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Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching

Kam W. Tang^{a,*}, Maiyai Taal^b

^aVirginia Institute of Marine Science, Gloucester Point, VA 23062, USA

^bDepartment of Marine and Environmental Science, Hampton University, Hampton, VA 23668, USA

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Abstract

Recent literature suggests that heterotrophic protists could improve the biochemical constituents of poor quality algae for subsequent use by higher trophic levels, a phenomenon dubbed “trophic upgrading”. We conducted experiments to test trophic upgrading effects on the omnivorous calanoid copepod *Acartia tonsa*. Two heterotrophic protists, *Oxyrrhis marina* and *Gymnodinium dominans*, were grown on three algal species of different nutritional qualities: *Dunaliella tertiolecta*, *Isochrysis galbana* and *Rhodomonas salina*. In parallel incubations, the copepods were fed the heterotrophic protists or the algae that the protists grew on. We measured the ingestion rate, egg production rate and egg hatching success of the copepods in each treatment. Comparison of the egg production efficiency (EPE; egg carbon production/carbon ingestion \times 100%) between parallel diet treatments allowed us to calculate the trophic upgrading index (TUI; ratio of EPE-heterotrophic protist to EPE-alga). The copepods ingested similar amount, or 20–30% more algae than heterotrophic protists. Daily rations ranged 50–122% of body C per day. Copepods feeding on *O. marina* that grew on *D. tertiolecta* had higher egg production rate and egg hatching success than copepods feeding on *D. tertiolecta* directly, and the resultant TUI between the two treatments was 19.7. Similarly, the copepod egg production was improved by *G. dominans* that grew on *D. tertiolecta* and the resultant TUI was 23.3. TUI was near 1 between *I. galbana* treatment and the parallel heterotrophic protist treatments, indicating no or little trophic upgrading effects on the copepod’s egg production. However, egg hatching success was significantly lower with *G. dominans* growing on *I. galbana*. *O. marina* that grew on *R. salina* induced a lower EPE relative to the alga itself, yielding a TUI of less than 1. Biochemical data showed that the heterotrophic protists contained eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) even when they fed on alga that lacked these essential fatty acids, which may explain some of the observed trophic upgrading effects and species-specific differences in our experiments. However, our data also suggested that dietary EPA and DHA could not be the sole factors regulating the

* Corresponding author. Tel.: +1 804 684 7602; fax: +1 804 684 7293.
E-mail address: kamtang@vims.edu (K.W. Tang).

copepod's reproductive output, and that egg production and egg hatching appeared to have different nutritional requirements and respond differently to the copepod's diets.

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1. Introduction

In aquatic ecosystems, the phytoplankton–zooplankton interface is a crucial and yet highly variable link that determines energy and material transfer within the food webs (McQueen et al., 1989; Brett and Goldman, 1996, 1997). The strength of trophic interactions between phytoplankton and zooplankton are moderated by many factors, such as toxicity (Turner and Tester, 1997), defense mechanisms (Agrawal, 1998) and nutritional quality of the phytoplankton (Gulati and Demott, 1997). While the common currencies in trophodynamic studies are carbon and nitrogen, many studies showed that dietary biochemical compounds, particularly essential fatty acids (EFA), have strong influence on zooplankton growth (Müller-Navarra, 1995a,b; DeMott and Müller-Navarra, 1997). Among the EFA, essential long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), such as docosahexanoic acid (DHA) and eicosapentanoic acid (EPA), play important roles in growth, reproduction and other physiological functions (e.g., Watanabe, 1993; Chu, 1996; Soudant et al., 2000). In the pelagic system, planktonic algae are the primary producers of DHA and EPA (Ackman et al., 1980; Sargent et al., 1987, 2002), and higher consumers such as zooplankton, shellfish and finfish must acquire them via trophic accumulation up the food chain (Sargent et al., 2002). Some harpacticoid copepods may modify n-3 precursors to EFA (Nanton and Castell, 1998), but such capability has not been shown in calanoid copepods, the dominant zooplankton in the marine pelagic environment. Planktonic algae can vary widely in their nutritional quality, with significant ramifications for growth and reproduction of the copepods that consume them. For example, algae such as *Dunaliella* and *Phaeocystis* spp. are poor in long-chain n-3 PUFAs, and adult calanoid copepods feeding on these algae have poor growth and fecundity (Koski et al., 1998; Tang et al., 2001; Turner et al., 2002). Prolonged feeding on *Dunaliella*

can cause female sterility and oocyte deterioration in the copepod *Calanus helgolandicus* (Lacoste et al., 2001). Copepod nauplii feeding on *Phaeocystis* also suffered from halted development and high mortality (Tang et al., 2001). In contrast, algae rich in long-chain n-3 PUFAs, such as the cryptophyte *Rhodomonas*, supports high egg production efficiency and naupliar growth rate (Tang et al., 2001). Copepods feeding on nutritionally rich vs. poor algal species exhibit different metabolic rate and body biochemistry (Roman, 1984, Thor et al., 2002). In situ egg production rate of copepods is also positively related to specific long chain (n-3) PUFAs in seston (Jónasdóttir et al., 1995).

Despite the fact that planktonic algae are major producers of EFA, the reproductive output of copepods may not depend directly on the nutritional quality of algae. Heterotrophic protists such as ciliates and dinoflagellates are ubiquitous members of the planktonic food web. Several studies showed that copepods may selectively ingest heterotrophic protists over algae (Dolan, 1991; Fessenden and Cowles, 1994; Zeldis et al., 2002). Many field studies also showed that heterotrophic protists contribute as much as 100% to a copepod's diet (Table 1). Thus, the nutritional content of heterotrophic protists is equally, if not more, important as that of algae in regulating zooplankton growth and production. In addition to being an important food source, the biochemical makeup of heterotrophic protists can differ from algae, making them an essential nutritional ingredient in a copepod's diet. For example, a recent study showed that omnivorous calanoid copepods failed to grow on *Dunaliella* sp. alone (Klein Breteler et al., 1999). However, when the copepods were fed *Oxyrrhis marina* that were raised on *Dunaliella* sp., they grew well and developed normally. Other studies also noted that heterotrophic protists as intermediate prey improve the nutritional composition from low quality algae, leading to higher egg production rate (Klein Breteler et al., 1999; Tang et al., 2001) and egg

Table 1
Contribution of heterotrophic protists to copepod diet as % carbon ration

% ration	Hetero. protist	Zooplankton	Region	Period	Reference
7.3–74	Dino., ciliates	Mixed copepods	South Georgia (Antarctic)	Austral summer	Atkinson (1994)
16–100	Ciliates	Mixed copepods	Oregon coast (USA)	Non-bloom	Fessenden and Cowles (1994)
3–41	Mixed	<i>Acartia tonsa</i>	Terrebonne Bay (USA)	Year-round	Gifford and Dagg (1988)
11–59	Dino., ciliates	<i>Neocalanus plumchrus</i>	Subarctic N. Pacific	June	Gifford and Dagg (1991)
20–75	Dino., ciliates	<i>Calanus</i> spp.	Greenland	Post-bloom	Levinsen et al. (2000)
90	Ciliates	<i>Oithona</i> spp.	Ross Sea	Austral summer	Lonsdale et al. (2000)
<1–10	Aloricate ciliates	<i>Acartia clausi</i> , <i>Centropages hamatus</i>	Temperate coastal waters	Summer	Tiselius (1989)
>50	Ciliates, nano.	Mixed copepods	Subantarctic (New Zealand)	Aug.–Oct.	Zeldis et al. (2002)

Dino.=dinoflagellates, nano.=nanoflagellates.

hatching success (Broglia et al., 2003) in copepods. In terms of carbon, heterotrophic protists could enhance the trophic transfer efficiency along the food chain by six-fold or higher (Tang et al., 2001). Thus, heterotrophic protists appeared to enhance the biochemical constituents of algal food for subsequent use at higher trophic levels. This phenomenon, dubbed “trophic upgrading” (Klein Breteler et al., 1999), challenges the traditional view of trophodynamics in the pelagic system in which heterotrophic protists are regarded merely a link for carbon transfer up the food chain and the production efficiency is expected to decline due to thermodynamic loss at each trophic step. Nevertheless, the underlying mechanisms of trophic upgrading are still not clear, although some evidence points to the involvement of EFA synthesis or alteration by heterotrophic protists. By feeding *O. marina* different food, Kleppel and Burkart (1995) and Kleppel et al. (1998) were able to produce *O. marina* of different fatty acid contents. Klein Breteler et al. (1999) and Broglia et al. (2003) suggested that, in addition to assimilating dietary EFAs, heterotrophic protists can synthesize EPA and DHA via elongation and desaturation of fatty acid precursors. The effectiveness of trophic upgrading by heterotrophic protists appears to vary among species; e.g., *Strombidium sulcatum* feeding on *Dunaliella* sp. did not improve the subsequent growth of juvenile copepods (Klein Breteler et al., 2004). Also, different reproductive parameters of a copepod may respond differently to trophic upgrading. For example, Broglia et al. (2003) noted that, while copepod’s egg viability was correlated with the prey’s EFA contents, no relationship was found between egg production and dietary EFA contents.

Most trophic upgrading studies tend to focus on the effects of one protist–one alga combinations, particularly with algae known to have poor to mediocre quality, such as *Dunaliella* sp., *Isochrysis* sp. and *Phaeocystis* sp. (e.g., Klein Breteler et al., 1999; Kleppel and Burkart, 1995; Tang et al., 2001). An aspect less studied is the species-specificity of trophic upgrading effects among heterotrophic protists. For example: Will different heterotrophic protists feeding on the same algal species result in the same trophic upgrading effects? Alternatively, will a heterotrophic protist species feeding on different algal species yield different nutritional effects on a higher predator? Broglia et al. (2003) tested *Gymnodinium dominans* growing on either *R. salina* or *Thalassiosira pseudonana*, and observed similar effects of the two *G. dominans* cultures on copepod egg production rates. However, their reported ingestion rates and egg production rates were very low (200–4000 cells copepod⁻¹ day⁻¹ and 3–8 eggs copepod⁻¹ day⁻¹), which may indicate food limitation. Also, parallel measurements of egg production rate with *T. pseudonana* treatment were not available; thus the trophic upgrading effect of *G. dominans* could not be evaluated. While the term trophic upgrading implies an improvement of nutritional effects due to heterotrophic protists feeding on poor quality algae, should one expect to see similar effects when a good quality alga is used? In addition, egg production and egg hatching appear to be affected differently by trophic upgrading, but available data are still scarce (Kleppel and Burkart, 1995; Broglia et al., 2003). In this study, we used three algal species, representing poor to high nutritional quality, and two heterotrophic protists in various combinations. Parallel treatments of hetero-

trophic protists and algae were tested for their effects on the reproductive output (egg production rate and egg hatching success) of copepods, which would allow us to evaluate the species-specific effectiveness of trophic upgrading among the protist treatments.

2. Materials and methods

Three planktonic unicellular algal species were used for the experiments: *Dunaliella tertiolecta* (CCMP 1320; chlorophyte), *Isochrysis galbana* (T-Iso; prymnesiophyte) and *Rhodomonas salina* (CCMP 1319; cryptophyte), representing respectively poor, intermediate and good food quality for marine copepods (e.g., Kleppel and Burkart, 1995; Klein Breteler et al., 1999; Tang et al., 2001). Algal cultures were maintained in f/2 medium of salinity 20‰ at 20 °C and a 12 h light:12 h dark photoperiod. The cultures were maintained in active growth by dilutions with fresh medium periodically and 2–3 days prior to experiments. Cell carbon contents of the algae were estimated based on size-to-carbon conversion (Strathmann, 1967; Table 2).

Two common estuarine heterotrophic protists were used: *O. marina* and *G. dominans* (*Gyrodinium dominans* in some literature). Both heterotrophic dinoflagellate species have been shown to upgrade

food quality for copepods when fed on poor quality algae (e.g., Klein Breteler et al., 1999; Tang et al., 2001). Protist cultures were maintained in f/2 medium of salinity 20‰ at 20 °C in dark. The protists were fed each of the three algal species; i.e. a total of 2 heterotrophic protist species × 3 algal species = 6 culture combinations were established for the study. The protist cultures were maintained for more than five cell divisions to assure equilibrium between the protist cell chemical contents and its algal food. In this paper, to distinguish the different protist cultures we use a letter in parentheses to indicate the algal food used to grow that protist culture; for example, “*O. marina* (D)” denotes *O. marina* that grew on *D. tertiolecta*. Just prior to the experiments, the protists were allowed to graze down most of the algal food in the culture flasks such that introduction of residual algal food to the experimental containers was negligible. Cell carbon contents of the heterotrophic protists were estimated based on size-to-carbon conversion (Menden-Deuer and Lessard, 2000; Table 2).

The omnivorous calanoid copepod species *Acartia tonsa* was collected from the York River, Virginia, and maintained in the laboratory at 20‰ and 20 °C (Table 2). A mixture of *R. salina* and *Thalassiosira weissflogii* (diatom) was added as food. The culture was regularly inspected for new nauplii, which were transferred to new containers to minimize cannibalism. We were able to maintain the copepods in laboratory conditions for months. Only active, intact female copepods with attached spermatophores were used for the experiments. The carbon contents were assumed to be 4.6 µg C copepod⁻¹ (Tang et al., 1999) and 45.7 ng C egg⁻¹ (Kiørboe et al., 1985).

All experiments were conducted in a walk-in environmental room at 20 °C with a 12 h light:12 h dark photoperiod. In each experiment, an algal treatment and the corresponding heterotrophic protist treatment were tested in parallel. For example, a *D. tertiolecta* treatment would be set up at the same time as a treatment with *O. marina* that had been growing on *D. tertiolecta*. A total of six sets of experiments (3 algal species × 2 heterotrophic protist species) were conducted. Each experiment involved an acclimation period and an incubation period. Aliquots of algal cultures or protist cultures were preserved in 2% acid Lugol's solution for cell counts, using a settling chamber and a Nikon TS-

Table 2
Species of algae, heterotrophic protists and copepod used in this study, their approximate sizes and estimated carbon contents

	Size (µm)	Carbon content (pg C cell ⁻¹)
<i>Planktonic alga</i>		
<i>Dunaliella tertiolecta</i> (CCMP 1320)	7.0	31.1
<i>Isochrysis galbana</i> (T-Iso)	4.2	8.2
<i>Rhodomonas salina</i> (CCMP 1319)	6.9	29.8
<i>Heterotrophic dinoflagellate</i>		
<i>Oxyrrhis marina</i>	20	516.3
<i>Gyrodinium dominans</i>	15	270.2
<i>Calanoid copepod</i>		
	Size	Carbon content (ng C ind ⁻¹)
<i>Acartia tonsa</i>	1 mm	4600
<i>Acartia tonsa</i> egg	85 µm	45.7

CCMP numbers refer to strain designations by the Center for Culturing of Marine Phytoplankton (Maine, USA).

See text for estimation of carbon contents.

100 inverted microscope (Gifford and Caron, 2000). Appropriate amounts of the cultures were then added to filtered 20‰ artificial seawater to attain a food concentration of approximately 300 ng C ml⁻¹, at which the ingestion rate of *A. tonsa* was expected to be at or near maximum (Tang et al., 2001). For acclimation, 30 female copepods were added to each 600 ml bottle with a food treatment (an alga or the corresponding heterotrophic protist). All bottles were sealed with parafilm to exclude air bubbles and were mounted onto a rotating plankton wheel (0.25 rpm) to maintain the food in suspension. After 24 h, the copepods were gently collected on a 200 µm sieve and transferred to new 600 ml bottles with freshly prepared media of the same food species. After a total of 48 h of acclimation, the copepods were recovered on a 200 µm sieve. Five active and intact copepods were then transferred to each 120 ml bottle containing freshly prepared medium of the same food species (in triplicate). Bottles without copepods were set up as controls (in triplicate). Aliquots of the food media were preserved in Lugol's solution for initial cell counts. All bottles were sealed air tight with parafilm and mounted onto the rotating plankton wheel. At the end of the 24 h incubation period, aliquots were drawn from each bottle and preserved for final cell counts. The initial and final cell count data were used to calculate the copepod's ingestion rates according to Frost (1972). The bottle content was gently poured through a 200 µm sieve to collect the copepods, and a 63 µm sieve to collect the eggs and nauplii. In cases where copepods were missing or mortality was observed, the initial and the final numbers of copepods were averaged for subsequent calculations. In most cases better than 80% of the copepods were recovered in the experiments. Eggs were further classified into eggs with normal appearance and empty egg shells (Tang and Dam, 2001). For the calculation of egg production rate, only normal eggs and nauplii were counted (Tang and Dam, 2001). Egg cannibalism is negligible for this copepod species (Dagg, 1977, Kleppel, 1992). The normal eggs were further incubated in 35 mm petri dishes with 20‰ filtered artificial seawater at 20 °C for 3 days in dark. At the end of 3 days, the number of newly hatched nauplii were recorded for calculating the cumulative egg hatching success (Tang et al., 1998).

3. Results

3.1. Filtration rates and ingestion rates

Mean filtration rates ranged 0.44 to 1.96 ml ind⁻¹ h⁻¹ across all experiments (Figs. 1–6). The filtration rate was significantly higher in the heterotrophic protist treatments than in the corresponding algal treatments in two cases: *O. marina* (*R*) and *G. dominans* (*I*) (*t*-tests, *p*<0.05). In terms of carbon, *A. tonsa* tended to ingest 20–30% more algae, or a similar amount of algae as the corresponding protists. One exception is *O. marina* (*R*), which was ingested twice as much as the corresponding *R. salina*. Assuming a body carbon content of 4.6 µg C ind⁻¹ for *A. tonsa* (Tang et al., 1999), the estimated ingestion rates were equivalent to a ration of 50–122% body C ind⁻¹ day⁻¹ across all experiments. The copepods derived a significantly higher daily ration from *O. marina* (*R*) than the corresponding *R. salina* (*t*-test, *p*<0.05). On the contrary, *A. tonsa* consumed a lower daily ration by feeding on *G. dominans* (*I*) than on the corresponding *I. galbana* treatment (*t*-test, *p*<0.05).

3.2. Egg production and egg hatching

Egg production rates were statistically equal between the algal treatments and the corresponding protist treatments, with the exception of *O. marina* (*D*) and *G. dominans* (*D*) treatments, which resulted in nearly 13–14 times higher egg production rates than the corresponding algal treatments (*t*-test, *p*<0.05; Figs. 1–6). Because the copepod ingestion rate differed among the different treatments, the reproductive effort was better evaluated by computing the egg production efficiency (EPE), which was defined as (Tang et al., 2001):

$$\text{EPE} = \frac{\text{Daily egg carbon production}}{\text{Daily carbon ingestion}} \times 100\% \quad (1)$$

where egg carbon content was calculated assuming 45.7 ng C egg⁻¹ (Kjørboe et al., 1985). Trophic upgrading index is here defined as the ratio between EPE with heterotrophic protist diet and EPE with the corresponding algal diet, such that:

$$\text{TUI} = \frac{\text{EPE}_{\text{het. protist}}}{\text{EPE}_{\text{alga}}} \quad (2)$$

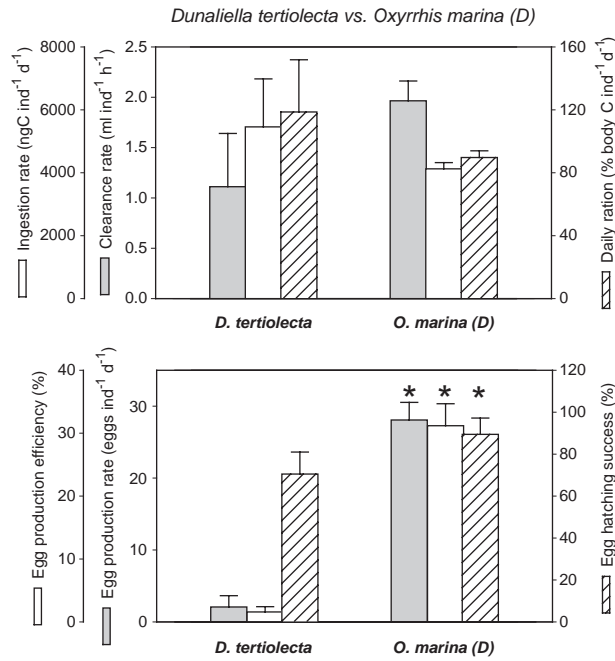


Fig. 1. *Dunaliella tertiolecta* vs. *Oxyrrhis marina* (D). Upper panel: *Acartia tonsa* clearance rates, ingestion rates and daily rations (mean+S.D., triplicate). Lower panel: *Acartia tonsa* egg production rate, egg production efficiency and egg hatching success (mean+S.D., triplicate). * $p < 0.05$, t -tests.

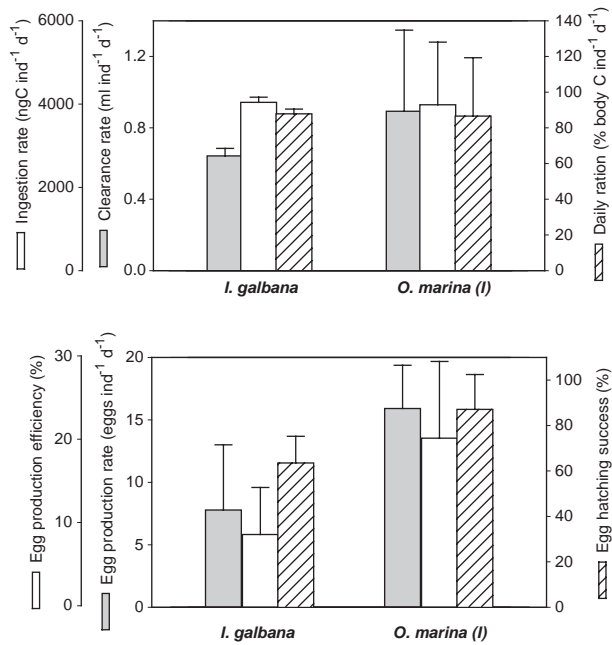


Fig. 2. *Isochrysis galbana* vs. *Oxyrrhis marina* (I). See Fig. 1 caption for explanation.

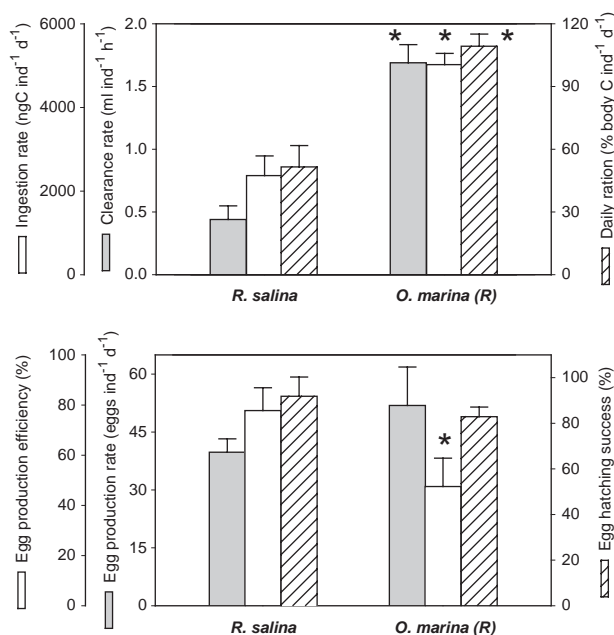


Fig. 3. *Rhodomonas salina* vs. *Oxyrrhis marina* (R). See Fig. 1 caption for explanation.

A TUI>1 indicates trophic upgrading by the heterotrophic protist, whereas a TUI<1 would indicate trophic downgrading. Comparison of TUI would indicate the relative effectiveness of trophic upgrading between treatments.

D. tertiolecta vs. *O. marina* (D)—EPE was significantly higher with *O. marina* (D) treatment (31.1%) than in the corresponding *D. tertiolecta* treatment (1.6%) (*t*-test, *p*<0.05), and the resultant TUI was 19.7.

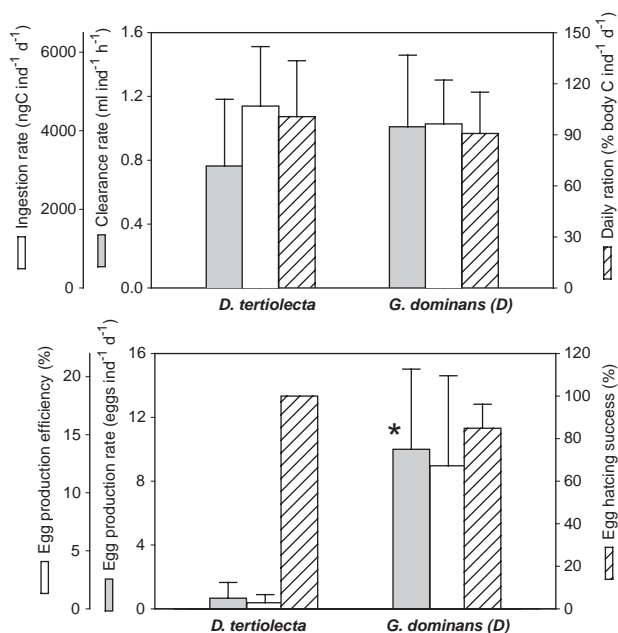


Fig. 4. *Dunaliella tertiolecta* vs. *Gymnodinium dominans* (D). See Fig. 1 caption for explanation.

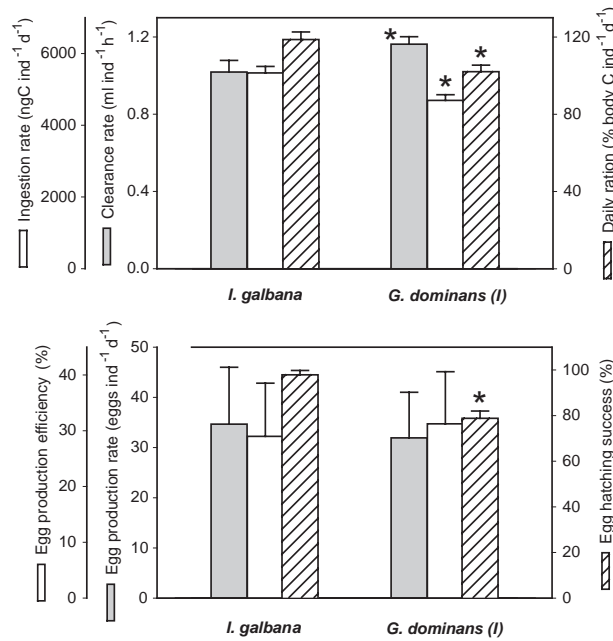


Fig. 5. *Isochrysis galbana* vs. *Gymnodinium dominans* (I). See Fig. 1 caption for explanation.

The mean egg hatching success was 70.5% for the *D. tertiolecta* treatment and was significantly higher for the *O. marina* (D) treatment, at 89.4% (*t*-test, *p*<0.05).

I. galbana vs. *O. marina* (I)—The mean EPE in the *O. marina* (I) treatment (20.3%) was not statistically different from that in the *I. galbana* treatment

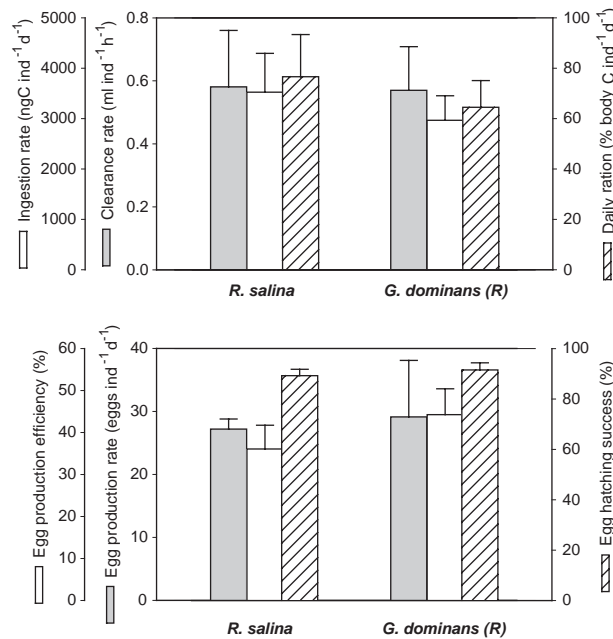


Fig. 6. *Rhodomonas salina* vs. *Gymnodinium dominans* (R). See Fig. 1 caption for explanation.

(8.7%). The TUI between these two treatments was 2.3. The mean egg hatching success for the algal treatment and the protist treatment were 63.5% and 87.1%, respectively.

R. salina vs. *O. marina* (*R*)—*A. tonsa* feeding on *O. marina* (*R*) had a significantly lower EPE (47.5%) than that feeding on *R. salina* (77.8%) (*t*-test, $p < 0.05$), yielding a TUI of 0.6. Despite the difference in EPE, the egg hatching success was not significantly different between the two treatments, at 91.8% for *R. salina* and 82.9% for *O. marina* (*R*).

D. tertiolecta vs. *G. dominans* (*D*)—*A. tonsa* feeding on *D. tertiolecta* had very low egg production rate, resulting in an EPE of 0.5%. In contrast, *G. dominans* (*D*) treatment yielded a higher EPE (12.3%), although the difference was not significant (*t*-test, $p = 0.059$). The calculated TUI, based on the mean EPE values, was 23.3. Mean egg hatching success was 100% and 84.9% for the algal treatment and the protist treatment, respectively.

I. galbana vs. *G. dominans* (*I*)—The two treatments yielded similar EPE, at about 30%, and a corresponding TUI of 1.1. However, egg hatching success was significantly lower in the *G. dominans* (*I*) treatment (78.8%) relative to the *I. galbana* treatment (97.9%) (*t*-test, $p < 0.05$).

R. salina vs. *G. dominans* (*R*)—EPE ranged from 36.1% to 44.3% between the two treatments with no significant difference, and the corresponding TUI was 1.2. Egg hatching success was also similar between the two treatments, at 89.2% for *R. salina* and 91.5% for *G. dominans* (*R*).

Comparison of algal treatments—The relative nutritional quality of the three algal diets, based on their respective EPE, was *D. tertiolecta* < *I. galbana* < *R. salina*. For the same algal species, the EPE also differed between experiments (*I. galbana* and *R. salina*; $p < 0.05$, *t*-tests).

Comparison of *O. marina* treatments—Among all *O. marina* treatments, EPE was significantly lower for *O. marina* (*I*), but not different between *O. marina* (*D*) and *O. marina* (*R*) (1-ANOVA followed by Tukey test, $p < 0.05$). When compared with the respective algal treatments, the calculated TUI was 19.7 for *O. marina* (*D*), 2.3 for *O. marina* (*I*) and 0.6 for *O. marina* (*R*) (Table 3).

Comparison of *G. dominans* treatments—Among the *G. dominans* treatments, significant difference in

Table 3

Trophic upgrading index (TUI) for the different algal–protist combinations in this study

	<i>D. tertiolecta</i>	<i>I. galbana</i>	<i>R. salina</i>
<i>O. marina</i>	19.7	2.3	0.6
<i>G. dominans</i>	23.3	1.1	1.2

EPE was detected only between *G. dominans* (*D*) and *G. dominans* (*R*) (1-ANOVA followed by Tukey test, $p < 0.05$). When compared with the respective algal treatments, the calculated TUI was 23.3 for *G. dominans* (*D*) and close to 1 for the other two *G. dominans* treatments (Table 3).

Comparison of the two protist species—Between the two protist species growing on *D. tertiolecta*, *O. marina* (*D*) induced a significantly higher EPE in *A. tonsa* than *G. dominans* (*D*) ($p < 0.05$, *t*-test), suggesting a better nutritional quality of *O. marina* (*D*). *O. marina* and *G. dominans* growing on the other two algal species did not appear to differ in their quality for *A. tonsa*, as indicated by their similar EPE.

4. Discussion

4.1. Nutritional quality of the algal species

D. tertiolecta consistently yielded the lowest EPE, confirming the poor quality of this alga for *A. tonsa* (Klein Breteler et al., 1999; Thor et al., 2002). The low EPE associated with *I. galbana* may be partly explained by the energetic cost of capturing such small algal cells (Kleppel et al., 1998). On the other hand, the high EPE associated with *R. salina* confirms the high nutritional value of this algal species for copepods (Tang et al., 2001). EPE also differed between experiments even for the same algal species (e.g., experiments with *I. galbana* and *R. salina*). Because the experiments were conducted with different cohorts of *A. tonsa*, the physiological conditions of the copepods might vary between experiments, resulting in different EPE with the same algal species.

4.2. Nutritional quality of *O. marina*

The EPE of *A. tonsa* was not different between *O. marina* (*D*) and *O. marina* (*R*), suggesting that both *D. tertiolecta* and *R. salina* resulted in *O. marina* of

similar quality. Nevertheless, when compared with the respective algal treatments, the difference in trophic upgrading effects becomes clear: *O. marina* (D) treatment had a higher TUI than the others, indicating the ability of *O. marina* to “upgrade” poor quality alga, such as *D. tertiolecta*, for *A. tonsa*. Relative to an intermediate quality alga such as *I. galbana*, the upgrading effect by *O. marina* was small, as indicated by a TUI close to 1. On the contrary, *O. marina* (R) had a TUI of 0.6, indicating trophic downgrading of food quality relative to the alga *R. salina*. Kleppel and Burkart (1995) and Kleppel et al. (1998) tested *A. tonsa* feeding on *I. galbana* or *O. marina* that grew on *I. galbana*. Despite the differences in experimental design (e.g., larger incubating bottle and higher food concentration), their measured copepod ingestion rates (4000–5000 ng C ind⁻¹ day⁻¹) and egg production rates (<20 eggs ind⁻¹ day⁻¹) were very similar to our results (Fig. 2), giving comparable egg production efficiencies (2–12% in Kleppel et al. vs. 8–20% in our study). However, Kleppel and Burkart (1995) reported a much lower egg hatching success (<12%) than our experiments (64–87%), possibly due to the short incubation time in their egg hatching measurements (≤24 h).

4.3. Nutritional quality of *G. dominans*

The nutritional qualities of *G. dominans* mirrored those of its algal food in that *G. dominans* (D) was poorer than *G. dominans* (R), with *G. dominans* (I) being of intermediate quality. Yet, when compared against the algal treatments, trophic upgrading effect appeared to be the strongest in *G. dominans* (D) (TUI=23.3), whereas the other two treatments appeared neutral (TUI close to 1). These results indicate that *G. dominans* could trophically upgrade poor quality alga such as *D. tertiolecta* for *A. tonsa*, but would not benefit the copepod in the case of intermediate or high quality alga. Broglio et al. (2003) tested *A. tonsa* feeding on *R. salina* vs. *G. dominans* that grew on *R. salina*. They reported an average carbon-based EPE of 21% with *R. salina* (among the treatments where egg hatching was also recorded) and 19% with *G. dominans*. These EPE values were lower than our results—36% and 44% for *R. salina* and *G. dominans* (R), respectively (Fig. 6). A notable difference is the low food concentration in the *G. dominans*

treatment used by Broglio et al. (2003), resulting in extremely low ingestion rate and egg production rate—200 cells copepod⁻¹ day⁻¹ and 3 eggs copepod⁻¹ day⁻¹ (in contrast to Fig. 6). Nevertheless, despite the differences in experimental food concentration, no trophic upgrading effects were noted on the copepod egg production or egg hatching in either study.

4.4. Quality differences between the two protist species

Although both *O. marina* (D) and *G. dominans* (D) treatments resulted in trophic upgrading, with TUI>>1, the EPE of *A. tonsa* was significantly higher with *O. marina* (D) than with *G. dominans* (D), indicating a species-specific differences between the two protist species in their nutritional quality for *A. tonsa*.

4.5. Nutritional requirements for egg production vs. egg hatching

Population recruitment of the copepod depends on both egg production and egg hatching. However, the two processes may have different nutritional requirements and may be affected by the copepod’s diets in different ways. In the present study, egg hatching success (EHS) was not affected by trophic upgrading with a few exceptions: EHS was significantly higher in the *O. marina* (D) treatment than in the corresponding *D. tertiolecta* treatment, suggesting that trophic upgrading by *O. marina* improved both egg production and hatching success in *A. tonsa*. While EPE was lower with *O. marina* (R), the corresponding EHS was not affected. On the other hand, EHS was significantly lower in *G. dominans* (I) although the EPE was not affected. These results show that EPE and EHS responded differently to trophic upgrading, suggesting that the two processes may be regulated by different chemical factors modified differently by the presence of heterotrophic protists.

4.6. Essential fatty acids as the smoking gun

What factors may explain the differences in EPE, TUI and EHS observed in our experiments? Many studies have pointed out the importance of essential

long-chain polyunsaturated fatty acids for zooplankton growth and reproduction (reviewed by Brett and Müller-Navarra, 1997). Several authors attributed trophic upgrading to modification of algal fatty acid by heterotrophic protists (Klein Breteler et al., 1999; Broglio et al., 2003). Available biochemical data suggest that changes in essential fatty acid profile across the algal–protist interface could explain some of our observations (Table 4). For example, the fatty acids EPA and DHA, essential for zooplankton growth and reproduction, are both absent in *D. tertiolecta*, which may explain the poor egg production rate of *A. tonsa* feeding on this alga (Figs. 1 and 4). Interestingly, *O. marina* and *G. dominans* growing on *D. tertiolecta* contained a substantial amount of DHA. Similar observations have been reported by Klein Breteler et al. (1999) who studied *O. marina* feeding on *Dunaliella* sp. The presence of DHA in *O. marina* (*D*) and *G. dominans* (*D*) in contrast to *D. tertiolecta* could explain the trophic upgrading effects of these protists on *A. tonsa* egg production. Also, comparison of the two protist species revealed that *O. marina* (*D*) was relatively more enriched with EPA and DHA than *G. dominans* (*D*) (Table 4), which may explain the higher egg production efficiency of *A. tonsa* feeding on *O. marina* (*D*) than on *G. dominans* (*D*). The alga *I. galbana* contained a small amount of EPA and DHA, which were present in higher amounts in *O. marina* and *G. dominans* growing on this alga. However, no trophic upgrading effects on egg production efficiency were detected in *A. tonsa* feeding on these protists (Figs. 2 and 5), suggesting that EPA and DHA may not be the sole factors that determine the egg production efficiency of the copepods. The relationship between the essential fatty acids and egg hatching is less clear. For example, *O. marina* (*D*) significantly improved the egg hatching success relative to *D. tertiolecta*, consistent with the difference in EPA and DHA contents between the protists and the alga. On the other hand, although *O. marina* (*I*) and *G. dominans* (*I*) contained higher EPA

and DHA contents than *I. galbana*, *O. marina* (*I*) did not improve egg hatching success and *G. dominans* (*I*) actually reduced egg hatching success relative to the alga. These observations suggest that egg production and egg hatching may have different nutritional requirements, and that EPA and DHA alone could not predict egg hatching success. The appearance of substantial amount of DHA in *O. marina* (*D*) and *G. dominans* (*D*) despite the lack of DHA in their diet begs for an explanation. There are at least three mechanisms by which heterotrophic protist may obtain DHA: (1) Bioaccumulation from its diet. However, DHA was not detectable in *D. tertiolecta*; thus bioaccumulation is unlikely an explanation in the present case. (2) De novo synthesis. Traditionally heterotrophs are believed incapable of de novo synthesis of essential fatty acids, including DHA. Unfortunately, we could not rule out or confirm de novo synthesis based on the available fatty acid data. (3) Modification of fatty acid precursors. Although *D. tertiolecta* lacks EPA and DHA, it contains other long chain fatty acids that could be modified by *O. marina* and *G. dominans* into DHA, a capability that has been demonstrated in some heterotrophs (Chu, 1996; Nanton and Castell, 1998; Tocher et al., 2001). We are presently investigating the detailed changes in biochemical composition, particularly essential fatty acids, across the different trophic levels (alga, heterotrophic protist, copepod); the results would reveal the biochemical mechanism by which the protists alter the fatty acid composition along the food chain.

4.7. The concept of trophic upgrading revisited

Many studies showed that heterotrophic protists can upgrade the nutritional constituents of algal food, as evidenced by the higher egg production or egg hatching in copepods feeding on the protists as trophic intermediaries. However, most of the previous experiments employed algae of known poor quality, particularly *Dunaliella* sp. In the present study, we tested different

Table 4
EPA and DHA contents (as % total fatty acids) in food species used in this study

	<i>D. tertiolecta</i>	<i>O. marina</i> (<i>D</i>)	<i>G. dominans</i> (<i>D</i>)	<i>I. galbana</i>	<i>O. marina</i> (<i>I</i>)	<i>G. dominans</i> (<i>I</i>)
EPA 20:5(n-3)	n/d	1.8±0.5	0.9±1.6	0.5±0.0	4.1±0.8	7.2±0.1
DHA 22:6(n-3)	n/d	27.1±7.1	13.8±1.6	9.4±0.4	17.8±2.9	29.9±1.5

Numbers are mean±S.D. of triplicate; n/d=not detectable. (Unpublished data from Adriana Veloza.)

combinations of two heterotrophic protists and three algal species of various food qualities. Trophic upgrading was evident in only the experiments with the poor quality alga *D. tertiolecta*. With an intermediate quality alga such as *I. galbana*, the trophic upgrading effect appeared to be neutral. On the contrary, relative to the good quality alga *R. salina*, the *O. marina* (R) treatment resulted in trophic downgrading as indicated by the significantly lower egg production efficiency in the copepod, and a TUI value of less than 1 (Table 3). The change from upgrading to downgrading by heterotrophic protists based on their algal diets suggests that the term “trophic upgrading” should be broadened to the more neutral term “trophic modification”. Trophic modification can be defined as the ability of heterotrophic protists to modify, either upgrade or downgrade, the nutritional constituents from algal diets, resulting in higher or lower physiological performance in higher consumers.

4.8. Implication for food web dynamics

Energy and carbon are the common currencies when calculating production efficiency across different trophic levels. Yet, biological production requires not merely energy and carbon, but a vast variety of biochemical compounds. Synthesis and alteration of biochemical compounds at the base of the food chain could have strong effects on the higher trophic levels. Some algal species are known to be of poor nutritional quality for zooplankton. However, many zooplankton species are omnivorous and may prey on heterotrophic protists, which are ubiquitous and often highly abundant in coastal waters. In this study, *O. marina* (D) and *G. dominans* (D) significantly enhanced the egg production efficiencies of the copepods relative to the ones feeding on the alga directly. In light of these and similar observations (e.g., Klein Breteler et al., 1999; Tang et al., 2001; Broglio et al., 2003), heterotrophic protists may mitigate and lessen the “poor quality” effects of the algae on the higher trophic levels. The contribution of heterotrophic protists to copepod diet in the field is well documented (Table 1). Dam et al. (1994) estimated that *A. tonsa* in Long Island Sound derived as much as 44% of its egg production from non-algal food. It is generally assumed that by preying on heterotrophic protists a copepod can diversify its diet and obtain a more balanced nutrition

(Kleppel, 1993). However, such feeding strategy is not necessarily beneficial as indicated by the *O. marina* (R) and *G. dominans* (I) treatments in our study, where the egg production efficiency or egg hatching success was actually decreased. As such, omnivory is more likely to be advantageous when the algal abundance is low (e.g., during non-bloom periods), or when the algal population is dominated by poor-quality species (e.g., *Phaeocystis* sp., cyanobacteria sp.). Although copepods are known to distinguish between algal cells of different nutritional qualities (Cowles et al., 1988; Butler et al., 1989), it is not clear if they can distinguish between heterotrophic protists of different chemical contents. In this paper, we present evidence that heterotrophic protists as intermediate prey could upgrade or downgrade algal food quality for copepods. The biochemical mechanisms behind the observed effects remain to be defined, although literature and available data point to the role of essential fatty acids such as EPA and DHA in the process. In this regard, some important but unresolved questions are whether heterotrophic protists are capable of de novo synthesis of EPA and DHA, or producing them via elongation and desaturation of fatty acid precursors, and what biochemical components other than EPA and DHA may affect copepod egg production and egg hatching. Integrating biochemistry and zooplankton ecology will prove necessary if we are to define the nutritional status of the pelagic environment, and to understand the trophodynamics among the plankton.

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