

Increasing zooplankton size diversity enhances the strength of top-down control on phytoplankton through diet niche partitioning

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Summary

1. The biodiversity-ecosystem functioning debate is a central topic in ecology. Recently, there has been a growing interest in size diversity because body size is sensitive to environmental changes and is one of the fundamental characteristics of organisms linking many ecosystem properties. However, how size diversity affects ecosystem functioning is an important yet unclear issue.

2. To fill the gap, with large-scale field data from the East China Sea, we tested the novel hypothesis that increasing zooplankton size diversity enhances top-down control on phytoplankton (H1) and compared it with five conventional hypotheses explaining the top-down control: flatter zooplankton size spectrum enhances the strength of top-down control (H2); nutrient enrichment lessens the strength of top-down control (H3); increasing zooplankton taxonomic diversity enhances the strength of top-down control (H4); increasing fish predation decreases the strength of top-down control of zooplankton on phytoplankton through trophic cascade (H5); increasing temperature intensifies the strength of top-down control (H6).

3. The results of univariate analyses support the hypotheses based on zooplankton size diversity (H1), zooplankton size spectrum (H2), nutrient (H3) and zooplankton taxonomic diversity (H4), but not the hypotheses based on fish predation (H5) and temperature (H6). More in-depth analyses indicate that zooplankton size diversity is the most important factor in determining the strength of top-down control on phytoplankton in the East China Sea.

4. Our results suggest a new potential mechanism that increasing predator size diversity enhances the strength of top-down control on prey through diet niche partitioning. This mechanism can be explained by the optimal predator-prey body-mass ratio concept. Suppose each size group of zooplankton predators has its own optimal phytoplankton prey size, increasing size diversity of zooplankton would promote diet niche partitioning of predators and thus elevates the strength of top-down control.

Key-words: biodiversity-ecosystem functioning, body size, size spectrum, taxa diversity, trophic interactions

Introduction

Understanding the basis of trophic structure and interactions has long been a central issue in ecology (McQueen,

Post & Mills 1986; Brett & Goldman 1997; Nicolle *et al.* 2011; Llope *et al.* 2012). The prevailing conceptual framework for understanding trophic structure is largely based on bottom-up (resource) and top-down (predation pressure) control principles (McQueen, Post & Mills 1986; Brett & Goldman 1996, 1997). The factors influencing

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variation of trophic interactions in aquatic ecosystems have been extensively studied. Some well-studied examples include the following: (i) the effects of nutrient enrichment (McQueen, Post & Mills 1986; Davis *et al.* 2010); (ii) trophic cascade phenomenon (Brett & Goldman 1996); (iii) the taxonomic diversity of predators (Duffy, Richardson & Canuel 2003; Jaschinski *et al.* 2009); (iv) and the role of temperature (Brown *et al.* 2004; Berlow *et al.* 2009). Both bottom-up and top-down controls have been found to affect trophic structure (Brett & Goldman 1997; Llope *et al.* 2012); however, their relative strength may vary across different ecosystems and time (Jeppesen *et al.* 2003). Further investigations on factors and mechanisms determining trophic structure and interactions are still necessary for a more complete understanding of the structure and function of ecosystems.

Recently, there has been a growing interest that organismal functional traits may play a critical role in affecting species interactions and other ecological processes (Wood *et al.* 2010; Flynn *et al.* 2011; Schneider, Scheu & Brose 2012). Body size may be one of the most important functional traits because it can affect the structure, stability and dynamics of food webs (Woodward *et al.* 2005; Brucet *et al.* 2006; Kohda *et al.* 2008; Barnes *et al.* 2010; Stouffer, Rezende & Amaral 2011; Heckmann *et al.* 2012). The importance of size settles on allometric relationships and size-based trophic dynamics that have been described in the early-mid-twentieth century (Elton 1927; Kleiber 1932; Platt & Denman 1977). Moreover, the increased attention devoted to research on size structure indicates that the size structure of phytoplankton, zooplankton and fishes in aquatic systems has been significantly affected by climate and human effects (such as fishing and eutrophication) (Daufresne, Lengfellner & Sommer 2009; Beaugrand, Edwards & Legendre 2010; Brucet *et al.* 2010; Genner *et al.* 2010; Morán *et al.* 2010; Emmrich *et al.* 2011; Yvon-Durocher *et al.* 2011). These size-based responses have even been suggested as the third universal response to global warming (Daufresne,

Lengfellner & Sommer 2009) and have led to investigations of how changes in size structure affect ecosystem functions (Ingram, Stutz & Bolnick 2011; Brose *et al.* 2012; Rudolf 2012; Toscano & Griffen 2012).

The slope of the normalized biomass size spectrum (NBSS) and size diversity are the two major metrics used to measure community size structure (Brown & Gillooly 2003; Quintana *et al.* 2008; Brucet *et al.* 2010; Yvon-Durocher *et al.* 2011). The slope of the NBSS represents the relative distribution of individual sizes; a flatter slope indicates a greater relative contribution of large organisms to the total biomass of the community (Brown & Gillooly 2003; White, Enquist & Green 2008; Yvon-Durocher *et al.* 2011). As larger-bodied zooplankton are more efficient at capturing their food (Hall & Threlkeld 1976), we hypothesized that flatter slopes of zooplankton NBSS (high ratio of large zooplankton) represent stronger top-down control on phytoplankton biomass. The other index, size diversity, was adapted from the Shannon diversity expression to quantify the continuous size distribution in a community (Brucet *et al.* 2006; Quintana *et al.* 2008) but has attracted relatively little attention in the ecological literature. Empirical evidence indicates the existence of optimal predator–prey size ratios in size-structured food webs (Barnes *et al.* 2010). According to the optimal-foraging theory, the optimum body-mass ratio determines the maximum attack rate of the consumers (Brose 2010). There is also empirical evidence showing that predators with a similar size have a similar diet range in a system disregarding their taxonomic classification (e.g. Poulet 1977; Berggreen, Hansen & Kiørboe 1988; Brucet *et al.* 2006), although exception exists (e.g. Boersma 1995; Compte *et al.* 2009). Based on size-based trophic selection, we formulate a hypothesis depicted as a conceptual diagram in Fig. 1. In our hypothesis, the community with low-size diversity can only forage on a rather narrow diet niche breadth (Fig. 1a); by contrast, the community with high-size diversity can have a rather evenly and widely distributed diet niche breadth (Fig. 1b,c). As such, we

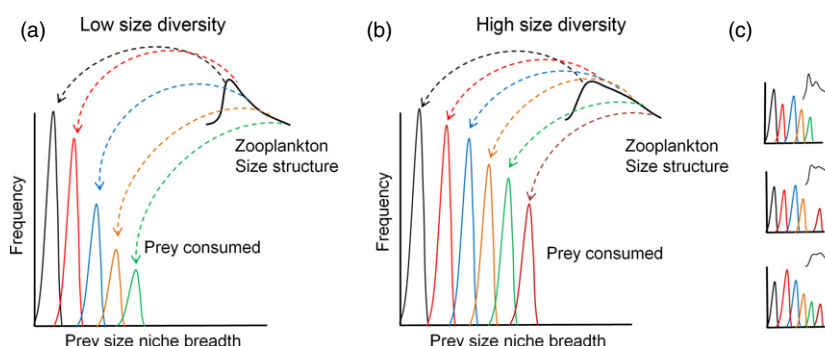


Fig. 1. Conceptual diagram summarizing our hypothesis: the mechanism of increasing predator size diversity enhancing the strength of top-down control on prey through diet niche partitioning. Panel (a) indicates that the community with a low-size diversity can only forage on a rather narrow diet niche breadth; by contrast in panel (b), the community with a high-size diversity can have a rather evenly and widely distributed diet niche breadth. The dash line represents conceptually the predation pressure of a given size class. Panel (c) shows other examples of increased size diversity with respect to panel (a).

propose this new hypothesis predicting that increasing diversity of predator size structure enhances the strength of top-down control in aquatic ecosystems.

We tested our hypothesis using plankton collected from the East China Sea (ECS), a region characterized by high spatial variation of nutrient availability, temperature conditions and plankton community composition (Gong *et al.* 2003; Wu, Shin & Chiang 2011). Plankton, at the base of aquatic food webs, represent an essential linkage in the trophic architecture of aquatic ecosystems (Llope *et al.* 2012). The nature and strength of interaction between primary producers and grazers determine many important ecosystem properties, such as ecosystem stability, carbon and nutrient recycling and accumulation, and ecosystem productivity (Gaedke & Straile 1994; Davis *et al.* 2010). Thus, understanding the factors and mechanisms determining planktonic trophic structure will improve our ability to predict the potential consequences of anthropogenic and environmental perturbations on the structure and function of aquatic ecosystems.

In this study, we tested a new hypothesis that zooplankton size diversity determines the trophic structure of zooplankton and phytoplankton in the ECS. In addition, we tested five conventional hypotheses based on zooplankton NBSS slope, nutrients, zooplankton taxa diversity, fish predation and temperature. Specifically, we tested the following hypotheses. H1: increasing size diversity of zooplankton enhances the top-down control of zooplankton over phytoplankton; H2: flatter zooplankton NBSS slope enhances the strength of top-down control (Hall & Threlkeld 1976); H3: nutrient enrichment lessens the strength of top-down control because it can weaken the grazing effect by increasing the dominance of predator-resistant prey (Davis *et al.* 2010); H4: increasing zooplankton taxa diversity enhances the strength of top-down control (Jaschinski *et al.* 2009); H5: increasing fish predation is linked to decreasing the strength of top-down control of zooplankton on phytoplankton as described by trophic cascade theory (Brett & Goldman 1996); H6: increasing temperature intensifies predator–prey interactions and thus strengthens top-down interactions through metabolic enhancement (Brown *et al.* 2004; Berlow *et al.* 2009).

Materials and methods

FIELD SAMPLING AND SAMPLE TREATMENTS

Zooplankton samples were collected in the East China Sea between 2006 and 2009, using a 330- μm mesh net with a mouth of 160 cm diameter (detailed in Fig. S1, Supporting Information). Zooplankton samples were digitized to extract the size information (e.g. body width and length) utilizing the ZooScan integrated system, and the scanned images were manually sorted into 24 taxonomic groups (Ye, Chang & Hsieh 2011). Zooplankton taxonomic diversity was computed as the Shannon diversity index from the 24 taxonomic groups. The biovolume of each zooplank-

ter was estimated as an ellipsoid using the length and width measured by the ZooScan integrated system. In total, we measured 134 156 zooplankton individuals for 126 samples belonging to 38 stations (Table S1).

The environmental variables, including water temperature, nitrate (NO_3), phosphate (PO_4), silicate (SiO_3), and chlorophyll-*a* concentrations, were sampled and analysed according to the standard methods (Fig. S1) (Gong *et al.* 2003, 2011). Each of these environmental variables was averaged over the depth that is consistent with the depth of zooplankton-net tows at each station. Fish larvae were obtained from the same 330- μm mesh net as the zooplankton samples and counted under a dissecting microscope. Fish larvae density was used as a proxy of predatory pressure on zooplankton (Ohman & Hsieh 2008), because population size of adult fish was not available. The fish larvae density was log10 transformed to achieve normality for the following analyses. Nitrate, phosphate and silicate were used to indicate nutrient status (San Martin *et al.* 2006).

SIZE STRUCTURE

For each sample, the zooplankton NBSS slope was estimated by the exponent of power-law frequency distributions, with a normalized logarithmic binning method (White, Enquist & Green 2008). The size diversity of zooplankton was calculated from the individual biovolume data (in the unit of μm^3) generally following the nonparametric method (Quintana *et al.* 2008), but without data standardization. We chose not to standardize the diversity to avoid creating an artificial correlation between size diversity and zooplankton total biomass (see justification in Data S1). The size diversity index (μ) is the continuous analogue of the Shannon diversity that is commonly used for species diversity. This measure takes the form of an integral involving the probability density function of the size distribution described by the following equation (Eqn.1):

$$\mu = - \int_0^{+\infty} p_x(x) \log_2 p_x(x) dx \quad \text{eqn 1}$$

Where $p_x(x)$ is the probability density function of size x . The probability density function used here followed the nonparametric kernel estimation (Brucet *et al.* 2010), while the optimal bandwidth in kernel estimation was determined according to Botev, Grotowski & Kroese (2010).

STRENGTH OF TOP-DOWN CONTROL

The zooplankton/phytoplankton biomass ratio was used as a proxy for estimating the strength of top-down predation pressure of zooplankton on phytoplankton (Jeppesen *et al.* 2003; Shurin & Seabloom 2005), as commonly used in research on trophic cascades (Brett & Goldman 1996). The zooplankton/phytoplankton biomass ratio was log10 transformed to achieve normality for the following analyses. Chlorophyll-*a* concentration was used as a proxy for phytoplankton biomass (Brucet *et al.* 2009). The dry weight of zooplankton and phytoplankton was estimated with conversion factors from the zooplankton biovolume and chlorophyll-*a* concentration, respectively (Brucet *et al.* 2009). Note that the conversion factors used in this study have no effects on analyses, as the same conversion was applied in all stations.

STATISTICAL ANALYSIS

To investigate the factors determining spatiotemporal variation of planktonic trophic structure in the ECS, we used the generalized linear mixed-effects model (GLMM) (Bolker *et al.* 2009). In all cases, the zooplankton/phytoplankton biomass ratio was considered as the response variable, and sampling cruises were included as the random-effect accounting for variation among cruises, such as seasonal effects. Including cruises as a random-effect variable removes the possibility that any relationship simply arises because of variation across seasons while using data from all cruises for the sake of increasing sample size. First, we tested the main hypothesis that zooplankton size diversity may affect the trophic structure of plankton (log10 transformed zooplankton/phytoplankton biomass ratio). Zooplankton size diversity was used as the fixed-effect variable in GLMM, considering random effects on the intercept and slope in modelling. Likewise, we tested additional hypotheses by coding the NBSS slope, nutrient (nitrate, phosphate or silicate), zooplankton taxa diversity, larval fish density and water temperature as the fixed-effect variable, respectively. Here, GLMMs were implemented using the lme4 library (Bates & Bolker 2011) in R (R Development Core Team 2011). The Markov chain Monte Carlo (MCMC) sampling method was used to estimate the *P*-value of GLMM with the MCMCglmm library (Hafield 2010) in R.

To further evaluate the possible combined effects of multiple factors on the zooplankton/phytoplankton biomass ratio, we searched for the best possible model using forward stepwise selection by considering all factors as the potential fixed-effect parameters. The best model was determined based on the Akaike's information criterion (AIC) (Akaike 1974). Changes of 10 units

or more in AIC values ($\Delta\text{AIC} \geq 10$) are generally considered as a significant improvement in the fitting of GLMMs for ecology and evolution studies (Bolker *et al.* 2009).

Moreover, to show the robustness of our results, we also analysed our data for each cruise separately for the relationship between zooplankton/phytoplankton biomass ratio and the selected variables to test our hypotheses. In these cruise-based analyses, we additionally included a spatial error model to partition out the spatial autocorrelation before making statistical inference. This spatial regression analysis was carried out using Open GeoDa (Ver. 1.0.1, available at <http://geodacenter.asu.edu/ogeda>) (Anselin, Syabri & Kho 2005). The summary statistics of zooplankton/phytoplankton biomass ratio, zooplankton size diversity, NBSS slope, zooplankton Shannon diversity, fish larvae density and the environmental variables are listed in the Table S2.

Results

Results of GLMMs support our main hypothesis that increasing size diversity of zooplankton enhances the strength of top-down control on phytoplankton (H1) and reveal that zooplankton size diversity is the most important factor determining the spatiotemporal dynamics of zooplankton/phytoplankton biomass ratio in the ECS (Fig. 2, Table 1). The model with zooplankton size diversity exhibited the best goodness of fit to explain the variation of plankton trophic structure ($\text{AIC} = 182.1$) and was significantly superior to the other models ($\Delta\text{AIC} \geq 22$). The hypotheses based on zooplankton size spectral slope (H2), nutrients (H3) and zooplankton taxonomic diversity

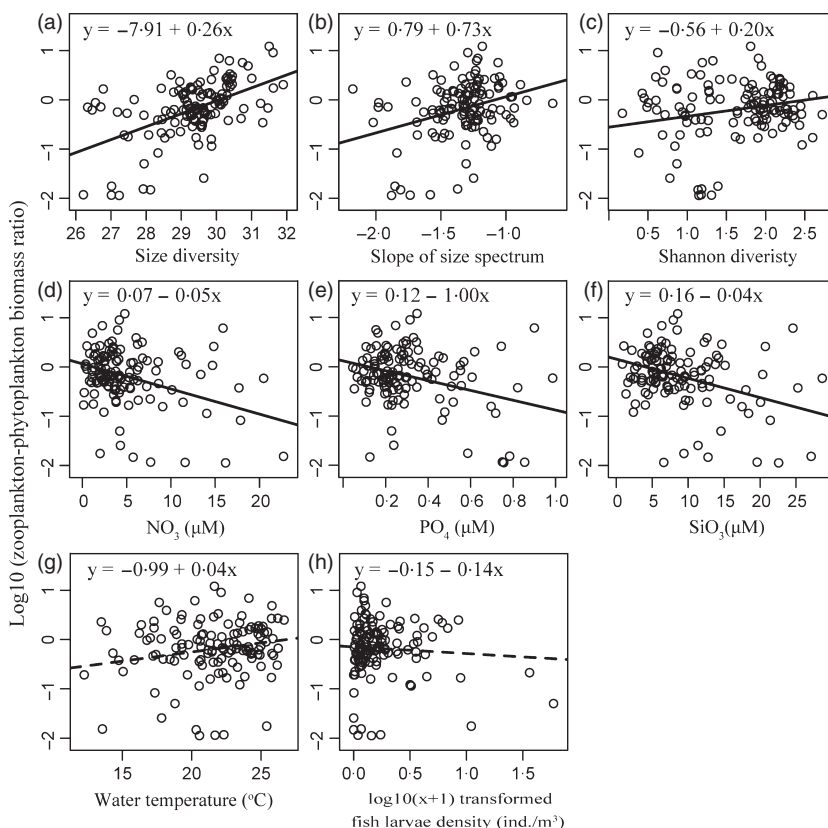


Fig. 2. Scatter plots illustrating the relationship between log10 transformed zooplankton-phytoplankton biomass ratio versus (a) zooplankton size diversity, (b) normalized biomass size spectrum (NBSS) slope of zooplankton, (c) zooplankton Shannon diversity, (d) NO_3^- , (e) PO_4^- , (f) SiO_3^- , (g) water temperature, and (h) log10 ($x + 1$) transformed fish larvae density in the East China Sea. The trend line represents the best-fit line according to generalized linear mixed-effects models (GLMMs) (Table 1). The solid line represents a significant ($P < 0.05$) and the dashed line represents nonsignificant ($P > 0.05$) regression.

Table 1. Results of the generalized linear mixed-effect model on investigating the effect of each factor on the spatiotemporal dynamics of plankton trophic structure (log10 transformed zooplankton/phytoplankton biomass ratio) in the East China Sea

Models	AIC	Slope			
		Estimate	SE	<i>t</i> value	<i>P</i> -value
Size diversity	182.1	0.263	0.046	5.737	<0.001
PO ₄	204.8	-0.998	0.321	-3.113	<0.001
SiO ₃	205.2	-0.039	0.013	-2.927	<0.001
NBSS slope	208.9	0.734	0.209	3.515	<0.001
NO ₃	209.0	-0.051	0.016	-3.086	<0.001
Shannon diversity	209.4	0.220	0.146	1.507	0.010
Water temperature	220.0	0.037	0.025	1.474	0.074
Log10(<i>x</i> + 1) transformed fish larvae density	222.5	-0.136	0.046	-0.763	0.400

Note that sampling cruises are considered as a random-effect variable. A lower value of Akaike's information criterion (AIC) represents better goodness of fit of the model. The *P*-value was estimated based on Markov chain Monte Carlo sampling. NBSS, normalized biomass size spectrum.

(H4) were also supported by GLMMs ($P \leq 0.01$, Table 1), but the other hypotheses based on fish predation (H5) and temperature (H6) were not supported ($P > 0.05$, Table 1).

While we found that zooplankton size diversity provided the best explanation for variation of plankton trophic structure in the ECS, we cannot rule out the contribution of other factors (Table 1) because some of the selected variables were significantly correlated (Table S3). Nevertheless, the further multivariate forward selection procedure demonstrated that the GLMM model including both zooplankton size diversity and PO₄ had the lowest AIC. However, the improvement was marginal ($\Delta AIC = 0.7$, Table 2) suggesting that the contribution from other factors might be very low (Table 2). Note that the spatial regression analysis for each cruise independently corroborated that zooplankton size diversity was the most important factor with the highest average explanatory power across cruises (Fig. 3).

Discussion

With the field data from the ECS, we provide significant evidence that increasing predator size diversity enhances the strength of top-down control in plankton food webs. In fact, zooplankton size diversity is the most important factor determining top-down control of zooplankton over phytoplankton biomass in the ECS (Table 1, Fig. 2). Although other three conventional hypotheses based on zooplankton NBSS slope (H2), nutrients (H3), zooplankton taxa diversity (H4) are also supported through univariate analyses, the results of multivariate analyses indicate that zooplankton size diversity alone best explains the variation of zooplankton/phytoplankton biomass ratio

Table 2. Results of the multivariate generalized linear mixed-effect model (GLMM) on investigating the factors that affect spatiotemporal dynamics of plankton trophic structure (log10 transformed zooplankton/phytoplankton biomass ratio) in the East China Sea

Fixed-Effect Variables	AIC
Two fixed-effect variables	
Size diversity, PO ₄	181.4*
Size diversity, NO ₃	183.5
Size diversity, SiO ₃	184.3
Size diversity, Shannon density	186.7
Size diversity, NBSS slope	190.5
Size diversity, Water temperature	192.2
Size diversity, log10(<i>x</i> + 1) transformed fish larvae density	191.7
Three fixed-effect variables	
Size diversity, PO ₄ , Shannon diversity	186.4
Size diversity, PO ₄ , NBSS slope	189.9
Size diversity, PO ₄ , SiO ₃	191.4
Size diversity, PO ₄ , NO ₃	193.3
Size diversity, PO ₄ , Water temperature	194.7
Size diversity, PO ₄ , log10(<i>x</i> + 1) transformed fish larvae density	193.0

The best GLMM was selected by using a step-forward procedure. Note that sampling cruises are considered as a random-effect variable. A lower value of Akaike's information criterion (AIC) represents better goodness of fit of the model. We report here only the results up to the level of three fixed-effect variables because AIC increases with more variables included.

*The final best model selected with the stepwise procedure. NBSS, normalized biomass size spectrum.

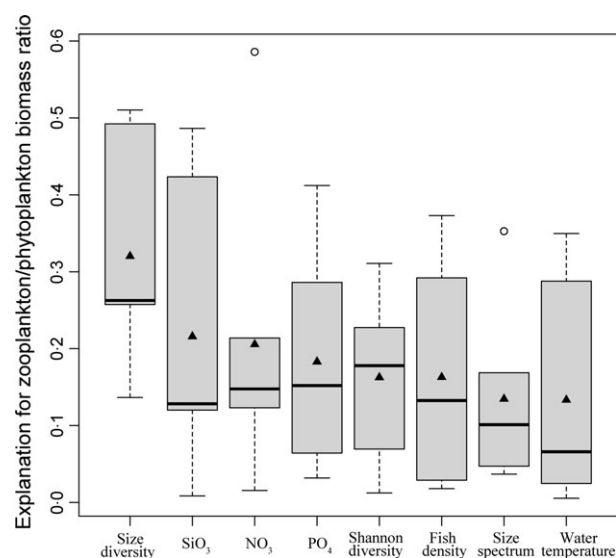


Fig. 3. Boxplot showing the explanatory power (R^2) for the spatiotemporal variation of log10 transformed zooplankton/phytoplankton biomass ratio by the zooplankton size diversity, SiO₃, NO₃, PO₄, zooplankton Shannon diversity, normalized biomass size spectrum (NBSS) slope of zooplankton, and log10 (*x* + 1) transformed fish larvae density in the spatial regression analysis. The dark horizontal lines represent the median, triangles represent the mean value, the box encloses the 25th and 75th percentiles, the whiskers represent the 5th and 95th percentiles, and circles represent the outliers.

(Table 2). In addition, results from spatial regression analysis carried out on a cruise-specific basis suggested that the most dominant factor varied among cruises; however, size diversity consistently explained a high fraction of that variation (Fig. 3) for 5 of 6 cruises (Table S4). Our finding suggests that size diversity may represent an important functional diversity metric and is useful for determining the structure and functioning of aquatic ecosystems.

In addition to commonly accepted ideas based on consumer (top-down) and resource (bottom-up) processes (Brett & Goldman 1997; Llope *et al.* 2012), our findings on zooplankton size diversity may suggest a novel mechanism that potentially explains trophic interactions in plankton food webs. That is, increasing predator size diversity enhances the strength of top-down control on prey through diet niche partitioning (Fig. 1). Indeed, size-structured food web theory hypothesizes that individuals with different sizes may not share a common energy source (Jennings & Mackinson 2003), and optimal-foraging theory suggests that the optimum body-mass ratio characterizes the maximum attack rate of consumers (Brose 2010). Both theories together imply that a community consisting of predators with greater overall variation in size could take greater advantage of opportunities for partitioning their resources (diet) niche.

We should emphasize that the concept of size diversity is adapted from the Shannon diversity index for measuring variation of community size structure (Brucet *et al.* 2006, 2010; Quintana *et al.* 2008). This concept is rooted in the Niche Diversification Hypothesis (Connell 1978), which suggests that systems of higher diversity could take greater advantage of the niche opportunities in an environment, and this allows diverse systems to capture a greater proportion of resources. While recent research has demonstrated that species diversity could improve resource utilization through niche partitioning (Cardinale 2011), we found that zooplankton taxa diversity exhibited weaker effects on planktonic trophic structure than zooplankton size diversity in the ECS. In fact, in real ecosystems, a predator exhibits size-based selection of its prey (Brooks & Dodson 1965). Separation of niches may exist among individuals of different body sizes even within the same species (Kohda *et al.* 2008), and trophic interactions have been found to depend more strongly on size constraints than taxonomy (Brucet *et al.* 2006; Badosa *et al.* 2007; Rall *et al.* 2011; Toscano & Griffen 2012). To this end, size diversity may better indicate diet niche partitioning than species diversity for zooplankton, as has already been suggested for shallow lake ecosystems (Brucet *et al.* 2006). Our results support recent empirical and theoretical studies emphasizing the use of size as the key niche dimension in food web research (Woodward *et al.* 2005; Stouffer, Rezende & Amaral 2011) and suggest that size diversity represents an important index of functional diversity linking community structure and trophic dynamics.

A significant positive relationship between the zooplankton/phytoplankton biomass ratio and NBSS slope was also found by GLMM ($P < 0.001$, Table 1). This finding is consistent with the hypothesis that a larger-bodied predator has stronger top-down control on its prey (Jaschinski *et al.* 2009; Jochum *et al.* 2012); however, the spatial regression analysis indicates that this relationship only holds for 2 of 6 cruises when considering the spatial autocorrelation (Table S4). Our results suggest that size diversity represents a better index of resource (diet) niche partitioning than the NBSS slope. NBSS slope quantifies the relative distribution of small and large organisms in a community. When the slope becomes steeper, it indicates that the system has shifted to a community dominated by small-organisms (Brown & Gillooly 2003; Jennings & Mackinson 2003). However, size diversity could measure the continuous distribution of body size as the size deviation (Quintana *et al.* 2008; Brucet *et al.* 2010). From this viewpoint, assuming predators of different sizes have their optimal diet niches, we could explain why zooplankton size diversity had stronger correlation with zooplankton/phytoplankton biomass ratio than did the zooplankton NBSS slope in our study (Fig. 1c).

It is worth to mention that the calculation of size diversity in our study is mathematically independent of zooplankton biomass (see Data S1). Thus, in a real ecosystem, even if we find a 'statistical' relationship between size diversity and biomass, it would be the result of size diversity increasing trophic transfer efficiency but not a result of a mathematical artefact. Indeed, in the East China Sea, we found a positive correlation between zooplankton size diversity and total zooplankton biomass (Pearson correlation $r = 0.246$, $P = 0.006$), although this correlation was weaker than the correlation between zooplankton size diversity and zooplankton/phytoplankton biomass ratio (Pearson correlation $r = 0.436$, $P < 0.001$). In fact, the positive correlation between size diversity and zooplankton biomass suggests that the zooplankton community with higher size diversity has higher efficiency of resource use; therefore, higher size diversity of zooplankton can increase zooplankton biomass. This is similar to the widely accepted plant diversity-productivity relationship (Tilman 1999; Tilman *et al.* 2001).

A significant positive correlation (Pearson correlation $r = 0.607$, $P < 0.001$) was found between the zooplankton size diversity and mean zooplankton body size. This may imply that the increased zooplankton size diversity in the ECS is to some extent contributed by the increasing proportion of large body size zooplankters (see Fig. S2). As larger-bodied zooplankton are more efficient at capturing their prey (Hall & Threlkeld 1976), such increase in mean body size of zooplankton community may have additional contribution for top-down control. This is consistent with the results based on the NBSS slope (Table 1 and Fig. 2b). However, separating such contribution from the size diversity effect is difficult with

our data. Further investigation using size-based field feeding experiments is needed to estimate the relative importance of these two effects.

We should add a caveat that our approach cannot fully address the potential contribution of zooplankton taxonomic diversity because the taxonomic resolution obtained from the plankton imaging system (i.e. ZooScan) is relatively coarse. However, we found that the zooplankton taxonomic diversity estimated from the ZooScan system exhibited a significant positive correlation with water temperature and increased from high to low latitudes in the ECS (Fig. S3 and Table S5), which is consistent with the general global pattern of latitudinal distribution of zooplankton diversity (Beaugrand, Edwards & Legendre 2010). In addition, our limited analyses for the 2006 cruise (comparison to zooplankton community data from Wu, Shin & Chiang (2011) indicated that the zooplankton taxa diversity computed from our ZooScan community data is significantly correlated with that computed from the order-level zooplankton community and species-level copepod community based on microscope counts (Fig. S4). This agreement suggests that our conclusion likely remains, even when high-resolution taxonomic data are used in the analysis. It is well known that obtaining highly resolved zooplankton taxonomic data is very challenging and time-consuming. Thus, the utility of plankton imaging systems has been suggested for a variety and environmental research questions (e.g. Morán *et al.* 2010; Ye, Chang & Hsieh 2011; Chang *et al.* 2012). We should also be cautious about the result on zooplankton predator pressure. We used larval fish density as a proxy for predation effects as used by Ohman & Hsieh (2008), and thus, our conclusion concerning trophic cascade remains preliminary.

Apart from zooplankton size structure, nutrients, fish predation and zooplankton taxa diversity, two other potential factors that may affect the zooplankton/phytoplankton biomass ratio in aquatic systems are not examined in our research: the contribution of food resources from the microbial loop (del Giorgio & Gasol 1995) and the change of phytoplankton size structure (Yvon-Durocher *et al.* 2011). On some occasions, the grazing control of zooplankton over phytoplankton may be weakened when additional food sources from the microbial loop (through bacteria to protists and then to mesozooplankton) are available (del Giorgio & Gasol 1995). Another possible scenario observed in mesocosm experiments indicates that shifting the phytoplankton size distribution towards smaller individuals resulted in an increased zooplankton/phytoplankton biomass ratio because smaller phytoplankton have rapid turnover rate and low standing biomass (Yvon-Durocher *et al.* 2011). We currently do not have the data with which to test these alternative hypotheses. However, those variations have been found to be mainly determined by temperature (Daufresne, Lengfellner & Sommer 2009; Yvon-Durocher *et al.* 2011). The fact that we found no significant

correlation between the zooplankton/phytoplankton biomass ratio and water temperature (Table 1, Fig. 2) suggests that temperature might not have strong effects in our system. Nevertheless, our mechanism is based on the assumption that phytoplankton community has a wide size spectrum for zooplankton to choose freely. In reality, phytoplankton size structure may have additional effects in determining the zooplankton/phytoplankton biomass ratio. We acknowledge that our conclusion is based on correlation analyses using mesozooplankton, and thus, further investigation on phytoplankton size structure and microzooplankton as well as feeding experiments is necessary to substantiate our proposed mechanism.

Our finding of the size diversity effect on trophic structure may have important management implications in aquatic ecosystems. Recent studies have demonstrated that Yangtze river floods to the ECS (Gong *et al.* 2011) or typhoon events (Lin *et al.* 2003) have increased primary production and suggested that these events may further enhance fisheries catches, based on an oversimplified assumption of fixed trophic transfer efficiency. However, if one uses the zooplankton/phytoplankton biomass ratio as a proxy for trophic transfer efficiency (Gaedke & Straile 1994), according to our findings, such assumptions of fixed efficiency are likely not to hold. That is, while primary production or phytoplankton biomass may be enhanced as a consequence of disturbance events, whether the energy can be transferred to higher trophic levels still critically depends on the size structure of the zooplankton community (see also Rykaczewski & Checkley 2008; Beaugrand, Edwards & Legendre 2010).

In summary, by testing our main hypothesis that zooplankton size diversity determines the trophic structure of zooplankton and phytoplankton in the ECS and five conventional hypotheses based on zooplankton NBSS slope, nutrients, zooplankton taxa diversity, fish predation and temperature, we found that zooplankton size diversity is the most important factor determining top-down control of zooplankton over phytoplankton biomass in the ECS. We propose a potential mechanism: increasing predator size diversity enhances the strength of top-down control on its prey through diet niche partitioning. This mechanism extends the current knowledge on top-down control in aquatic ecosystems and may have important management implications. Our findings reveal the importance of considering size diversity in ecological research and the feasibility of this approach by using automatic imaging systems (e.g. Morán *et al.* 2010; Ye, Chang & Hsieh 2011; Chang *et al.* 2012).

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Data Accessibility

The data set used in this paper is available in the Dryad data repository: doi:10.5061/dryad.pt354.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Sampling stations in the East China Sea. Zooplankton samples were collected in the East China Sea from 2006 to 2009, by using a 330-µm mesh ORI net (Ocean Research Institute plankton net) with a mouth of 160-cm diameter.

Fig. S2. Schematic indicating that either increasing the proportion of small body size zooplankters (community b) or large body size zooplankters (community c) can increase the size diversity compared with community a.

Fig. S3. Correlation between zooplankton taxa diversity with water temperature (A) and latitude (B) for the six cruises (2006–2009) in the East China Sea.

Fig. S4. Comparisons of Shannon diversity estimated from the ZooScan data versus the diversity estimated from the order-level zooplankton community data (A) and from the species-level copepod community data (B).

Fig. S5. Schematic indicating that size diversity is independent of zooplankton biomass.

Fig. S6. Schematic indicating that size diversity is independent of zooplankton mean body size.

Fig. S7. Scatter plot illustrating a positive relationship between log10 transformed zooplankton biomass and geometric mean of zooplankton body size.

Data S1. Argument for mathematical independence of size diversity versus zooplankton biomass and mean zooplankton body size

Table S1. Check lists for stations sampled during each cruise. The geographic location of each site can be found in Fig S1.

Table S2. Summary statistics for variables investigated for the six cruises in the East China Sea.

Table S3. Pearson correlation coefficients (upper triangle) and pairwise partial correlation coefficients for each pair of variables after accounting for all the other variables (lower triangle) the

selected factors explaining the spatiotemporal dynamics of plankton trophic structure.

Table S4. Results of spatial regression analysis on investigating the individual effect of each factor on the spatiotemporal dynamics of plankton trophic structure (log10 transformed zooplankton-phytoplankton biomass ratio) in the East China Sea for each

cruise with the spatial auto-correlation taken into consideration.

Table S5. Results of the generalized linear mixed-effect model on investigating the relationship between zooplankton Shannon diversity versus water temperature and latitude in the East China Sea.