodomonas salina is considered a good-quality alga due to its ability to support high egg production and naupliar growth rates in copepods (Tang et al. 2001). *Acartia tonsa* that were fed *R. salina* had high concentrations of EPA, DHA, and α-linolenic acid. The high content of ($n-3$) EFAs in the profile of *R. salina*, as well as in the copepods that fed on it, indicate an efficient transfer of essential nutrients at the phytoplankton–zooplankton interface.

Long chain $n-3$ PUFAs are vital to marine organisms for proper growth and development (Brett et al. 1997). Planktonic algae are viewed as the primary producers of LC$n-3$PUFAs, while organisms in higher trophic levels obtain these nutrients mainly through bioaccumulation. However, evidence that heterotrophic protists may act as modifiers and/ or producers of LC$n-3$PUFAs is mounting. In this study, *Oxyrrhis marina* (OM-DT) and *Gyrodinium dominans* (GD-DT) enhanced the amount of DHA and EPA when feeding on nutritionally poor algae. Heterotrophic dinoflagellates are ubiquitous in marine ecosystems and a valuable component in the diet of zooplankters. The interactions between planktonic algae and heterotrophic protists may be key factors regulating the production and subsequent transfer of EFAs in marine food webs (Brett and Muller-Navarra 1997). In the present study we also showed that the EFA content of *Acartia tonsa* depended on its diet. Assuming that *A. tonsa* did not produce any EPA or DHA and that the increase or decrease is linear, feeding on *D. tertiolecta* resulted in a loss of 12.28% EPA and 8.62% DHA day^{-1} compared to the initial contents in *A. tonsa*, and feeding on OM-DT resulted in a loss of 11.09% EPA and a bioaccumulation of 29.35% DHA day^{-1}. This dependency could have important repercussions for the transfer of EFAs to higher trophic levels since changes can occur quite rapidly. DHA demand is high in marine fish, especially larvae and juveniles, which require DHA for optimum growth and development (Watanabe 1993; Bell and Sargent 1996; Rainuzzo et al. 1997). Poor transfer of this fatty acid to developing fish could lower productivity and result in failed recruitment. Further research is necessary to determine if trophic upgrading is common among marine heterotrophic protists and if so, what biochemical mechanisms (e.g., de novo synthesis and/or modification of precursors) allow the production of EFAs and other biochemical compounds in heterotrophic protists, and how changes in EFA content at the base of the food chain affect production in higher trophic levels.

Acknowledgements This study was supported by NOAA-CMER awards NA03NMF4550382 and NA04NMF4550390, and by Sigma Xi Grants In Aid of Research. The authors are grateful for Dr. Eric Lund's advice and help in lipid analysis, GC operation and editing the revised version of this manuscript. The authors thank Mrs. Georgetta Constantin for assistance in lipid analyses. Contribution no. 2698 by the Virginia Institute of Marine Science, College of William and Mary.

References

Ackman RG, Tocher DS, McLachlan J (1968) Marine phytoplankter fatty acids. J Fish Res Bd Can 25:1603–1620

Arashkevich EG (1977) Duration of food digestion in marine copepods. Pol Arch Hydrobiol 24 : (Suppl) 431–438

Atkinson A (1994) Diets and feeding selectivity among the epipelagic copepod community near South Georgia in summer. Polar Biol 14:551–560

Barclay WR, Meager KM, Abril JR (1994) Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. J Appl Phycol 6:123–129

Bell MV, Sargent JR (1996) Lipid nutrition and fish recruitment. Mar Ecol Prog Ser 134:315–316

Bligh EG, Dyer WG (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911–917

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biol 38:483–499

Broglio E, Jónasdóttir SH, Calbet A, Jakobsen HH, Saiz E (2003) Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. Aquat Microb Ecol 31:267–278

Chu F-LE, Greaves J (1991) Metabolism of palmitic, linoleic, and linolenic acids in adult oysters, *Crassostrea virginica*. Mar Biol 110:5229–236

Chu F-LE, Ozkizilcik S (1995) Lipid and fatty acid composition of striped bass (*Morone saxatilis*) larvae during development. Comp Biochem Physiol 111:665–674

Desvilletes C, Bourdier G, Breton JC (1997) On the occurrence of a possible bioconversion of linolenic acid into docosahexaenoic acid by the copepod *Eucyclops serrulatus* fed on phytoplankton. J Plankton Res 19:273–278

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnol Oceanogr 40(5):860–867

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

Fraser AJ, Sargent JR (1989) Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar Chem 27:1–18

Gifford DJ, Dagg MJ (1991) The microzooplankton–mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Murkukawa. Mar Microb Food Webs 5:161–177

Goad LJ (1981) Sterol biosynthesis and metabolism in marine invertebrates. Pure Appl Chem 51:837–852

Graeve M, Kattner G, Hagen W (1994) Diet induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97–110

Gurr MI, Harwood JL, Frayn KN (Eds) (2002) Lipid biochemistry. Blackwell , Oxford

Harvey HR, Ederington MC, McManus GB (1997) Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina*: shifts in response to changes in diet. J Eukaryot Microbiol 44:189–193

Jónasdóttir SH (1994) Effect of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Mar Biol 101:67–81

Jónasdóttir SH, Kiørboe T (1996) Copepod recruitment and food composition: do diatoms affect hatching success? Mar Biol 125:743–750

Kattner G, Krause M, Trahms J (1981) Lipid composition of some typical North Sea copepods. Mar Ecol Prog Ser 4:69–74

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135:191–198

Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence of trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274:199–208

Kleppel GS, Burkart CA (1995) Egg production and the nutritional environment of *Acartia tonsa*: the role of food quality in copepod nutrition. ICES J Mar Sci 52:297–304

Kleppel GS, Burkart CA, Houchin L (1998) Nutrition and their regulation of egg production in the calanoid copepod *Acartia tonsa*. Limnol Oceanogr 43: 1000–1007

Koski M, Klein Breteler W, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). Mar Ecol Prog Ser 170:169–187

Lacoste A, Poulet SA, Cueff A, Kattner G, Ianora A, Laabir M (2001) New evidence of the copepod maternal food effects on reproduction. J Exp Mar Biol Ecol 259:85–107

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204:65–77

Marty Y, Delaunay F, Moal J, Samain JF (1992) Change in the fatty acid composition of *Pecten maximus*. J Exp Mar Biol Ecol 163:221–34

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569–579

Metcalfe LD, Schmitz AA (1961) The rapid preparation of fatty acid esters for gas chromatography analysis. Anal Chem 33:363–364

Moreno JJ, de Moreno JEA, Brenner RR (1979) Fatty acid metabolism in the calanoid copepod *Paracalanus parvus*: 1. polyunsaturated fatty acids. Lipids 14:313–322

Morris RJ, McCartney MJ, Robinson GA (1983) Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. J Exp Mar Biol Ecol 70:249–262

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E, Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427:69–72

Nanton DA, Castell JD (1998) The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae. Aquaculture 163:249–259

Norsker NH, Støttrup J (1994) The importance of dietary HUFAs for fecundity and PUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture 125:155–166

Park S, Brett MT, Müller-Navarra DC, Shin SC, Liston AM, Goldman CR (2003) Heterotrophic nanoflagellates and increased essential fatty acids during *Mycrocistis* decay. Aquat Microb Ecol 33:201–205

Rainuzzo JR, Reitan KI, Olsen Y (1997) The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103–115

Sargent JR (1976) The structure, metabolism, and function of lipids in marine organisms. In: Malins C, Sargent JR (eds) Biochemical and biophysical perspectives in marine biology. Academic Press, London, pp 149–212

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: Longhurst AR (eds) Analysis of marine ecosystems. Academic Press, London, pp 491–533

Soudant P, Marty Y, Moal J, Robert R, Quere C, Le Coz JR, Samain JF (1996) Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143:361–378

Soudant P, Le Coz JR, Marty Y, Moal J, Robert R, Samain JF (1998) Incorporation of microalgae sterols by scallop *Pecten maximus* (L.) larvae. Comp Biochem Physiol 119A:451–457

Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12:411–418

Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg production of the calanoid copepod *Acartia tonsa* Dana. J Exp Mar Biol Ecol 141:87–105

Sul DG, Erwin JA (1997) The membrane lipids of the marine ciliated protozoan *Parauronema acutum*. Biochim Biophys Acta 1345:162–171

Tang KW, Taal M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. J Exp Mar Biol Ecol 318:85–98

Tang KW, Dam HG, Visscher PT, Fenn TD (1999) Dimethylsulfoniopropionate (DMSP) in marine copepods and its relation with diets and salinity. Mar Ecol Prog Ser 179:71–79

Tang KW, Jakobsen HH, Visser AW (2001) *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: Feeding, growth and trophic interactions among grazers. Limnol Oceanogr 46:1860–1870

Watanabe T (1993) Importance of docosahexaenoic acid in marine larval fish. J World Aquacult Soc 24:152–161

Zhukova NV, Kharlamenko VI (1999) Sources of essential fatty acids in the marine microbial loop. Aquat Microb Ecol 17:153–157