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Quantitative significance of n-3 essential fatty acid contribution by heterotrophic protists in marine pelagic food webs

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ABSTRACT: To assess the contribution of n-3 essential lipids by heterotrophic protists in the pelagic food webs, we examined the kinetics and efficiency of long-chain n-3 essential fatty acid (LCn-3EFA) production of 2 common heterotrophic protists, *Oxyrrhis marina* and *Gyrodinium dominans,* fed an alga (*Dunaliella tertiolecta*) deficient in both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). *D. tertiolecta* was rapidly ingested and consumed by the heterotrophic protists. Growth rates ranged from 0.77 to 0.82 and 0.78 to 0.92 d^{-1} in *O. marina* and *G. dominans*, respectively. LCn-3EFA production in the 2 heterotrophic protists reached the highest levels at the highest protist cell density in the feeding experiments, equivalent to a production of 1.9 ± 0.3 µg EPA and 22.3 ± 6.4 µg DHA by *O. marina* and 4.7 ± 0.5 µg EPA and 16.5 ± 1.1 µg DHA by *G. dominans* per mg algal carbon consumed. Both protists contained much higher levels of DHA, a nutrient critical for neural and visual development for marine organisms at higher trophic levels, compared to the good food quality alga *Rhodomonas salina*. This suggests that the LCn-3EFA contribution by heterotrophic protists to the pelagic food webs is quantitatively significant and may be crucial for the production and recruitment of species at higher trophic levels, particularly at times of blooms dominated by algal species deficient in LCn-3EFAs or when the primary producer standing stock is dominated by pico- and nanoplankton during non-bloom periods.

KEY WORDS: Heterotrophic protists · Algae · Essential fatty acids · Nutrient upgrading · Docosahexaenoic acid · Eicosapentaenoic acid · Phytoplankton-heterotrophic protist interface

INTRODUCTION

Microzooplankton, mainly ciliates and heterotrophic dinoflagellates, contribute a substantial portion of the zooplankter food ration throughout a wide geographical range, particularly during non-bloom periods (Tang & Taal 2005). Heterotrophic protists are nutritionally diverse, consisting of both purely heterotrophic and mixotrophic species. They feed on a wide range of microbes, micro-, pico- and nanoplankton, and detritus, and are in turn consumed by larger animals such as copepods, the most dominant animal group in the marine planktonic system. They serve as an intermediate link between the microbial loop and higher trophic levels and play a role in top-down regulation of the *in situ* algal and bacterial biomass, thus affecting community composition and production.

Lipids contribute to cellular structure and provide stored fuel for all living organisms. They are involved in many biological and biochemical processes and serve as precursors of physiologically active metabolites such as eicosanoids. Polyunsaturated fatty acids (PUFAs) are one of the major constituents of structural lipids. The long-chain n-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaneoic acid (EPA) and the long-chain n-6 PUFA arachidonic acid (AA) (Ackman et al. 1980, Sargent et al. 1987, 2002) are essential for many animals, since they cannot synthesize them de novo or in sufficient amounts, and so must rely on diet as the primary source of these biochemicals. The

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importance of these essential fatty acids as membrane components and for the maintenance of proper physiological functions, and DHA particularly for visual and neural development in marine organisms at higher levels of the trophic hierarchy, is well recognized (M. V. Bell et al. 1995, Navarro et al. 1997, J. G. Bell et al. 1999). In the pelagic system, planktonic algae are the primary producers of DHA, EPA and AA (Ackman et al. 1980, Sargent et al. 1987, 2002). Higher consumers such as calanoid copepods, shellfish and finfish cannot synthesize essential fatty acids de novo and must acquire them via trophic accumulation up the food chain (Sargent et al. 2002).

Due to their prominence as algal predators and zooplankton prey, heterotrophic protists play a significant role in channeling energy and nutrients across the phytoplankton-zooplankton interface. Recently the ecological benefits and implications of essential nutrient upgrading and transfer by heterotrophic protists at the phytoplankton-zooplankton interface in the pelagic food webs have attracted much interest (e.g. Klein Breteler et al. 1999, Tang & Taal 2005, Veloza et al. 2006). The emerging attention stems from laboratory observations of better growth and development and higher fecundity of calanoid copepods fed heterotrophic protists previously raised on poor food quality algae (i.e. deficient or low in DHA and EPA) than the copepods fed the poor food quality algae alone. For example, a recent study showed that while omnivorous calanoid copepods failed to grow on the essential fatty acid-deficient *Dunaliella* sp. alone (Klein Breteler et al. 1999), they grew well and developed normally when fed the heterotrophic protist *Oxyrrhis marina* raised on *Dunaliella* sp. Other studies also noted that utilizing heterotrophic protists as intermediate prey improves the fatty acid composition from low quality algae, leading to higher egg production (Klein Breteler et al. 1999, Tang et al. 2001) and egg hatching success (Broglio et al. 2003) in copepods. Lipid analysis of the protist predators and the algal prey revealed that the essential lipids EPA and DHA and sterols which are not present in the prey are present in the predators, and that the predators have elevated levels of these compounds when fed algae that do contain them (Klein Breteler et al. 1999, Broglio et al. 2003, Veloza et al. 2006). Apparently, heterotrophic protists can 'upgrade' the biochemical constituents such as long-chain n-3 essential fatty acids (LCn-3EFAs) of algal food. Although the mechanisms and chemical processes of upgrading are not known, it is clear that as intermediaries certain heterotrophic protists contribute to the pool of essential fatty acids subsequently available to copepods and higher trophic levels. Presently, the kinetics and efficiency of trophic upgrading of essential fatty acids by heterotrophic protists are unclear and have not been examined. Such data are critically needed to evaluate the potential contribution of essential nutrients by heterotrophic protists as trophic intermediaries in the plankton, on which the ocean food chain depends, and the nutritional status of the pelagic system as a whole. The assimilation and/or production dynamics of LCn-3EFAs at the algae-heterotrophic protist interface may ultimately affect productivity at higher trophic levels. Also, the biochemical process of upgrading in heterotrophic protists appears to vary with species (Veloza et al. 2006). To assess the contribution of essential fatty acids by heterotrophic protists in the pelagic food webs, we examined the kinetics and efficiency of essential fatty acid production of 2 common heterotrophic protists, *O. marina* and *Gyrodinium dominans,* fed an essential fatty acid-deficient alga *Dunaliella tertiolecta*.

MATERIALS AND METHODS

Algae and protist cultures. The chlorophyte *Dunaliella tertiolecta* (CCMP 1320), the cryptophyte *Rhodomonas salina* (CCMP 1319), the bacillariophyte *Chaetoceros calcitrans* (CCMP 1315) and the prasinophyte *Tetraselmis suecica* (PLY 305) were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and all, except *C. calcitrans*, cultured in f/2 culture medium prepared from artificial sea water (ASW) of salinity 20. *C. calcitrans* was cultured in f/2 culture medium containing silicon (Si). *D. tertiolecta* was chosen as the prey for the heterotrophic protist feeding experiments, because it lacks the essential fatty acids EPA and DHA, but contains high levels of precursors to EPA and DHA, e.g. 18:3(n-3) and 18:4(n-3). *R. salina*, *C. calcitrans* and *T. suecica* were used to determine EPA and DHA content in algae that contain only EPA (*T. suecica*) or both EPA and DHA (*R. salina*, *C. calcitrans*). *D. tertiolecta* cultures used in feeding experiments were grown in 1 l round bottom flasks and the other 3 species were cultured in 250 ml Erlenmeyer flasks with aeration in a walk-in environmental room at 19°C in a 12 h light:12 h dark cycle. Medium was refreshed in all algal culture flasks every 3 to 5 d. To determine the fatty acid composition of *D. tertiolecta,* cultures that were used for all of the feeding experiments and those species cultivated for determination of EPA and DHA contents were sampled (n = 3) for later analysis of fatty acid composition.

The heterotrophic dinoflagellates *Oxyrrhis marina* Dujardin and *Gyrodinium dominans* Hulbert were obtained from the Shannon Point Marine Center and cultured in f/2 medium prepared from ASW of salinity 20 and fed solely on *Dunaliella tertiolecta* cultures.

Both *O. marina* and *G. dominans* cultures used for feeding experiments were maintained in 300 ml bottles on a rotating plankton wheel at 1.5 rpm in a walk-in environmental room at 19°C in the dark.

Expt 1. *Oxyrrhis marina* fed *Dunaliella tertiolecta*. Prior to the start of the feeding experiment, *Oxyrrhis marina* cultures were last fed *Dunaliella tertiolecta* at a prey:predator ratio of 100:1 and maintained on a plankton wheel in 300 ml glass bottles in the dark for 4 d prior to the start of all experiments to ensure that all prey had been consumed and metabolized prior to the commencement of this feeding experiment. At the start of each feeding experiment, 3.0×10^5 cells of prey-depleted *O. marina* (n = 3), 1.5×10^7 cells of *D. tertiolecta* (n = 3) and a sample containing 1.5×10^5 cells of prey-depleted *O. marina* plus 1.5×10^7 cells of *D. tertiolecta* were collected to determine the initial fatty acid composition and contents. To determine the kinetics (production per unit time) and efficiency (production per unit of carbon ingestion) of LCn-3EFA production by the heterotrophic protist *O. marina* fed LCn-3EFA-deficient algae, prey-depleted *O. marina* (1.5×10^5 cells) were inoculated with the LCn-3EFA-deficient alga *D. tertiolecta* (1.5×10^7 cells) in a 300 ml glass bottle containing fresh f/2 medium (n = 9 bottles). The 9 bottles containing the mixture of prey-depleted protists and algae were maintained on a rotation plankton wheel at 1.5 rpm at 19°C in the dark to prevent algal growth. One, 2, and 3 d after inoculation, protist-algal mixture from 3 to 4 culture bottles was harvested for fatty acid analysis. At each sampling, prior to the harvest, triplicates of 2.0 ml aliquots of the protist-algal mixture were taken from each culture bottle to measure cell densities of the prey and predator in the mixture. Cell densities of *O. marina* and *D. tertiolecta* were determined by direct cell count of fixed cells with Lugol's solution in a 1.0 ml Sedgewick Rafter chamber using an inverted microscope (Nikon, phase contrast, ELWD 0.3). Protists, algae and protist-algal mixture were collected on acetone-washed GF/F using mild water aspiration only when necessary. Collected sample filters were stored in glass screw cap test tubes at −20°C for no more than 4 wk prior to lipid extraction and analysis. This experiment was repeated once to determine the reproducibility of the results and additional samples were harvested at Day 4 after inoculation to determine if *O. marina* cultures had truly reached stationary phase by Day 3.

Expt 2. *Gyrodinium dominans* fed *Dunaliella tertiolecta*. The experimental conditions and the ratio of predator to prey were the same as those described for Expt 1. *Gyrodinium dominans* cultures were last fed *Dunaliella tertiolecta* at a prey:predator ratio of 100:1 and maintained on a plankton wheel at 1.5 rpm in 300 ml glass bottles in continuous darkness 4 d prior to the commencement of the feeding experiment, and at the start of the feeding experiment, samples of prey-depleted *G. dominans*, *D. tertiolecta* and a mixture of prey-depleted *G. dominans* and *D. tertiolecta* were collected for analysis of initial fatty acid profiles. The *G. dominans*-*D. tertiolecta* cultures were harvested (n = 3 bottles per sampling) at Days 1, 2, 3 and 4 after inoculation for cell counts and later lipid analysis. To verify the reproducibility of the results, this experiment was repeated once.

Fatty acid analysis. Lipids were extracted from the GF/F that contained harvested cells by the method of Bligh & Dyer (1959). Extracted lipid samples were dried under a stream of nitrogen and resuspended in 1.5 ml KOH-saturated methanol, 2.5 ml methanol and 0.5 ml distilled water and heated at 80°C for 1 h for saponification. After cooling, 0.5 ml distilled water was added to each sample and the sterols and other unsaponifiable neutral lipids (e.g. hydrocarbon and fatty acid alcohols) were recovered by 3 extractions with 1.5 ml of hexane:ether (9:1) and stored at −80°C for future analysis. The sample tubes containing the remaining lipids were then acidified by the drop-wise addition of concentrated HCl to a pH < 2. The free fatty acids were then recovered with 3 pooled extractions of 1.5 ml hexane:ether (9:1). The hexane:ether fractions containing the free fatty acids were each washed with 2 ml of water to remove any traces of KOH or HCl prior to derivatization of the samples.

Free fatty acid fractions were spiked with 20 µg of 23:0 internal standard and derivatized to fatty acid methyl esters (FAMEs) using methanolic BF_3 (Metcalfe & Schmitz 1961). The FAMEs were extracted with carbon disulfide (Marty et al. 1992) which was evaporated under a stream of nitrogen. Samples were then redissolved in hexane for gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis. FAMEs were analyzed on a Varian model 3800 GC-FID, using a DB-WAX capillary column (25 m × 0.32 mm; 0.2 µm film thickness; J&W Scientific). The column was temperature-programmed from 60 to 150°C at 30°C min^{-1} and 150 to 220°C at 2°C min^{-1}, with injector and detector temperatures of 230 and 250°C, respectively; the flow rates of compressed air and hydrogen were 300 and 30 ml min^{-1}. Helium was used as the carrier gas (1.5 ml min^{-1}). Identification of FAMEs was based on the comparison of their retention times with those of authentic standards and confirmed by GC-MS. The quantity of each component was calculated based on the internal standard.

Carbon content measurements of heterotrophic protists and algae. Algal and protist carbon concentrations were determined using a procedure modified from Smith et al. (2000). Briefly, algae and protists

were filtered through combusted (450°C for 2 h) Whatman GF/F filters under low vacuum. The filters were placed in a desiccator containing a Petri dish filled with concentrated HCl to remove inorganic carbonates; afterwards they were placed in combusted glass tubes, capped with combusted aluminum foil, and dried at 60°C. Samples were analyzed using a Carlo-Erba Model 252 elemental analyzer.

Statistical analyses. The significance of the changes in cell density (cells ml^{-1}), contents of individual fatty acids and total fatty acids (µg), fatty acid production per algal carbon ingestion (µg fatty acid mg algal C^{-1}) over time were analyzed by 1-way ANOVA. DHA and EPA concentrations in heterotrophic protists and algae (µg fatty acid mg C^{-1}) were also compared using 1-way ANOVA. When results were significant ($p < 0.05$), Tukey's test was employed to discriminate the means.

RESULTS

Fatty acid profiles of *Dunaliella tertiolecta*, prey-depleted *Oxyrrhis marina* and *Gyrodinium dominans*

Total saturated fatty acids accounted for 30.6 ± 4.0, 52.0 ± 11.7 and 39.0 ± 1.3% of the total fatty acids in the inocula of *Dunaliella tertiolecta*, prey-depleted *Oxyrrhis marina* and *Gyrodinium dominans*, respectively (Table 1). The major saturated fatty acids in *D. tertiolecta*, prey-depleted *O. marina* and *G. dominans* were 16:0 and 18:0. Relatively high amounts of the monounsaturated fatty acids 18:1(n-9) and 18:1(n-7) were present in both the prey *D. tertiolecta* and the prey-depleted protists. *D. tertiolecta* contained significant amounts of 16 and 18 n-3 series fatty acids, with 16:4(n-3) accounting for 10.5 ± 1.1% and 18:3(n-3) for 25.1 ± 2.3%, while the amounts of these fatty acids in prey-depleted *O. marina* and *G. dominans* were very minor or non-detectable. Both 20:5(n-3) (EPA) and 22:6(n-3) (DHA) were detected in prey-depleted protists, but not in *D. tertiolecta*. Prey-depleted *O. marina* contained much higher DHA (~13%) than prey-depleted *G. dominans* (<1%).

Kinetics of essential fatty acid production and transfer at the phytoplankton-protist interface

Oxyrrhis marina fed *Dunaliella tertiolecta*

Predator-prey dynamics. The pattern of grazing and growth of *Oxyrrhis marina* in both experiments was similar. In both feeding experiments in which prey-depleted *O. marina* cultures were fed *Dunaliella tertiolecta* for 3 to 5 d, *D. tertiolecta* was rapidly ingested and consumed by *O. marina*. By Day 1, *D. tertiolecta* concentrations dropped from the inoculation density of 50 000 cells ml^{-1} to 4171 ± 759 and 4617 ± 2313 cells ml^{-1} in Expts 1 and 2,

Table 1. Fatty acid (FA) composition and content of prey-depleted *Oxyrrhis marina* and *Gyrodinium dominans* and the algal prey, *Dunaliella tertiolecta*, used for feeding experiments. All values are mean ± SD (n = 3). Inoculum masses of fatty acids are based on using 1.5×10^7 *D. tertiolecta* cells and 1.5×10^5 cells of either *O. marina* or *G. dominans* per replicate 300 ml bottle. FAME = fatty acid methyl ester; Mono-unsat. = monounsaturates; Trace < 0.1 µg; nd = non-detectable; Total FA includes unreported FA < 0.1 µg

FAME	Prey-depleted *Oxyrrhis marina*		Prey-depleted *Gyrodimium dominans*		*Dunaliella tertiolecta*	
	µg inoculum^{-1}	wt %	µg inoculum^{-1}	wt %	µg inoculum^{-1}	wt %
14:0	0.2 ± 0.0	1.9 ± 0.6	2.0 ± 0.3	5.1 ± 0.4	1.3 ± 0.3	0.7 ± 0.1
16:0	3.6 ± 0.3	31.5 ± 6.9	9.1 ± 1.9	23.1 ± 1.1	49.6 ± 10.8	26.5 ± 3.1
16:1(n-9)	0.1 ± 0.0	1.1 ± 0.3	2.3 ± 1.5	6.2 ± 3.8	1.4 ± 0.1	0.8 ± 0.2
16:1(n-7)	0.3 ± 0.0	2.4 ± 0.5	0.7 ± 0.2	1.6 ± 0.4	nd	nd
16:3(n-3)	Trace	Trace	0.1 ± 0.1	0.3 ± 0.2	5.2 ± 2.0	2.7 ± 0.2
16:4(n-3)	0.1 ± 0.1	0.6 ± 0.5	nd	nd	20.2 ± 7.5	10.5 ± 1.1
18:0	2.1 ± 0.2	18.7 ± 4.4	3.3 ± 1.1	8.2 ± 1.3	6.3 ± 0.5	3.4 ± 0.8
18:1(n-9)	0.5 ± 0.2	4.0 ± 0.7	5.7 ± 3.2	13.8 ± 5.6	13.5 ± 2.3	7.3 ± 1.3
18:1(n-7)	0.9 ± 0.1	7.5 ± 1.4	1.8 ± 0.6	4.4 ± 0.9	4.1 ± 0.6	2.2 ± 0.4
18:2(n-6)	0.1 ± 0.1	0.6 ± 0.5	3.4 ± 4.0	7.7 ± 8.3	10.2 ± 2.4	5.4 ± 0.5
18:3(n-6)	nd	nd	Trace	Trace	5.1 ± 1.4	2.7 ± 0.3
18:3(n-3)	0.2 ± 0.2	1.9 ± 0.8	0.2 ± 0.0	0.5 ± 0.0	47.5 ± 13.2	25.1 ± 2.3
18:4(n-3)	nd	nd	Trace	Trace	2.1 ± 0.7	1.1 ± 0.0
20:1(n-9)	nd	nd	0.3 ± 0.2	0.7 ± 0.4	nd	nd
20:5(n-3)	0.1 ± 0.1	0.6 ± 0.5	Trace	Trace	nd	nd
22:6(n-3)	1.5 ± 0.2	12.9 ± 2.3	0.1 ± 0.1	0.3 ± 0.4	nd	nd
Saturates	5.9 ± 0.5	52.0 ± 11.7	15.2 ± 3.0	39.0 ± 1.3	57.2 ± 11.6	30.6 ± 4.0
Mono-unsat.	1.7 ± 0.3	15.1 ± 1.5	11.0 ± 3.8	27.5 ± 5.5	19.0 ± 2.9	10.3 ± 1.9
Total	11.8 ± 3.0		39.1 ± 8.0		192.0 ± 65.1	

respectively. These values were less than 10% of the level at inoculation. By Day 2, *D. tertiolecta* cell concentrations were below 100 cells ml^{-1} in Expt 1 and 350 ± 50 cells ml^{-1} in Expt 2. In contrast *O. marina* cells proliferated rapidly on Days 1 and 2, then entered a stationary phase by Day 3 in both feeding experiments. *O. marina* concentrations on Day 3 were 3000 ± 743 and 2823 ± 35 cells ml^{-1} in Expts 1 and 2, respectively. The cell concentrations (cells ml^{-1}) of the predator, *O. marina*, and the prey, *D. tertiolecta*, calculated from Expt 1 are shown in Fig. 1. *O. marina* growth rates (μ, d^{-1}), calculated from the linear portion of the natural log of cell counts regressed against time, were 0.77 d^{-1} and 0.82 d^{-1} in Expts 1 and 2, respectively.

These results suggest that when *Oxyrrhis marina* at a concentration of 500 cells ml^{-1} are fed *Dunaliella tertiolecta* at an initial predator:prey ratio of 1:100 that *O. marina* reaches a maximum cell density of 3000 ± 743 cells ml^{-1} and 2823 ± 35 cells ml^{-1} by Day 3. After accounting for *O. marina* in the inoculum, this corresponds to a prey:predator conversion ratio of 20 to 28 *D. tertiolecta* consumed per *O. marina* produced.

Changes in total fatty acid content and composition. Since the trend of changes in total fatty acid contents and fatty acid composition over time at the *Dunaliella tertiolecta–Oxyrrhis marina* interface were similar in the 2 feeding experiments, only results from Expt 1 are reported below. Total fatty acid content decreased over the course of the feeding experiment, from 140.1 ± 20.9 μg in the initial mixture of *O. marina* cultures fed *D. tertiolecta* to 92.4 ± 20.7 μg by Day 3, a 35% reduction (Table 2). Similarly, total saturated fatty acids decreased by 35% (40.6 ± 3.7 to 26.5 ± 1.4 μg). The 16 and 18 n-3 series fatty acids originating in the *D. tertiolecta* prey decreased by >88% over the 3 d feeding. While the decrease in 18:1(n-9) accounted for the decrease in the total monounsaturated fatty acids, the decrease in 18:3(n-3) accounted for the major decrease in 16 and 18 n-3 series fatty acids over time. The essential fatty acids 20:5(n-3) and 22:6(n-3) detected in the initial *O. marina-D. tertiolecta* mixed culture originated from the prey-depleted *O. marina* (Table 1). Consistent with the increased production of *O. marina* over the 3 d feeding experiment (Fig. 1), the 2 essential fatty acids 20:5(n-3) and 22:6(n-3) increased 3-fold and almost 10-fold, respectively (Table 2).

Table 2. Micrograms of fatty acids in *Oxyrrhis marina* cultures fed *Dunaliella tertiolecta*. All values are mean ± SD (n = 3). Different letters denote significant differences across sampling dates at the $p < 0.05$ level. O.m. = *Oxhyrris marina*; D.t = *Dunaliella tertiolecta*. FAME = fatty acid methyl ester; Mono-unsat. = monounsaturates; Total FA includes unreported FA < 0.1 μg

FAME	O. m. + D.t. initial	Day 1	Day 2	Day 3
14:0	1.1 ± 0.1^{c}	3.0 ± 0.3^{a}	1.6 ± 0.4^{b,c}	1.9 ± 0.1^{b}
16:0	34.6 ± 1.5^{a}	30.4 ± 1.0^{a}	20.9 ± 4.9^{b}	21.4 ± 1.6^{b}
16:1(n-9)	0.9 ± 0.0^{b}	0.9 ± 0.1^{b}	1.3 ± 1.5^{a,b}	3.4 ± 0.8^{a}
16:1(n-7)	0.7 ± 0.1^{a,b}	1.0 ± 0.1^{a}	0.6 ± 0.1^{b}	0.6 ± 0.1^{b}
16:3(n-3)	3.2 ± 0.2^{a}	2.4 ± 0.2^{b}	0.4 ± 0.1^{c}	0.6 ± 0.1^{c}
16:4(n-3)	12.1 ± 0.9^{a}	9.7 ± 1.2^{b}	1.8 ± 0.7^{c}	0.4 ± 0.1^{c}
18:0	4.9 ± 2.1	5.8 ± 3.2	3.1 ± 0.3	3.2 ± 0.6
18:1(n-9)	9.2 ± 0.3^{a}	6.7 ± 0.4^{b}	4.3 ± 0.6^{c}	5.8 ± 0.7^{b}
18:1(n-7)	3.8 ± 0.2^{a,b}	4.4 ± 0.6^{a}	3.3 ± 0.6^{a,b}	2.9 ± 0.2^{b}
18:2(n-6)	7.0 ± 0.3^{a}	5.2 ± 0.2^{b}	2.4 ± 1.4^{c}	1.3 ± 0.1^{c}
18:3(n-6)	3.4 ± 0.1^{a}	3.1 ± 0.2^{a}	1.2 ± 0.6^{b}	0.4 ± 0.1^{b}
18:3(n-3)	33.3 ± 1.8^{a}	27.6 ± 0.9^{b}	10.3 ± 3.6^{c}	3.6 ± 1.0^{d}
18:4(n-3)	1.3 ± 0.0^{a}	1.5 ± 0.3^{a}	0.5 ± 0.2^{b}	0.2 ± 0.2^{b}
20:1(n-9)	0.1 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.4 ± 0.0
20:5(n-3)	0.3 ± 0.1^{b}	0.4 ± 0.1^{b}	0.7 ± 0.3^{b}	1.2 ± 0.2^{a}
22:6(n-3)	1.6 ± 0.2^{c}	6.4 ± 0.4^{b,c}	10.7 ± 2.9^{b}	14.4 ± 3.7^{a}
Saturate	40.6 ± 3.7^{a}	39.1 ± 3.9^{a}	25.6 ± 4.6^{b}	26.5 ± 1.4^{b}
Mono-unsat.	14.5 ± 0.6^{a}	13.1 ± 1.0^{a}	9.6 ± 1.3^{b}	13.0 ± 1.6^{a}
Total	140.1 ± 20.9^{a}	132.9 ± 15.2^{a,b}	76.9 ± 8.0^{b}	92.4 ± 20.7^{b}
D.t. × 10^4	1500	125.1 ± 22.8	nd	nd
O.m. × 10^4	15	30.6 ± 2.0	70.7 ± 4.6	90 ± 22.3

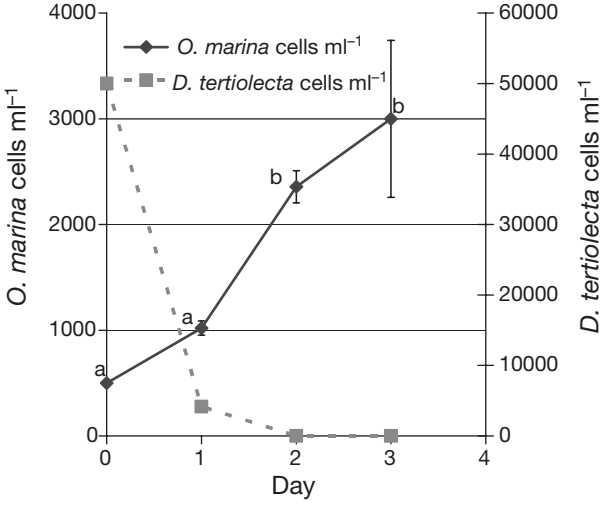

Fig. 1. *Oxyrrhis marina* fed *Dunaliella tertiolecta*. Growth (cells ml^{-1}) and predation dynamics (cells ml^{-1}). Values are mean ± SD (n = 3). Different letters denote significant differences across sampling dates at the $p < 0.05$ level

Gyrodinium dominans fed *Dunaliella tertiolecta*

Predator–prey dynamics. The grazing and growth responses of *Gyrodinium dominans* fed *Dunaliella tertiolecta* were similar in both experiments. In both feed-

Table 3. Content of fatty acids (µg per culture) in *Gyrodinium dominans* cultures fed *Dunaliella tertiolecta* across sampling dates. All values are mean ± SD (n = 3). Different letters denote significant differences at the $p < 0.05$ level. G.d. = *Gyrodinium dominans*; D.t. = *Dunaliella tertiolecta.* FAME = fatty acid methyl ester; nd = non-detectable; Total FA includes unreported FA < 0.1 µg

FAME	G.d. + D.t. initial	Day 1	Day 2	Day 3	Day 4
14:0	2.3 ± 0.4^{c}	2.7 ± 0.3^{c}	5.2 ± 1.6^{a,b}	6.5 ± 1.1^{a}	3.4 ± 0.4^{b,c}
16:0	54.4 ± 3.3^{a}	45.0 ± 2.7^{a,b}	41.0 ± 7.0^{b}	21.2 ± 2.5^{c}	12.3 ± 1.4^{c}
16:1(n-9)	3.7 ± 1.0	2.3 ± 0.6	4.0 ± 3.1	4.8 ± 0.7	1.5 ± 0.1
16:1(n-7)	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.2^{a}	0.7 ± 0.1^{a,b}	0.4 ± 0.04^{b}
16:3(n-3)	5.3 ± 0.6^{a}	3.9 ± 0.2^{b}	2.3 ± 0.4^{c}	nd	0.1 ± 0.1^{d}
16:4(n-3)	18.4 ± 2.1^{a}	14.8 ± 1.2^{b}	7.0 ± 0.4^{c}	nd	nd
18:0	4.0 ± 0.3^{a}	3.2 ± 0.5^{ab}	3.5 ± 0.5^{a}	3.1 ± 0.7^{ab}	2.0 ± 0.2^{b}
18:1(n-9)	23.7 ± 1.1^{a}	15.2 ± 1.1^{b}	13.0 ± 3.1^{b}	6.4 ± 0.9^{c}	3.0 ± 0.1^{c}
18:1(n-7)	3.7 ± 0.2^{b}	5.0 ± 0.2^{a}	4.8 ± 0.5^{a}	3.4 ± 0.4^{b}	2.7 ± 0.2^{b}
18:2(n-6)	15.1 ± 1.2^{a}	10.5 ± 0.6^{b}	8.1 ± 1.0^{c}	1.0 ± 0.3^{d}	0.4 ± 0.1^{d}
18:3(n-6)	5.3 ± 0.6^{a}	4.6 ± 0.2^{a}	2.8 ± 0.3^{b}	0.1 ± 0.2^{c}	nd
18:3(n-3)	55.2 ± 6.3^{a}	34.0 ± 1.6^{b}	19.8 ± 1.8^{c}	1.3 ± 0.5^{d}	0.5 ± 0.2^{d}
18:4(n-3)	1.7 ± 0.2^{b}	3.9 ± 0.2^{a}	1.9 ± 0.3^{b}	nd	nd
20:1(n-9)	0.3 ± 0.0	0.2 ± 0.1	0.3 ± 0.3	0.4 ± 0.1	0.2 ± 0.1
20:5(n-3)	0.4 ± 0.1^{c}	1.3 ± 0.1^{b}	2.4 ± 0.3^{a}	1.5 ± 0.5^{b}	0.7 ± 0.1^{c}
22:6(n-3)	0.9 ± 0.1^{d}	4.8 ± 0.4^{b}	8.2 ± 0.6^{a}	4.5 ± 0.6^{b}	2.9 ± 0.2^{c}
Saturates	63.4 ± 3.1^{a}	53.0 ± 3.7^{a}	52.3 ± 10.2^{a}	33.4 ± 4.6^{b}	18.5 ± 2.0^{c}
Mono-unsat.	32.8 ± 1.2^{a}	24.1 ± 2.3^{a}	23.6 ± 7.5^{a}	16.9 ± 2.1^{b}	8.0 ± 0.1^{c}
Total	217.7 ± 11.8^{a}	176. 7± 13.8^{ab}	149.2 ± 32.2^{b}	75.6 ± 8.5^{c}	39.9 ± 9.0^{c}
# D.t. (× 10^4)	1500	854.7 ± 122.9	225.3 ± 56.1	0.5 ± 1.0	nd
# G.d. (× 10^4)	15	20.8 ± 1.7	75.8 ± 5.5	141.3 ± 12.6	149.3 ± 21.0

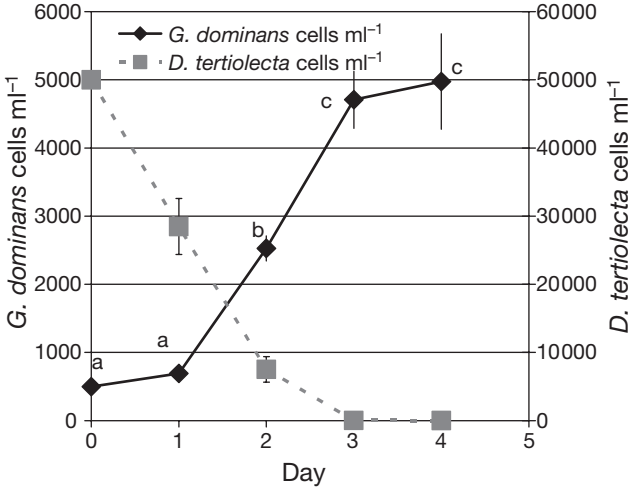

Fig. 2. *Gyrodinium dominans* fed *Dunaliella tertiolecta*. Growth (cells ml^{-1}) and predation dynamics (cells ml^{-1}). Values are mean ± SD (n = 3). Different letters denote significant differences across sampling dates at the $p < 0.05$ level

ing experiments, concomitant with rapid proliferation of *G. dominans*, the algal prey was grazed and consumed rapidly by the protist. The predation and growth dynamic of Expt 1 is illustrated in Fig. 2. Maximum prey consumption was concomitant with maximum predator growth on Days 3 and 4 (Fig. 2). The prey concentration dropped from an initial 50 000 cells ml^{-1} to 17 ± 29 cells ml^{-1} in Expt 1 and in Expt 2 to almost 0 cells ml^{-1} 3 d after the start of the feeding experiments. The protist growth reached a plateau, the stationary phase, in both experiments on Day 3 at the time of food exhaustion. At that time, the protist concentration was 4975 ± 701 cells ml^{-1} in Expt 1 on Day 4. The highest protist concentration in Expt 2 was on Day 3, 2552 ± 574 cells ml^{-1}, but it declined to 2316 ± 664 cells ml^{-1} on Day 4. The protist growth rates were 0.92 d^{-1} and 0.78 d^{-1} in Expts 1 and 2, respectively. Cultures started to crash on Day 5 due to the continuous depletion of prey. After subtracting the initial number of *G. dominans* in the inoculum, this corresponds to a prey:predator conversion ratio of 11 to 25 *D. tertiolecta* consumed per *G. dominans* produced.

Changes in total fatty acid content and composition. The data obtained from Expt 1 are reported here. Total, saturated and monounsaturated fatty acids decreased significantly over time (Table 3). The decrease in total fatty acids was more than 5-fold (82%, from 217.7 ± 11.8 µg in the initial mixed culture of *Gyrodinium dominans* and *Dunaliella tertiolecta* to 39.9 ± 9.0 µg) by Day 4, and in total saturated and monounsaturated fatty acids was approximately 3.4-fold (71%, from 63.4 ± 3.1 to 18.5 ± 2.0 µg) and 4-fold (76%, from 32.8 ± 1.2 to 8.0 ± 0.1 µg), respectively, by Day 4. The decrease in *D. tertiolecta*-derived 16 and 18 n-3 series fatty acids was dramatic. By Day 4 these fatty acids were almost completely diminished (a loss of 94%). The decreases in 16:0 and 18:1(n-9) accounted for the decreases in total saturated and total monounsaturated fatty acids, respectively, whereas, the decreases in 16:4(n-3) and 18:3(n-3) contributed to the major decreases in 16 and 18 n-3 series fatty acids over time. The 2 essential fatty acids 20:5(n-3) and 22:6(n-3) increased significantly over time, but unlike in the *O. marina* fed *D. tertiolecta* experiment, the increases in 20:5(n-3) and 22:6(n-3) were not parallel to the increase in the predator's cell density. These 2 essential fatty acids increased up to 6.7- and 8.7-fold, respectively, at Day 2, then their quantity gradually decreased afterward, although the protist cell density continued to rise on Days 3 and 4. The highest predator density was at Day 4, but the

amounts of 20:5(n-3) and 22:6(n-3) at that day were 70 and 65% less, respectively, than the quantities recorded on Day 2. The essential fatty acids 20:5(n-3) and 22:6(n-3) detected in the initial *G. dominans*-*D. tertiolecta* mixed culture were from the prey-depleted *G. dominans* (Table 1).

Carbon content of heterotrophic protists and algal species

The carbon content of *Oxyrrhis marina*, *Gyrodinium dominans*, *Dunaliella tertiolecta*, *Rhodomonas salina*, *Chaetoceros calcitrans* and *Tetraselmis suecica* measured using a Carlo-Erba Model 252 elemental analyzer were 336.1 ± 45.1 (n = 10), 282.7 ± 47.9 (n = 14), 38.6 ± 0.1 (n = 4), 65.0 ± 2.2 (n = 4), 8.3 ± 0.1 (n = 4) and 50.0 ± 4.9 pg C cell^{-1} (n = 4), respectively (Tables 4 & 5).

Essential fatty acid production by heterotrophic protists and trophic transfer efficiencies

Lipid production and trophic transfer efficiencies were calculated using carbon content values derived from direct measurement (Table 4). The essential fatty acids EPA 20:5(n-3) and DHA 22:6(n-3), which are not found in the algal diet, reached highest levels at Day 3 in *Oxyrrhis marina* (Fig. 1); and at Day 2 in *Gyrodinium dominans* (Fig. 2). Production calculated according to carbon content derived from direct algal measurement was equivalent to 1.9 ± 0.3 µg EPA and 22.3 ± 6.4 µg DHA by *O. marina* and 4.7 ± 0.5 µg EPA and 16.5 ± 1.1 µg DHA by *G. dominans* per mg algal carbon consumed (Table 4). The carbon transfer efficiencies calculated from carbon content values derived from direct measurements were 43.5 ± 12.9 and 34.9 ± 1.8%, respectively, at the *Dunaliella tertiolecta*–*O. marina* interface and the *D. tertiolecta*–*G. dominans* interface (Table 4).

EPA and DHA concentrations in heterotrophic protists and algae

No DHA or EPA was detected in *Dunaliella tertiolecta* (Table 1), while the other 3 algal species contained either EPA and DHA (*Chaetoceros calcitrans* and *Rhodomonas salina*) or only EPA (*Tetraselmis suecica*) (Table 5). *R. salina* had higher levels of both DHA and EPA than *C. calcitrans*. *Oxyrrhis marina* and *Gyrodinium dominans* had concentrations of 47.6 ± 3.7 and 38.4 ± 3.6 µg DHA mg C^{-1}, respectively, and 4.0 ± 0.5 and 11.1 ± 1.4 µg EPA mg C^{-1}, respectively (Table 5). Both heterotrophic protists had significantly higher DHA levels and DHA:EPA ratios than the 2 algal species containing both EPA and DHA. The DHA:EPA ratios in *O. marina* and *G. dominans* were 11.9 and 3.5, respectively, and the values for *R. salina* and *C. calcitrans* were much lower at 0.8 and 0.1, respectively.

Table 4. Essential fatty acid production per unit algal carbon ingestion (µg fatty acid (mg algal C)$^{-1}$) and trophic transfer efficiencies. Carbon content values were derived from direct measurement of cell culture carbon content of *Dunaliella tertiolecta*, *Oxyrrhis marina* and *Gyrodimium dominans*. Values are mean ± SD (n = 3, except for cell carbon content)

	Dunaliella tertiolecta	*Oxyrrhis marina* (Day 3)	*Gyrodinium dominans* (Day 2)
EPA	–	1.9 ± 0.3	4.7 ± 0.5
DHA	–	22.3 ± 6.4	16.5 ± 1.1
Total fatty acids	–	139.3 ± 35.7	225.1 ± 72.7
Carbon content (pg C cell^{-1})	38.6 ± 0.1 (n = 4)	336.1 ± 45.1 (n = 10)	282 ± 47.9 (n = 14)
% Trophic transfer of algal C	–	43.5 ± 12.9	34.9 ± 1.8

Table 5. Essential fatty acids per unit carbon of *Oxyrrhis marina*, *Gyrodinium dominans* and 4 species of marine algae (µg fatty acid mg^{-1} C). Essential fatty acid content of *O. marina* and *G. dominans* are from day of maximal EFA content in kinetics experiments (Days 3 and 2 for *O. marina* and *G. dominans*, respectively, n = 3). Essential fatty acid contents of algal species are from the same samples used for carbon determination (n = 4 for carbon and fatty acid analyses). Carbon determinations for *O. marina* and *G. dominans* (n = 10 and n = 14, respectively) were conducted on prey-depleted protists. All values are mean ± SD. Different letters denote significant differences in EPA and DHA among the algal species at the $p < 0.05$ level

	Oxyrrhis marina	*Gyrodinium dominans*	*Dunaliella tertiolecta*	*Chaetoceros calcitrans*	*Tetraselmis suecica*	*Rhodomonas salina*
EPA	4.0 ± 0.5^{c}	11.1 ± 1.4^{b}	nd	20.6 ± 1.1^{a}	22.0 ± 1.7^{a}	22.6 ± 2.3^{a}
DHA	47.6 ± 3.7^{a}	38.4 ± 3.6^{b}	nd	2.1 ± 0.5^{d}	nd	17.9 ± 1.9^{c}
pg C cell^{-1}	336.1 ± 45.1	282 ± 47.9	38.6 ± 0.1	8.3 ± 0.1	50.0 ± 4.9	65.0 ± 2.2

DISCUSSION

Heterotrophic protists occupy an important trophic position in marine food webs because of their nutritional and energetic contributions to higher consumers. While phytoplankton is the primary carbon source, the contribution of heterotrophic protists (i.e. ciliates and heterotrophic dinoflagellates) to the diet of calanoid copepods, the dominant zooplankton in the marine environment, is considered to be equal to algae, particularly in non-bloom periods (Tang & Taal 2005).

The abundance and succession in the field, growth and grazing, predator-prey relationship, efficiency of carbon transfer and nutrient regeneration in terms of nitrogen and phosphorus on several representative heterotrophic protist species, including *Gyrodinium dominans* and *Oxyrrhis marina*, the 2 common estuarine species, have previously been investigated (Goldman et al. 1989, Nakamura et al. 1992, 1995, Davidson et al. 1995, Tang et al. 2001, Kim & Jeong 2004).

The grazing and growth responses of the 2 tested protists in the present study corroborated with those observed in previous studies. Although there were some variations between replicates and among feeding experiments, the grazing and growth patterns were similar between the feeding experiments in which the protists *Gyrodinium dominans* or *Oxyrrhis marina* were fed the LCn-3EFA-deficient algal prey *Dunaliella tertiolecta*. The calculated growth rates of 0.77 and 0.82 d^{-1} in *O. marina* and 0.77 and 0.92 d^{-1} in *G. dominans* in the feeding experiments fed with the prey *D. tertiolecta* were similar to the reported growth rates of *G. dominans* (0.88 d^{-1}) fed the red tide flagellate *Chattonella antiqua* (Nakamura et al. 1992) and *O. marina* (0.80 d^{-1}) fed *D. tertiolecta* (Goldman et al. 1989). The predator's grazing/feeding rates were not measured in the present study; nevertheless, as described in previous studies (Goldman et al. 1989, Nakamura et al. 1992), both protists rapidly ingested and consumed the algal prey for growth (Figs. 1 & 2). The carbon transfer efficiency at the algal-protist interface appears quite high: 44% from *D. tertiolecta* to *O. marina* and 35% from *D. tertiolecta* to *G. dominans*.

Calanoid copepods, the primary predators of auto- and hetero-microprotists, are the dominant zooplankton on which the production of animals at the upper end of the food chain depends. The importance of copepods to fisheries is well known (Cushing 1995, Xie & Yang 2000, Thiel 2001) and their nutritional quality (i.e. LCn-3EFA and sterol content) is one of the important factors controlling the development and growth of fish and therefore their recruitments. Larvae of many fish species are dependent on local zooplankton production for growth and subsequent recruitment into the fishery. For example, the Chesapeake Bay striped bass *Morone saxatilis* recruitment is characterized by high annual variability and is strongly related to zooplankton abundance (Rutherford et al. 1997). However, in dynamic environments such as estuaries and coastal zones, the nutritional characteristics of the food environment are highly variable. The abundance and composition of the plankton community can vary significantly in space and in time (Jónasdóttir 1994, Galois et al. 1996, Lavaniegos & Lopez-Cortes 1997, Napolitano et al. 1997). As a consequence, the nutritional differences may create significant variability in zooplankters' abundance and their biochemical composition. This may lead to spatial and temporal fluctuations in food quality and availability for larval and juvenile fish.

While the LCn-3EFA contents of copepods are critical for the growth and wellbeing of larval and juvenile fish, the physiological fitness and nutritional value of calanoid copepods are in turn dependent on the chemical compositions of the microplankton they consume (St. John & Lund 1996, St. John et al. 2001). The effects of food quality on the growth, reproduction and neonatal development of copepods have been studied extensively (e.g. Jónasdóttir et al. 1995, Jónasdóttir & Kiørboe 1996, Koski et al. 1998, Tang et al. 2001, Turner et al. 2002, Tang & Taal 2005, Ju et al. 2006). It has become apparent that both the quality and quantity of food in the diet affect copepod production, and ultimately, production at higher trophic levels. Essential fatty acids are among the critical nutrients that determine copepod production and nutritional quality. Because of the inability of calanoid copepods and their predators to synthesize EPA and DHA de novo, the trophic significance of nutrient upgrading by herbivorous protists is highly regarded, particularly at times when the phytoplankton bloom is dominated by poor food quality species. The quantitative significance of nutrient upgrading and transfer to higher trophic levels via protists is unclear and has not been examined, although knowing to what extent nutritionally critical essential lipids are transferred from the phytoplankton-heterotrophic protist interface to zooplankton has obvious implication for the nutritional ecology of the pelagic food webs. The present study is the first examination of the kinetics and efficiency of essential fatty acid assimilation and production at the phytoplankton-heterotrophic protist interface.

As previously reported (Klein Breteler et al. 1999, Veloza et al. 2006), no EPA or DHA, only abundant linolenic acid, 18:3(n-3), was detected in *Dunaliella tertiolecta.* The present study demonstrated that *Oxyrrhis marina* and *Gyrodinium dominans* effectively produce the essential fatty acids EPA and DHA, and much of the prey item fatty acids were catabolized for either energy or as a source for making essential fatty acids

and/or other cellular components. The amounts of DHA and EPA produced by the 2 protist species were significant compared to the amounts in the initial algal-protist mixtures. The smaller increase of both EPA and DHA over time in *G. dominans* might suggest that this protist was not as effective as *O. marina* in making these 2 essential fatty acids. However, when looking at the production of EPA and DHA per unit of algal carbon ingestion (µg lipid mg algal C^{-1}) and lipid concentrations (µg lipid mg protist C^{-1}) calculated based on carbon content derived from direct measurements (Tables 4 & 5), these 2 protists were equally effective in making these 2 fatty acids. The dramatic drop of DHA, 55% of the Day 2 level on Day 3 and continuing on Day 4 in *G. dominans*, while cell density continued to increase (~46%), is surprising. However, this observed phenomenon was consistent across the 2 feeding experiments conducted on *G. dominans*. The decline of these 2 essential fatty acids is possibly an indication of the consumption of lipids for energy due to food limitation since the algal prey cell number plummeted to near 0 on Days 3 and 4, probably reaching the point of no return, though all the protists appeared to be viable when cell counts were conducted.

While both EPA and DHA are essential membrane components, their physiological roles differ somewhat. EPA is the precursor of several eicosanoids, which are signal molecules playing a role in modulating many biological and biochemical processes (Tocher et al. 1996, Sargent et al. 2002). DHA is required for the proper function of rhodopsin in retinas and for neural development in many organisms, including humans (M. V. Bell et al. 1995, Navarro et al. 1997, J. G. Bell et al. 1999, Gurr et al. 2002, Sargent et al. 2002). However, the physiological functions of EPA and DHA in heterotrophic protists are presently not known. The finding of much lower levels of EPA compared to DHA in the 2 protists after feeding on the algal prey *D. tertiolecta* is consistent with results reported previously. Our previous study noted that when fed on phytoplankton rich in EPA and DHA, both *O. marina* and *G. dominans* preferentially accumulated DHA over EPA, and regardless of the diets, both protists had less EPA than DHA (Veloza et al. 2006). Klein Breteler et al. (1999) and Broglio et al. (2003) also reported that heterotrophic dinoflagellates tended to be richer in DHA than in EPA.

The mechanisms and biochemical processes involved in trophic upgrading in protists are presently unclear. Several mechanisms have been hypothesized: (1) assimilation/incorporation from dietary sources, (2) modification of dietary precursors and (3) de novo synthesis. Thus far, only the freshwater nanoflagellate *Paraphysomonas* sp. (Bec et al. 2006) and the marine zooflagellate *Bodo* sp. (Zhukova & Kharlamenko 1999) appear to be capable of synthesizing both EPA and DHA, and the heterotrophic dinoflagellate *Crypthecodinium cohnii* has the ability to synthesize DHA (Barclay et al. 1994, De Swaaf et al. 2003a, 2003b). A significant drop in 18:3(n-3) and 18:4(n-3), the likely precursors for EPA and DHA, over time, parallel to the substantial increases of EPA and DHA, suggests the conversion of these dietary fatty acids to EPA and DHA via elongation and desaturation. However, we would not preclude the possibility of de novo synthesis of EPA and DHA and the utilization of 18:3(n-3) as an energy source during proliferation. A recent report indicated that a nanoflagellate, *Paraphysomonas* sp., grown on sterol and essential fatty acid-deficient cyanobacteria is capable of producing EPA, DHA and various sterols (Bec et al. 2006). Along the same line, in a separate study, we found that when fed a parasitic protozoan, *Perkinsus marinus*, which lacks EPA, DHA and n-3 fatty acids (Soudant & Chu 2001, E. D. Lund & F.-L. E. Chu unpubl. data), to the 2 protists, both *G. dominans* and *O. marina* contained fair amounts of EPA and DHA. The EPA and DHA concentrations (µg fatty acid per mg of *P. marinus* carbon consumed) in these 2 protists after feeding on the parasitic protozoan for 2 d were 4.1 ± 0.8 µg EPA mg C^{-1} for *G. dominans* and 4.0 ± 0.3 EPA mg C^{-1} for *O. marina* and 13.2 ± 2.7 µg DHA mg C^{-1} for *G. dominans* and 36.7 ± 1.7 µg DHA mg C^{-1} for *O. marina* (E. D. Lund & F.-L. E. Chu unpubl. data). However, no LCn-3EFAs were detected in previous studies in *O. marina* grown on yeast, which lacks EPA, DHA and other PUFAs (Kleppel & Burkart 1995, Kleppel et al. 1998). *O. marina* and *G. dominans* may employ the polyketide synthases (PKS) rather than the fatty acid synthases (FAS) for PUFA synthesis. If this is the case, it is unlikely that these 2 protists synthesize EPA and DHA via elongation and desaturation of their precursors, 18:3(n-3) and 18:4(n-3). Thus far, however, only *Crypthecodinium cohnii* has been shown to use polyketide pathways to synthesize DHA using a 2-C substrate such as acetate as building blocks (De Swaaf et al. 2003b) and the desaturation process in the biosynthesis of DHA is still not completely clear (De Swaaf et al. 2003b). Presently, how *cis* double bonds are generated in the PKS pathway for PUFA synthesis and whether desaturases are active in synthesis of DHA are not known (Metz et al. 2001, De Swaaf et al. 2003b). Therefore, whether *O. marina* and *G. dominans* elongate and desaturate 18:3(n-3) for EPA and DHA or produce EPA and DHA employing PKS pathways remains to be confirmed with adequate tracer studies.

Although the benefits of trophic upgrading and its potential impact on the pelagic ecosystem have long been noted, their actual contribution has not been quantified. Our results provide direct evidence to sup-

port the notion that their contribution of LCn-3EFA to the pelagic ecosystem is important. Considering the amount of LCn-3EFA production per mg algal carbon consumed (Table 4) and the LCn-3EFA content in the protists (Table 5) compared to those contained in the algal species *Chaetoceros calcitrans*, *Tetraselmis suecica* and particularly *Rhodomonas salina,* which is considered to be good food for copepods, certain heterotrophic protists contribute significantly to the essential fatty acid pool in pelagic food webs. Protists were fed with limited algal rations for a short period of time (up to 4 d) in the present study. We believe that the production of essential lipids observed in our study would be continued in an environment with a continuous food supply. High ratios of dietary DHA:EPA are considered beneficial for the development and growth of marine fish, especially in larval and juvenile stages (Watanabe 1993, Sargent 1995, Rainuzzo et al. 1997). Our recent study showed that copepods (*Acartia tonsa*) fed *Oxyrrhis marina* previously grown on *Dunaliella tertiolecta* had a much higher DHA:EPA ratio (4.8) than those fed *R. salina* (1.3) (Veloza et al. 2006). High predation/consumption of heterotrophic protists may result in high ratios of DHA:EPA in consumers such as copepods, which are an important food item for larval and juvenile fish, and in nature, as indicated earlier, their abundance is strongly correlated with fish recruitment (Rutherford et al. 1997). Thus, heterotrophic protists are not only capable of trophically upgrading poor quality food including low food quality algae, bacteria and parasitic protists, but also enhancing the DHA level and DHA:EPA ratio of their consumers.

In summary, our results unveil that heterotrophic protists contribute significantly to the pelagic food webs, not only a carbon source, but significant amounts of n-3 essential fatty acids, DHA in particular. This underscores the nutritional importance of trophic upgrading by heterotrophic protists on the marine pelagic ecosystem.

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