

Effect of mesozooplankton feeding selectivity on the dynamics of algae in the presence of intermediate grazers — a laboratory simulation

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ABSTRACT: An indirect increase in algal abundance that is induced by mesozooplankton predation on intermediate trophic level grazers (microzooplankton) is among the factors that shape the structure of a marine planktonic food web (copepods/cladocerans–ciliates–algae). Marine mesozooplankton include species with diverse feeding strategies, and hence they play different roles in trophic interactions. In this study, we simulated a simple pelagic food web in the laboratory using 3 copepod species and 1 cladoceran as top predators to test the hypothesis that different species with different feeding behaviors will cause different impacts on prey communities. Our results showed that among the 3 crustacean mesozooplankton species, *Parvocalanus crassirostris* was the most carnivorous species that caused the strongest cascading effect, which led to an increase in algal density as the rate of the cascading effect exceeded the direct consumption of algal prey by intermediate consumers. In contrast, the marine cladoceran *Penilia avirostris* generally caused a decline in algal density because it created no indirect positive effect on algae since it was incapable of capturing the intermediate grazers. *Temora turbinata* fed on ciliates and algal prey at similar rates so that the direct consumption of the algal prey was balanced by the indirect trophic cascade effect. The strength of the cascade effect induced by *Acartia erythraea* was significantly enhanced by increasing the densities of ciliates. The mechanism was due to a switch in preying behavior from suspension feeding to ambush feeding. Our results imply that mesozooplankton omnivory is important in maintaining the stability of the community structure of microplankton because the effects of direct consumption and the cascading effect balanced each other due to the broad feeding strategies of predators.

KEY WORDS: *Parvocalanus* · *Temora* · *Acartia* · Marine cladocerans · Grazing · Trophic cascade

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INTRODUCTION

The hypothesis of trophic cascades predicts a negative relationship between 2 adjacent trophic levels and a positive relationship between non-adjacent levels in a food chain (Carpenter et al. 1985, Pace et al. 1999, Chase 2000). For example, in a simple 3-level food chain, predators can cause an indirect effect on primary producers by preying on intermediate grazers. When the predation effect is intense, predators may facilitate the biomass of primary pro-

ducers as the grazing mortality from herbivores decreases. Similarly, removal of predators results in a decrease of primary producers.

Trophic cascades on phytoplankton induced by higher trophic levels such as fish have been reported in marine pelagic food webs (Shiomoto et al. 1997, Daskalov 2002, Frank et al. 2005, Daskalov et al. 2007), although their strengths are usually not as remarkable and predictable as those in freshwater systems, despite their similar food web structures (Shurin et al. 2002, Stibor et al. 2004, Borer et al.

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2005). However, marine top-down trophic effects are largely dampened at the interface between phytoplankton and mesozooplankton (Micheli 1999, Baum & Worm 2009), although the mechanism is still not fully understood (Stibor et al. 2004, Sommer & Sommer 2006, Sommer 2008). One striking difference between marine and freshwater planktonic food webs is that the degree of herbivory is far lower in marine mesozooplankton (dominated by copepods) than in their freshwater counterparts (dominated by cladocerans). In fact, omnivory and carnivory are common in marine copepods, which may explain why marine mesozooplankton overall play a relatively minor role in daily removal of primary production, compared to microzooplankton (such as ciliates and heterotrophic dinoflagellates) in marine planktonic ecosystems (Calbet 2001, Calbet & Landry 2004). On the other hand, because microzooplankton make up a large portion of the diet of copepods, the extra link of copepods–microzooplankton becomes an important pathway for energy and material transportation and eventually connects to the classic fish food chain (Kleppel 1993, Fessenden & Cowles 1994, Calbet & Saiz 2005). Consequently, a cascading effect on phytoplankton occurs when their major grazers (such as ciliates) are intensively consumed by copepods, especially for small-sized phytoplankton (Froneman 2002, Sommer & Sommer 2006). Studies have shown that strong cascading effects induced by mesozooplankton influenced the lower trophic levels of the pelagic microbial food web, by indirectly affecting the biomass of heterotrophic nanoflagellates (grazers of bacteria), and also influenced the activities and diversity of bacteria (Zöllner et al. 2003, 2009).

Mesozooplankton show highly diverse feeding behaviors, ranging from purely herbivorous at one end of the spectrum to purely carnivorous at the other end. The composition of marine mesozooplankton is highly variable at both spatial and temporal scales, especially in coastal areas where species with different feeding modes coexist. In order to understand the overall impact of mesozooplankton grazing on phytoplankton biomass, it is essential to elucidate how species of different feeding modes affect the trophic structure in the planktonic microbial food web.

Not only is the mesozooplankton community composed of species with a great variety of feeding modes, but individual species, especially some coastal copepods such as acartiids, are also able to switch their feeding behavior (e.g. from suspension feeding to ambush feeding) according to the food composition in the environment (Gifford & Dagg 1988, Kiør-

boe et al. 1996). When phytoplankton biomass is high, acartiids primarily graze on phytoplankton. When phytoplankton is scarce, they switch to more predatory feeding mode by consuming more microzooplankton, and consequently exert a larger indirect positive effect than direct grazing mortality on phytoplankton. Chen & Liu (2011) showed that the strength of trophic cascades on diatoms induced by *Acartia erythraea* was increased with increasing ingestion rate on heterotrophic dinoflagellates.

Therefore, the aim of this study was to demonstrate how different mesozooplankton species with different feeding behaviors (relatively carnivorous, omnivorous, or herbivorous feeding) affect the dynamics of algae in the presence of intermediate grazers. We constructed simple experimental food chains in the laboratory to quantify the direct and indirect trophic effects of different marine mesozooplankton species with different feeding behaviors, using a ciliate (*Strombidium sulcatum*, equivalent spherical diameter, ESD = 24.8 μm) as the intermediate grazer and algae (*Dunaliella* sp., ESD = 6.7 μm) as the primary producer. The predators we chose are primarily suspension feeders with different degrees of carnivory, including 2 copepod species (*Parvocalanus crassirostris* and *Temora turbinata*) and 1 cladoceran (*Penilia avirostris*). In addition, we studied how a change in the feeding behavior of *Acartia erythraea* along a gradient of ciliate abundance affected the relative strength of the cascading effect and direct consumption on phytoplankton. Current methods to estimate the rates of mesozooplankton herbivory are based on the changes in phytoplankton abundance or its biomarkers, e.g. chlorophyll concentration, without considering the complex trophic interaction. Quantifying the cascade effect will allow us to correct the bias in estimating mesozooplankton ingestion rates and better understand the mechanisms that stabilize the planktonic food webs in marine ecosystems. Results of this study will help to predict how phytoplankton abundance changes if the dominant mesozooplankton species are known.

MATERIALS AND METHODS

Preparation of experimental organisms

The green alga *Dunaliella* sp. was maintained in regular f/2 medium, under a 14:10 h light:dark cycle of approximately 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at 24°C. Cultures were transferred every 3 to 4 d to keep them at exponential growth phase. The ciliate *Strombid-*

ium sulcatum was initially maintained in filtered seawater with rice grains as a nutrient source for bacteria. Before the experiments, the ciliates were transferred to filtered and autoclaved seawater with growing *Dunaliella* culture as food. Ciliates grown on a *Dunaliella* diet were transferred weekly to fresh medium with more than 10 generations before feeding experiments were conducted. To determine cell concentrations, triplicate 1 ml aliquots and triplicate 10 ml aliquots were taken from culture flasks and fixed with 5% acid Lugol's solution for algae and ciliates, respectively. Cell numbers of *Dunaliella* sp. and *S. sulcatum* were then counted under an inverted microscope in a 96-well plate and a 24-well plate, respectively. To determine cellular carbon content, duplicate *Dunaliella* subsamples with a known volume and concentration from culture flasks were filtered onto pre-combusted GF/C glass-fiber filters. Ciliates were first separated from their prey by filtering through a 10 μm polycarbonate membrane filter, and were then washed off the filter using autoclaved filtered seawater and onto pre-combusted GF/C filters. Carbon content of both *Dunaliella* and ciliates was then measured using a Perkin-Elmer CHN elemental analyzer. ESDs of *Dunaliella* sp. (6.7 μm) and *S. sulcatum* (24.8 μm) were determined by a Z2 particle counter (Beckman-Coulter).

Copepods and cladocerans were collected by net tows with a 200 μm mesh from the pier off Porter Shelter, Hong Kong, during the summer of 2010. Mixtures of samples in the cod-end of the plankton net were transferred to a 10 l PC carboy containing filtered seawater enriched with both *Dunaliella* and *Strombidium sulcatum*. Sufficient numbers of targeted animals were sorted under a dissecting microscope and acclimated in the target food for 1 d.

Ciliate feeding experiment

A ciliate feeding experiment was conducted to establish a regression curve between the ciliate ingestion rate and algal concentrations, which would allow us to differentiate the ciliate grazing rates between bottles in the presence and absence of top predators. This difference in ciliate grazing rates was used to calculate the cascading effect induced by copepod predation on ciliates (see also Chen & Liu 2011). Before the feeding experiments were conducted, the regular transfer of the ciliate culture was stopped to reduce the residual food in the culture. Cell numbers of both ciliates and algae in the ciliate culture and algae in the algal culture were recorded

(see above) to determine the initial concentrations of feeding experiments. A known volume of ciliate stock culture was added to 5 treatments containing different concentrations of algae (104, 200, 500, 750, 1000 cells ml^{-1}) in 170 ml PC bottles. The established concentrations of ciliates were the same among treatments (15 ciliates ml^{-1}), and each treatment was run in triplicate with duplicate controls (no ciliates). About 10 ml of f/2 medium were added to each experimental bottle to support the growth of the algae. The bottles were then filled fully with autoclaved filtered seawater, capped, and incubated in a walk-in culture room with conditions the same as in the culture maintenance. After a 48 h incubation, subsamples were taken from each bottle, fixed, and counted to determine the final concentrations of both grazers and prey. The density changes of grazers and prey were used to calculate the clearance rate (F , $\text{ml predator}^{-1} \text{d}^{-1}$) and ingestion rate (I , cells ciliate $^{-1} \text{d}^{-1}$) of ciliates according to Frost (1972) and Heinbokel (1978):

$$I = F \times \bar{A} = (g/\bar{C}) \times \bar{A} \quad (1)$$

where \bar{A} (mean concentration of algae) and \bar{C} (mean concentration of ciliates) are calculated from the changes of densities and growth rates:

$$\bar{A} = \frac{A_{\text{end}} - A_{\text{initial}}}{\ln A_{\text{end}} - \ln A_{\text{initial}}} \quad \text{and} \quad \bar{C} = \frac{C_{\text{end}} - C_{\text{initial}}}{\ln C_{\text{end}} - \ln C_{\text{initial}}},$$

respectively; A_{end} (C_{end}) and A_{initial} (C_{initial}) are the concentrations of algae (ciliates) at the end and the beginning of the incubation, respectively; g is the grazing rate of ciliates, which is calculated from the difference of algal net growth rate between controls and treatments by assuming that the decrease of algal net growth rate in the treatments was caused by grazing mortality. Since ingestion rate is a functional response (hyperbolic increase) to food concentrations, the ingestion rates of ciliates at different mean concentrations of algae were then fitted to a Michaelis-Menten equation:

$$I = I_{\text{max}} \times \bar{A} / (K_{\text{d}} + \bar{A}) \quad (2)$$

where I_{max} is the potential maximum ingestion rate (cells ciliate $^{-1} \text{d}^{-1}$), while K_{d} is the food concentration when ingestion was half-saturated. The value of I_{max} and K_{d} were then obtained by iteratively fitting the observational data to Eq. (2). Substituting Eq. (2) into Eq. (1), the ciliate grazing rate (g , d^{-1}) can be established as a function with both mean concentrations of grazer and prey as the following equation:

$$g = (\bar{C}/\bar{A}) \times I = \bar{C} \times I_{\text{max}} / (K_{\text{d}} + \bar{A}) \quad (3)$$

This equation was then applied to estimate the ciliate grazing rates in the following feeding experiments in the presence of mesozooplankton predators in which mean concentrations of both ciliates and algae can be measured.

Mesozooplankton feeding experiments

Two mesozooplankton feeding experiments were carried out. The first experiment was conducted to study the effects of mesozooplankton identity with different degrees of omnivory on trophic interactions. *Parvocalanus crassirostris*, *Temora turbinata*, and *Penilia avirostris* were chosen because they co-dominated in the study area during the study period. After a 1 d acclimation, triplicate 10 individuals of all species were sorted from the mixture and subsequently added to 600 ml PC bottles containing *Dunaliella* sp. (3519 cells ml⁻¹) and *Strombidium sulcatum* (20 ciliates ml⁻¹). To support the growth of algae and to reduce the indirect effect of nutrient recycling by predators, sufficient nutrients (10% of f/2 medium) were added to each bottle. Duplicate bottles containing the same concentrations of algae and ciliates and f/2 medium but no predators were also prepared and served as controls for all treatments. After filling the bottles with autoclaved filtered seawater, they were incubated for 48 h under the same conditions as used for culture maintenance.

The second experiment was conducted to study the effect of food composition on the feeding mode of *Acartia erythraea*. This species was abundant in Port Shelter under conditions of either high or low phytoplankton biomass. Triplicate 10 adult female acartiids were sorted and added to 5 treatments with varying concentrations of *Strombidium sulcatum* (5, 15, 30, 60, 100 ciliates ml⁻¹) and *Dunaliella* sp. (1911 to 3136 cells ml⁻¹) in 600 ml PC bottles. Five duplicates of controls without acartiids were prepared corresponding to each treatment. Other conditions were the same as in the first experiment.

At the end of the incubation, triplicate 1 ml aliquots and triplicate 10 ml aliquots were sampled from all of the experimental bottles and fixed with 5% acid Lugol's solution to determine the final concentration of algae and ciliates, respectively.

The final concentrations of ciliates in treatments (C_t) and controls (C_c) were then applied to calculate the grazing rates (G_c , d⁻¹) and clearance rates (F_c , ml ind.⁻¹ d⁻¹) by comparing the difference between controls and treatments according to equations of Frost (1972):

$$G_c = [\ln (G_c/G_t)] / t \quad (4)$$

$$F_c = G_c \times V/n \quad (5)$$

$$I_c = F_c \times \bar{C} \quad (6)$$

where t is the duration of the incubation, V is the volume of the incubation bottle, and n is the number of top predators in the bottles. The ingestion rates (I_c , ciliates ind.⁻¹ d⁻¹) on ciliates were calculated from the clearance rates (F_c) and mean concentrations of ciliates (\bar{C}) during the incubation.

Since the grazing mortality of algae in mesozooplankton treatments was caused by both meso- and microzooplankton, the traditional equation for grazing rate of mesozooplankton (Eq. 4), which ignores the cascading effect, is not accurate because the grazing rate (G_a) is not equal to the log-response of algae in the presence of mesozooplankton by directly comparing the difference in the algal net growth rates between controls ($k_c = k_{\text{intrinsic}} - g$) and predation treatments ($k_t = k_{\text{intrinsic}} - G_a - g'$; where g' is the ciliate grazing rate on algae in the presence of mesozooplankton). This was because a cascading effect induced by predation on ciliates caused a reduction of ciliate grazing rate in predation treatments ($g - g'$). Thus, the factor 'cascading effect' ($g - g'$) needs to be included in order to calculate the direct grazing rate of mesozooplankton on algae, according to the following equation:

$$G_a = k_c - k_t + g - g' = -[\ln (A_t/A_c)]/t + g - g' \quad (7)$$

where A_c and A_t are the final concentrations of algae in the controls and treatments, respectively. The ciliate grazing rates (g and g') can be estimated from the established standard equation (Eq. 3) obtained from the previous ciliate feeding experiments. The term $[\ln (A_t/A_c)]/t$ is the log-response of algae (the change in density) in the presence of mesozooplankton, which indicates the balance of the effects between direct grazing mortality and benefit from indirect trophic interactions:

$$[\ln (A_t/A_c)]/t = g - g' - G_a \quad (8)$$

When the cascading effect ($g - g'$) is greater than the direct grazing mortality (equal to the grazing rate of mesozooplankton on phytoplankton, G_a), the log response is positive, indicating an overall beneficial effect for algal density induced by the presence of a predator. When the log response is negative, predators directly cause a decrease in algal density.

Clearance rates and ingestion rates of mesozooplankton on algae were then calculated from the estimated grazing rates and the mean concentrations of algae, i.e. Eqs. (5) and (6), respectively. To allow a

comparison of mesozooplankton consumption on both prey and to calculate feeding selectivity, the cell-specific ingestion rates were transferred to carbon-specific ingestion rates based on factors obtained from CHN analysis. The feeding selectivity index (α) was calculated by comparing the frequency distribution of specific prey in the environment (mean concentrations) and in the diet (ingestion rates) (Chesson 1978). The index varies between 0 and 1, with $\alpha_i > 0.5$ indicating feeding preference and $\alpha_i < 0.5$ indicating avoidance. In this study, we used feeding selectivity to define feeding behavior. The degree of mesozooplankton carnivory and herbivory depends on the selectivity index of mesozooplankton on ciliates and algae, respectively (ranging from 0 to 1).

Data analysis

The data curve-fitting and single regression analyses between factors were conducted in Sigmaplot 11.0. Regression relationships between ingestion rate of ciliates and prey concentrations were analyzed by a Michaelis-Menten equation. Mean values between treatments were compared using independent *t*-tests. The assumption of homogeneity of variances was satisfied for independent *t*-tests by using Levene's test. The correlation between the cascading effect and the number of ciliates ingested by predators was tested by Pearson's correlation index. The normality of the data was confirmed by using a Kolmogorov-Smirnov test with SPSS 20 (Park 2008).

RESULTS

Functional response of ciliate ingestion rate to algal concentrations

When *Strombidium sulcatum* fed on *Dunaliella* sp., its ingestion rate increased hyperbolically with increasing prey mean concentrations (Fig. 1), suggesting a functional response of ciliate ingestion to changes in algal density. The positive relationship between ciliate ingestion rate and mean prey concentration was significant ($r^2 = 0.90$, $p < 0.0001$) when the data were fitted to a Michaelis-Menten equation. Thus, this data set served as a standard curve for ciliate ingestion rate for feeding experiments where ingestion rate was not easily directly measured. The estimated maximum ingestion rate (I_{\max}) and half-saturation concentration (K_d) determined from the standard equation were 38 cells ciliate⁻¹ d⁻¹ and 1093 cells ml⁻¹, respectively.

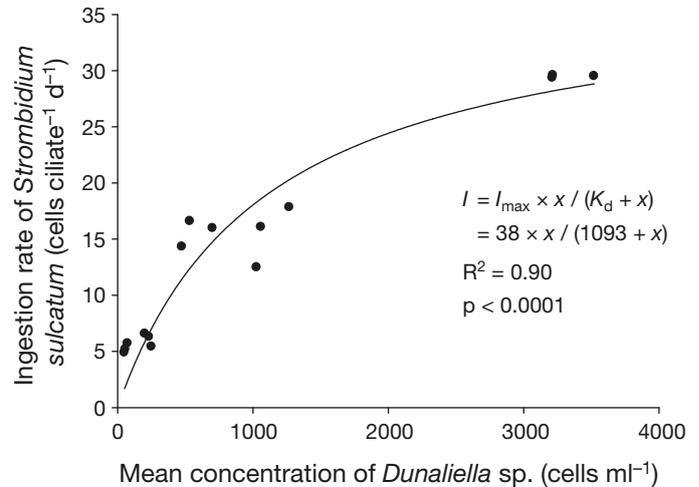


Fig. 1. Functional response of ciliate ingestion rate to prey concentration. Data are fitted by the Michaelis-Menten equation: $I = I_{\max} \times x / (K_d + x)$, where I (cells ciliate⁻¹ d⁻¹) is the ingestion rate of ciliates, I_{\max} is the maximum ingestion rate, and K_d is the half-saturated concentration

Clearance rate, ingestion rate, and selectivity index of different mesozooplankton

Clearance rates (Fig. 2A), ingestion rates (Fig. 2B), and selectivity indices (Fig. 3) of the 3 mesozooplankton species demonstrated clear differences in their feeding habits. The mean clearance rates of the 3 predators on ciliates ranged from 3 to 48 ml predator⁻¹ d⁻¹, while the rates on algae ranged from 7 to 17 ml predator⁻¹ d⁻¹. Comparing rates between the 2 prey, the mean clearance rate of *Parvocalanus crassirostris* on ciliates was 6-fold higher than that on algae (independent *t*-test, $t = 3.332$, $p = 0.029$, $df = 4$). *Temora turbinata* had similar clearance rates on the 2 prey (independent *t*-test, $p > 0.05$, $df = 4$), while *Penilia avirostris* had significantly higher clearance rates on algae than on ciliates (independent *t*-test, $t = 2.862$, $p = 0.046$, $df = 4$).

Mean carbon-specific ingestion rates of the 3 mesozooplankton predators ranged from 2.7 to 20.4 $\mu\text{g C predator}^{-1} \text{d}^{-1}$ on ciliates and from 3.9 to 6.8 $\mu\text{g C predator}^{-1} \text{d}^{-1}$ on algae. Similar to the pattern of clearance rates, the ingestion rates on ciliates were also significantly different among mesozooplankton. *Parvocalanus crassirostris* ingested a significantly higher amount of ciliates than *Temora turbinata* (independent *t*-test, $t = 3.024$, $p = 0.039$, $df = 4$) and *Penilia avirostris* (independent *t*-test, $t = 5.618$, $p = 0.005$, $df = 4$) did.

The selectivity indices were apparently different among the 3 species. Based on the index, *Parvocala-*

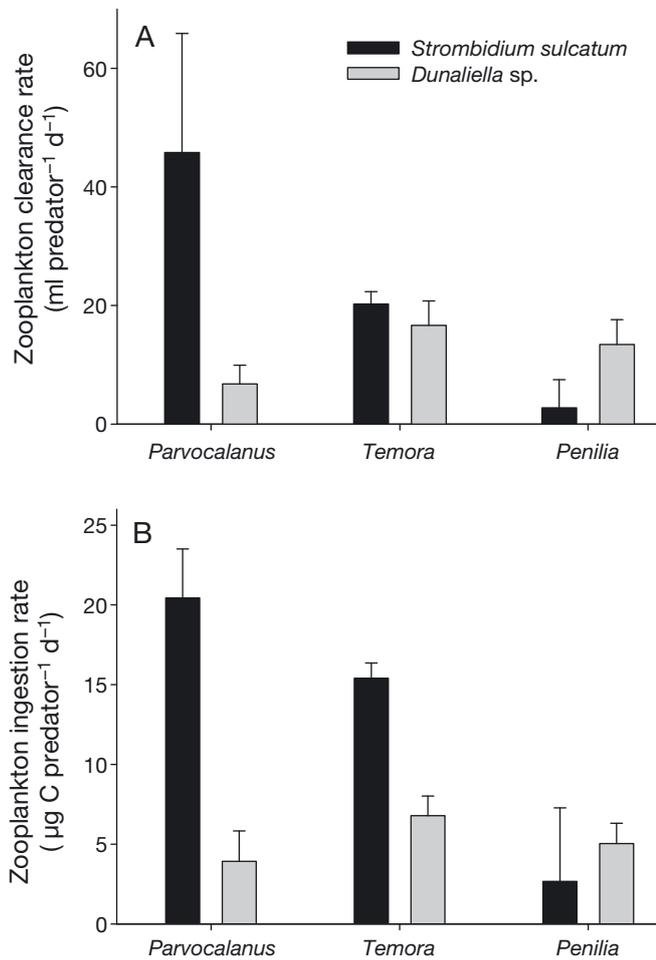


Fig. 2. (A) Clearance rates and (B) carbon-specific ingestion rates of different zooplankton (*Parvocalanus crassirostris*, *Temora turbinata*, *Penilia avirostris*) on the ciliate *Strombidium sulcatum* (black bars) and the green alga *Dunaliella* sp. (grey bars). Error bars show 1 SD among triplicate treatments. Cellular carbon contents of *S. sulcatum* and *Dunaliella* sp. were 20.25 and 0.15 ng C cell⁻¹, respectively

crassirostris significantly preferred ciliates to algae (independent *t*-test, $t = 79.196$, $p < 0.001$, $df = 4$), indicating that this species had a higher degree of carnivory and hence a higher ingestion rate on ciliates than the other 2 species. *Temora turbinata* did not show a significant preference for each prey, as the selectivity indices on ciliates and on algae were similar, indicating that this species was a passive omnivorous feeder whose ingestion rates were determined by prey availability. On the other hand, *Penilia avirostris* significantly preferred algae to ciliates (independent *t*-test, $t = 5.215$, $p = 0.006$, $df = 4$), suggesting that it was an herbivorous species which is not able to feed on ciliates effectively.

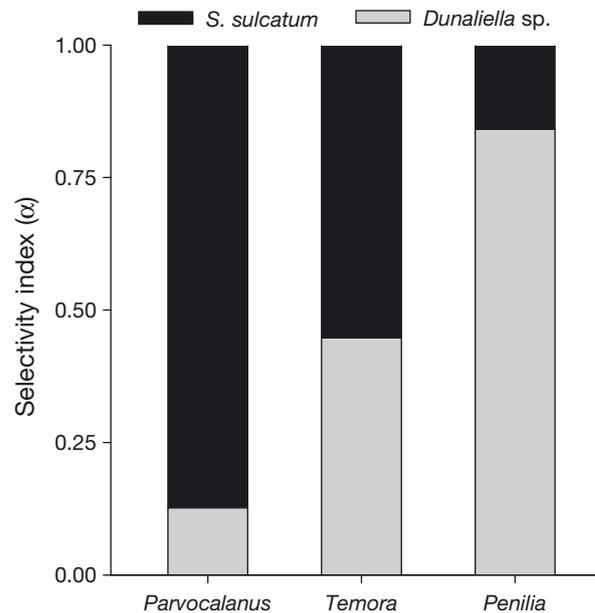


Fig. 3. Selectivity indices (α) of 3 mesozooplankton species feeding on the ciliate *Strombidium sulcatum* (black bars) and the green alga *Dunaliella* sp. (grey bars). The selectivity index was calculated by comparing the frequency distribution of specific prey in the environment and in the diet (Chesson 1978). An index value > 0.5 indicates feeding preference

Cascading effect of different mesozooplankton

The direct and indirect effects of mesozooplankton on algae were measured by grazing mortality and the net rate of reduced microzooplankton grazing (Fig. 4), respectively. Based on different net effect, which is the balance between the cascading effect and the direct grazing mortality (negative form of mesozooplankton grazing rate), the 3 mesozooplankton species played significantly different roles in changing the algal density. The cascading effect induced by *Parvocalanus crassirostris* on *Dunaliella* was 2-fold higher than the direct grazing mortality. Thus, this species caused an overall increase in algae with a rate of 0.2 d⁻¹. In contrast, *Penilia avirostris* caused a strong decline in algae by direct grazing and induced no indirect increase in algae. The small negative cascading effect was probably due to the change in the functional response of the ciliate ingestion rate to algal density through a competition effect. *Temora turbinata* caused a small overall decline in algae density because of the relatively high clearance rate on algae, although a moderate trophic cascading effect was induced.

Overall, the algal log response to mesozooplankton and the cascading effects were significantly corre-

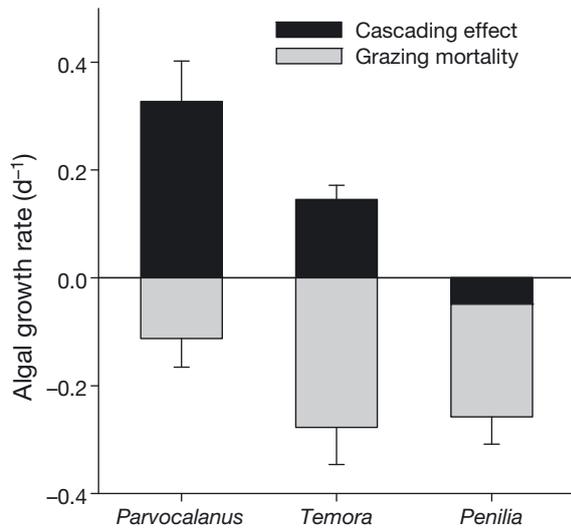


Fig. 4. Effect of different zooplankton on algae, expressed as the rate of algal growth. Grazing mortality (grey bars) caused the decrease on algal growth rate, while cascading effects (trophic cascades) induced by feeding on intermediate grazers (black bars) caused the increase on algae. Error bars indicate 1 SD among triplicates

lated with feeding selectivity indices (Fig. 5), and were positive with the selectivity index on ciliates and negative with the index on algae. These results indicated that the different ability of different mesozooplankton species to affect algal density was determined by their feeding behaviors.

Ingestion rate and cascading effect of *Acartia erythraea* with varying ciliate concentrations

Compared to the other 3 species at similar prey concentrations, the ingestion rate of *A. erythraea* was similar to *Parvocalanus crassirostris*, which had a high selectivity index on ciliates. The mean ingestion rate of *A. erythraea* on ciliates (ranging from 8.2 to 28.0 $\mu\text{g C predator}^{-1} \text{d}^{-1}$) increased hyperbolically with increasing ciliate concentrations (Fig. 6), while the rates on algae decreased towards 0. Results indicated that *A. erythraea* can shift its diet composition based on food availability and composition. It had a high degree of carnivory, with a feeding selectivity index on ciliates >0.75 when ciliates were abundant.

Corresponding to the increase in ingestion rates with increasing ciliate concentrations, the cascading effect induced by *Acartia erythraea* was also significantly increased, ranging from 0.16 to 0.55 d^{-1} (Fig. 7). Direct grazing mortality of algae (0–0.33 d^{-1}) was overall lower than the rate of the cascading

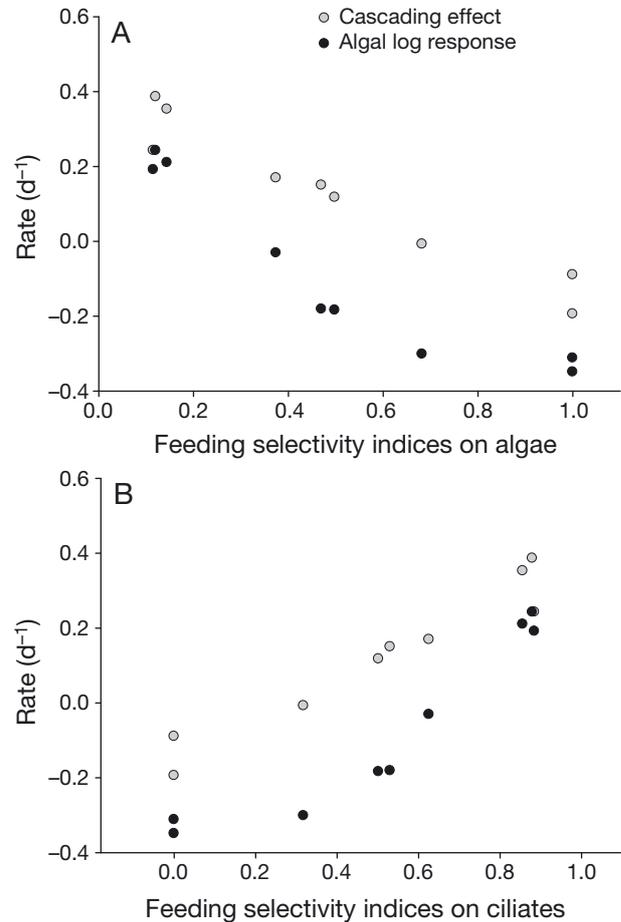


Fig. 5. Relationships between crustacean zooplankton feeding selectivity indices and the rates of algal log response (black circles) and cascading effect (grey circles): (A) rates vs. selectivity index on algae; (B) rates vs. selectivity index on ciliates. Linear regression tests: $R^2 = 0.935$, $p < 0.0001$ (cascading effect) and $R^2 = 0.873$, $p = 0.0002$ (algal log response)

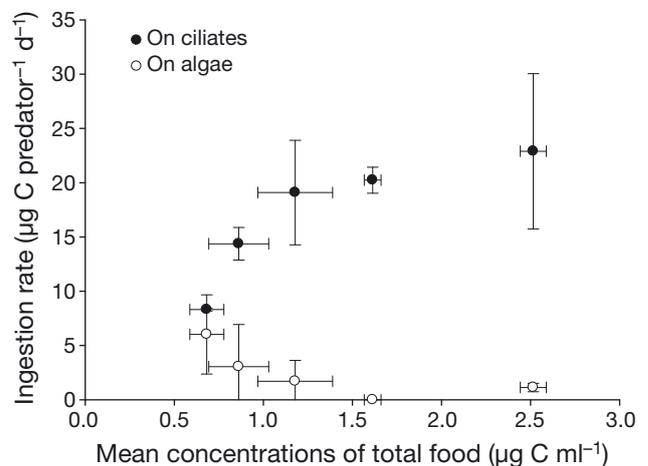


Fig. 6. *Acartia erythraea*. Ingestion rates on ciliates (filled circles) and algae (open circles) with varying initial ciliate concentrations but constant algal concentration. Bi-directional error bars indicate SD among triplicates

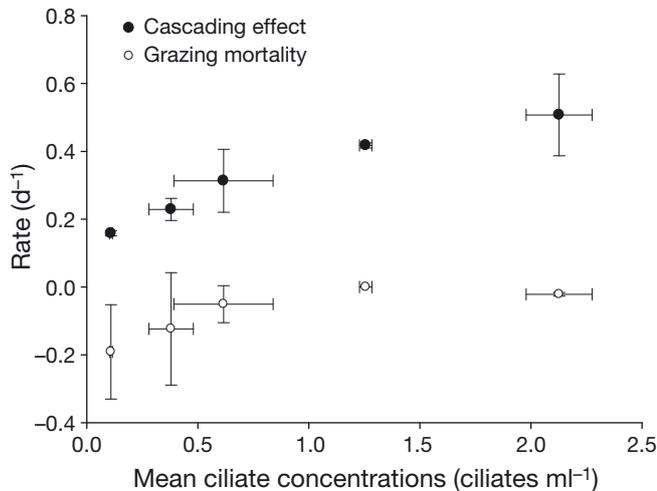


Fig. 7. *Acartia erythraea*. Cascading effect (filled circles) and grazing mortality (open circles) caused by *A. erythraea* feeding on algae at varying concentrations of ciliates. Bi-directional error bars indicate SD among triplicates

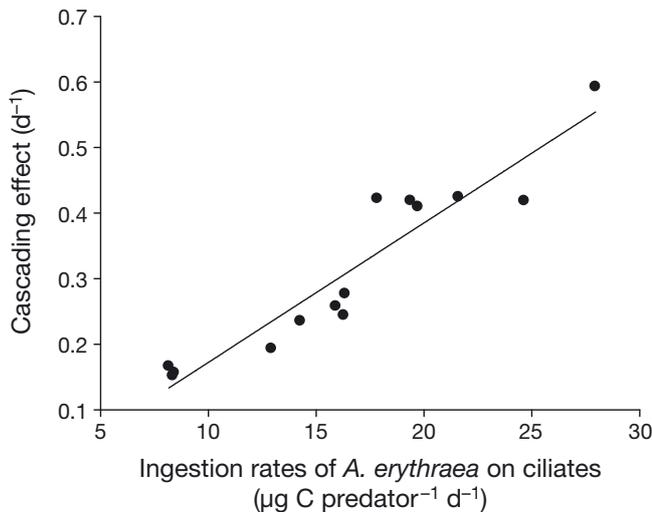


Fig. 8. *Acartia erythraea*. Relationship between the cascading effect on algal prey and copepod ingestion rate on ciliates. Linear regression: $R^2 = 0.888$, $p < 0.0001$

effect (Fig. 7). Overall, the net effect (algal log response) of this species on algal density change was positive, except for the treatment with the lowest ciliate concentration in which algae contributed a significant part of the diet of *A. erythraea* causing a decrease of algal density. A strong positive correlation between a cascading effect and the number of ciliates ingested by *A. erythraea* (Fig. 8) suggested that the strength of the trophic cascade was enhanced by the predator's carnivorous feeding behavior.

DISCUSSION

Because of the extra link of mesozooplankton–microzooplankton in the interface between mesozooplankton and phytoplankton, the relationship among components of marine planktonic food webs is complex, consisting of both direct and indirect interactions (Calbet & Landry 1999). As one of the major indirect interactions, the increase in phytoplankton biomass through reduced grazing of microzooplankton induced by mesozooplankton predation on the microzooplankton has been generally overlooked in previous studies because of the previously undetermined change in the ciliate functional response to algal density, as well as the difficulty of direct quantification of such a change. By differentiating the microzooplankton grazing rates between controls (no mesozooplankton) and treatments (in the presence of mesozooplankton), which were calculated based on a standard equation established from additional ciliate feeding experiments, this study demonstrated that different mesozooplankton species were able to induce a significantly different magnitude of trophic cascade. Several studies have also made efforts to solve the underestimation of mesozooplankton feeding rate due to trophic cascades (Nejstgaard et al. 2001, Liu et al. 2005a,b, Klaas et al. 2008, Chen & Liu 2011). Chen & Liu (2011) demonstrated that the cascading effect induced by copepods (via predation on heterotrophic dinoflagellates) can be as strong as, or even greater than, the direct grazing rates on diatoms, resulting in an insignificant net effect or positive effect on diatoms. Their simulations, together with the findings of our study, suggest that strong indirect cascading effects on phytoplankton induced by copepods through predated on microzooplankton (heterotrophic dinoflagellates and ciliates) is one of the reasons for the weak control of phytoplankton biomass by mesozooplankton. These results support the notion that the presence of intermediate trophic levels and the complex interactions among marine planktonic food webs are the most important biological factors that weaken the transmission of top-down control from higher trophic levels to phytoplankton (Sommer 2008).

One of our key results is that the type of mesozooplankton species determines the strength of the cascading effect in marine planktonic food webs and simultaneously determines the net effect of mesozooplankton on controlling algal density. The mechanism of the indirect trophic interaction observed in this study was primarily mediated by predator dietary habits. The nature of the cascading effect

related to predator identity and its feeding behaviors (hunting mode) have also been explicated in other systems, such as streams and ponds (reviewed by Schmitz et al. 2004). The 3 mesozooplankton species used in this study are primarily suspension-feeders with different abilities to capture motile and large prey (e.g. Turner et al. 1988, Gismervik 2006). We found significant relationships between the rates of the cascading effect or log response of algal density and the feeding habits of mesozooplankton expressed as a selectivity index (Fig. 5). These relationships are consistent with the study of Chen & Liu (2011), who found that trophic cascades increased with increasing feeding preference of copepods on heterotrophic dinoflagellates.

It is easy to understand why strong cascading effects induced by predators are a result of high levels of carnivory. However, dominant species of coastal mesozooplankton are purely carnivorous. Many species are more or less omnivorous and different species have different degrees of carnivory. Thus, the top-down effect of a predator or a diverse zooplankton assemblage depends primarily on the degree of carnivory. Based on its higher clearance rate, ingestion rate, and the feeding selectivity index on ciliates compared to those on algae, *Parvocalanus crassirostris* had the highest degree of carnivory among the 3 experimental species when it was fed with a mixture of ciliates and green algae. Although they were not solely carnivorous predators, their presence caused an apparent net increase in algal biomass (Fig. 4). Whether suspension feeders can cause such an apparent trophic cascading effect is primarily due to size-selective feeding. In our experiments, the sizes of the 2 prey were strikingly different, so that ciliates were strongly selected because of the larger size. In natural seawater, such an indirect increase should be restricted mainly to small particles since most marine calanoid copepods primarily select their food based on size, and large particles (>20 μm), including both heterotrophs and autotrophs, are directly controlled by mesozooplankton assemblages dominated by such copepods (Calbet & Landry 1999, Liu et al. 2005a). Thus, size-selective feeders generally produce 2 distinct and opposite top-down controls on small and large phytoplankton (Stibor et al. 2004, Sommer & Sommer 2006). Therefore, the net effect of the mesozooplankton assemblage on the total phytoplankton community depends on the balance between the consumption rate on large phytoplankton and indirect increase of small phytoplankton. For example, Dagg et al. (2009) reported that dominant copepods generally consumed

a minor proportion of the total phytoplankton production, but their high clearance rates on large phytoplankton and a simultaneous strong trophic cascade on small phytoplankton led to the dominance of small phytoplankton in the subarctic Pacific Ocean.

The feeding selectivity indices of *Temora turbinata* for its 2 prey were similar (Fig. 3), indicating that the feeding size spectrum of food items in this species was broad, and hence it was a typical omnivorous species. Compared to *Parvocalanus crassirostris*, *T. turbinata* exerted a lower cascading effect and higher direct grazing mortality on algae. Overall, this species caused a decline in algal density because its direct grazing rate exceeded the cascading effect (Fig. 5). The results indicated that high omnivory could mask the net role of predators on the dynamics of primary producers, since the grazing pressure from intermediate grazers was partially released, but the direct grazing pressure from top predators increased. At the same time, the abundance of intermediate grazers will eventually be determined by both food competition and predation due to omnivorous predators.

In contrast, due to extremely low consumption on ciliates, the marine cladoceran species *Penilia avirostris* in our experiments caused no indirect increase in algae but a decline of algal density. Although the top-down controls of marine cladocerans on the prey community has received much less attention because of their restricted distribution (e.g. coastal regions with a low level of eutrophication), previous studies suggested that marine cladocerans primarily affect small particles through suspension feeding and they lack the ability to capture large particles, especially motile protists (Turner et al. 1988, Atienza et al. 2006). Therefore, they play a remarkably different role in a marine planktonic food web compared to copepods that prefer large particles. Nevertheless, the prey size spectrum of marine cladocerans differs from that of freshwater cladocerans (e.g. *Daphnia*) and is rather narrow with a lower size limit >2 μm , and thus they are inefficient at ingesting picoplankton and bacteria (Katechakis & Stibor 2004, Atienza et al. 2006). Instead, marine cladocerans may cause indirect positive effects on pico-sized particles by exerting strong top-down controls on heterotrophic flagellates (Turner et al. 1988, Atienza et al. 2006).

In addition, a small 'negative' cascading effect on algal density was observed in the experiment with *Penilia avirostris*. We believe that such a phenomenon is the result of food competition between the predator and the intermediate grazer when prey concentration is low (Chen & Liu 2011). A competition

effect may occur when the abundance of producers is reduced to a critical level by both intermediate grazers and predators. Under such a condition, the intermediate grazers quickly respond and increase their clearance rate relative to the control with no top predators.

Another mechanism causing an increase in the cascading effect is the change in the feeding mode to ambush feeding from suspension feeding of some mesozooplankton species. Our results showed that a switch in prey behavior of marine copepods in responding to food composition could significantly influence both direct and indirect trophic interactions. The primary reason is that the feeding efficiency of consumers is a functional response to the change in prey densities. We observed a significant increase in copepod ingestion rate on ciliates in responding to an artificial increase in ciliate abundance, while ingestion rate on algae was significantly reduced (Fig. 6). Correspondingly, such a behavior-mediated switch in prey resulted in an increase in the cascading effect and a decrease in the grazing mortality on algae (Fig. 7). Such a behavior-mediated indirect effect plays a very important role in stabilizing the base trophic level by avoiding an over-cropping by intermediate grazers. The ability of marine copepods to switch prey in response to prey composition has been demonstrated by numerous studies (e.g. Landry 1981, Kiørboe et al. 1996, Gismervik 2006), while the implication to change trophic interactions and stabilize ecosystems has not received much attention. The hypothesis that predators feeding on 2 or several trophic levels may cause opposite trophic effects that stabilize the ecosystem has been proposed by previous studies including experimental demonstrations and theoretical simulations (e.g. Moran et al. 1996, Schmitz et al. 1997, Persson 1999). In marine systems, the prey-switching behavior of dominant copepods may provide a mechanism to protect prey species of low density, while simultaneously suppressing the bloom of dominant species (Kiørboe et al. 1996). However, the indirect effect of acartiids seems to be generally positive for algae even when algae are much more abundant than the intermediate grazers because of nutritionally-driven feeding selectivity of copepods towards heterotrophic protozoans (Chen & Liu 2011). Hence, we may also need to consider food quality as a potential factor influencing the trophic interactions (Mackay & Elser 1998).

Our simplified laboratory simulation represents a particular situation in the natural environment. In natural seawater, the mesozooplankton community is dominated by 1 or several species during a particular

season or at a location. Since the type of mesozooplankton and their flexible feeding behavior determine how an individual predator influences the dynamics of prey populations, the top-down effect of an assemblage depends on its dominant feeding preference and prey composition. For instance, when the assemblage of mesozooplankton is dominated by species such as *Acartia* which has a high feeding selection on microzooplankton, the cascading effect will be strong and hence favor the growth of small algae that are not grazed by mesozooplankton. On the other hand, when marine cladocerans such as *Penilia* dominate the assemblage, the grazing pressure on small algae will be intense.

Overall, mesozooplankton are so diverse that both direct and indirect controls of mesozooplankton on the phytoplankton community will be dampened by increasing the diversity of feeding behavior and selectivity because various feeding behaviors will broaden the whole feeding size spectrum of predators. Through complex trophic interactions, a highly diverse assemblage of grazers (with multiple feeding behaviors in a functional group) may have a null effect on the biomass of the lower trophic level, as direct consumption is balanced by indirect effects of predation. Due to the increased functional redundancy and weakened trophic effect, increasing the diversity of predator feeding behavior will consequently stabilize the system because the niche of an extinct species will soon be able to be occupied by others (Steiner et al. 2005). Such a stabilization effect of predators with diverse feeding behaviors has also been studied in various systems, especially when predators engage in omnivory (Bruno & O'Connor 2005), cannibalism, or intraguild predation (Finke & Denno 2004). On the other hand, if the grazer community is dominated by a special group with apparent feeding selectivity, a cascading effect will certainly be induced in favor of algal populations that possess a strong defense against predation (such as chain-forming algae or toxic species) and they will ultimately be dominant in the prey assemblage. In contrast, prey that are preferred by predators will be vulnerable to grazing and eventually become extinct. Consequently, a shift in the prey community structure is caused by the domination of mesozooplankton species.

CONCLUSIONS

The interaction between mesozooplankton and their algal prey is complex due to the presence of intermediate grazers, which are also important food

for mesozooplankton. Mesozooplankton feeding on the intermediate grazers can indirectly increase the density of algae and enhance the complexity in marine planktonic food webs. The strength of the cascading effect is dependent on the feeding modes of the predators, more specifically, the ability and efficiency of predators consuming the intermediate grazers. In marine copepods and cladocerans, size-selective feeding and prey switch feeding behavior leads to different levels of the indirect effects, and such a behavior is probably driven by nutritional requirements. Because different species have a different effect on prey density (both direction and strength), the net effect of a mesozooplankton assemblage on a phytoplankton community depends on its dominant feeding preference. Our results imply that mesozooplankton omnivory and the increasing diversity of feeding behavior will dampen the effect of predation on the lower trophic levels.

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