What controls microzooplankton biomass and herbivory rate across marginal seas of China?

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Abstract

Microzooplankton (2-200 μm) are the primary herbivores and nutrient regenerators in the marine food web, but their importance is often underestimated, and the quantitative relationships between environmental factors and the biomass and herbivory rate of microzooplankton remain obscure. To fill this gap, we conducted 224 dilution experiments to measure microzooplankton biomass and herbivory rate across a vast area of the marginal seas of China. To gain the potential mechanisms controlling microzooplankton herbivory, we also use a model that combines Metabolic Theory of Ecology and functional responses of grazing to quantify the effects of temperature, phytoplankton biomass, and microzooplankton biomass on microzooplankton grazing rate. We estimate an activation energy of 0.51 eV of microzooplankton and found that the Holling III function best described the functional response of microzooplankton grazing with a maximal ingestion rate of 4.76 d^{-1} at 15 °C and a half-saturation constant of 0.27 μM N. We also find that microzooplankton biomass scales with phytoplankton biomass with an exponent of 0.77, consistent with the general 3/4 scaling law found in other ecosystems. This scaling relationship is accompanied by a shift from ciliates to heterotrophic dinoflagellates with increasing phytoplankton biomass. Our results provide empirical patterns that will be vital to parameterize and validate marine ecosystem models, particularly in China seas.

Introduction

Microzooplankton, defined as heterotrophic and mixotrophic organisms with a size range of 20-200 μm, primarily consist of phagotrophic protists, including ciliates, dinoflagellates, and mesozooplankton nauplii (Sieburth et al. 1978). As the primary consumers of marine phytoplankton, microzooplankton consume ~59%-75% daily primary production, much larger than mesozooplankton (Calbet and Landry 2004; Calbet 2008). Microzooplankton are also pivotal regenerators of nutrients which fuel primary production and food sources for metazoans (Calbet 2008).

Despite their ecological importance, microzooplankton remain understudied. Routine oceanographic observations seldom monitor microzooplankton biomass or herbivory rate, although the dilution technique, an elegant method of measuring microzooplankton herbivory rate, has been developed for almost four decades (Landry and Hassett 1982). The number of observations of microzooplankton herbivory rate is around 1600 globally (Chen et al. 2012; Schmoker et al. 2013), far less than that of primary productivity (>50000; Buitenhuis et al. 2013). This makes validating and optimizing the grazing function of microzooplankton difficult in ocean ecosystem models. Indeed, microzooplankton have been rarely validated by observational data in mainstream ocean models (but *see* Buitenhuis et al. 2010). Promoted by such gap, Buitenhuis et al. (2010) suggested that "the most effective progress which can be made in defining the role of microzooplankton in global biogeochemical cycles is to make microzooplankton biomass a standard oceanographic measurement, in particular during dilution grazing experiments".

We lack a thorough understanding of the relationship between microzooplankton biomass and environmental variables such as prey (phytoplankton) biomass and temperature. The slope of microzooplankton biomass against phytoplankton biomass reflects whether microzooplankton are under bottom-up or top-down control (Yuan and Pollard 2018). If

69 microzooplankton are mostly controlled by bottom-up factors, microzooplankton biomass
70 should increase in proportion with phytoplankton biomass. Conversely, if microzooplankton
71 are mostly regulated by top-down effects by their predator (i.e., mesozooplankton), increases
72 in phytoplankton biomass should lead to negligible increases of microzooplankton biomass.
73 However, empirical evidence is mixed as to the biomass relationship between
74 microzooplankton and phytoplankton. Hatton et al. (2015) have found that logarithmic
75 predator biomass increases linearly with logarithmic prey biomass with the slope close to 3/4
76 in different ecosystems. Nevertheless, a universal scaling exponent has been challenged
77 (Yuan and Pollard 2018). For instance, zooplankton biomass was almost constant with
78 increasing phytoplankton biomass in eutrophic environments (Irigoien et al. 2004; Yuan and
79 Pollard 2018). As such, it is still uncertain whether the relationship between
80 microzooplankton biomass and phytoplankton biomass should be linear or curvilinear on a
81 log-log scale.
82 There are also uncertainties as to whether temperature should affect
83 microzooplankton biomass relative to phytoplankton biomass. Because the metabolic rate of
84 zooplankton is believed to increase with temperature faster than that of phytoplankton, the
85 biomass ratio of microzooplankton to phytoplankton should decrease with increasing
86 temperature (Chen et al. 2012). However, this metabolic asymmetry has recently been
87 challenged (Chen and Laws 2017). Thus, it remains unclear whether the microzooplankton-
88 to-phytoplankton biomass ratio should vary with temperature or not.
89 In addition, we also lack clearly defined grazing functions of microzooplankton,
90 especially for natural microzooplankton assemblages (Sandhu et al. 2019). Microzooplankton
91 grazing rate is a complex function of the characteristics of predator and prey, such as prey
92 quality and concentration, predator concentration and feeding strategies, and predator-prey
93 size (Hansen et al. 1997). Among these factors, prey concentration is considered as major

94 determinants of microzooplankton grazing rate in many biogeochemical / ecosystem models
95 due to its relatively easy access (Buitenhuis et al. 2010; Laufkotter et al. 2016). The effect of
96 prey concentration on microzooplankton specific grazing rate (i.e. per capita zooplankton per
97 unit time) is described as the functional response. In general, the ingestion rate increases with
98 increasing prey concentration linearly or nonlinearly to a plateau and the corresponding
99 clearance rate decreases as prey concentration increases (Gentleman et al. 2003; Sandhu et al.
100 2019). Temperature is another factor that affects microzooplankton grazing activities and
101 should be incorporated into the grazing functions (Hansen et al. 1997; Buitenhuis et al. 2010).
102 Although the grazing functions of microzooplankton have been intensively studied in the
103 laboratory (Hansen et al. 1997), few studies clearly quantified the effects of prey
104 (phytoplankton) concentration and temperature simultaneously on natural microzooplankton
105 assemblages (but *see* Buitenhuis et al. 2010), which should be worth more effort.
106 In this study, we fill these gaps by assembling a dataset consisting of 224
107 microzooplankton biomass and herbivory rates that we measured through dilution
108 experiments during 9 cruises across China seas including the South China Sea, the East China
109 Sea, and the Yellow Sea. These data expand previous studies of Chen et al. (2009, 2013), Su
110 et al. (2014), Chen and Liu (2015), Zheng et al. (2015), and Liu et al. (2019) and cover the
111 gradient from eutrophic coastal waters to oligotrophic oceanic waters. This rich dataset
112 covering the gradient from eutrophic coastal waters to oligotrophic oceanic waters provides
113 us with the opportunity to address the following questions: (1) What are the most important
114 factors affecting microzooplankton biomass? Specifically, what are the quantitative
115 relationships between microzooplankton biomass and environmental variables such as
116 phytoplankton biomass and temperature? (2) What are the most important factors affecting
117 microzooplankton community grazing rates? We will examine the empirical relationships
118 between microzooplankton biomass and grazing rate with phytoplankton biomass and

119 temperature. Then we will contrast these empirical relationships against theoretical
120 predictions. We also investigate the role of mesozooplankton biomass and microzooplankton
121 community via the fraction of heterotrophic dinoflagellates in the total community (Sherr and
122 Sherr 2007) in affecting microzooplankton biomass and grazing rate. Our analysis will
123 deepen the mechanistic understanding of what controls microzooplankton biomass and
124 grazing rate in the ocean. The algorithms we have developed will also benefit parameterizing
125 and validating ocean ecosystem models, particularly in China seas.
126
127 **Methods and Materials**
128 **Field data**
129 *Field sampling and plankton sample analysis*
130 Microzooplankton samples and accompanying environmental data were collected
131 during 9 oceanographic cruises across the marginal seas of China including the South China
132 Sea, the East China Sea, and the Yellow Sea (Table S1, Fig. 1). Samples were also collected
133 during whole-year monthly observations at three coastal stations adjacent to the northern
134 South China Sea (Table S1, Fig. 1). The stations covered a broad environment of China seas
135 ranging from very eutrophic coastal waters to oceanic offshore waters in different seasons.
136 The data were geographically classified into four subsets based on the study areas: the South
137 China Sea, the East China Sea, the Yellow Sea, and Coastal stations. We grouped the data of
138 monthly observations as one subset (Coastal stations) because the sampling stations were
139 very close to the land and substantially affected by the river input (Fig. 1).
140 We collected in situ data of temperature and Chlorophyll *a* (Chl *a*) associated with
141 microzooplankton samples. Water temperature and salinity were measured at each station by
142 Seabird CTD probes during the cruises and a YSI multi-probe sensor (6600 or EXO2) during
143 monthly observations. Surface seawater was collected using normal Niskin bottles attached to

144 a CTD rosette system or an acid-washed plastic bucket. To measure Chl *a* concentration,
145 aliquots of 100 mL to 500 mL seawater were filtered onto 25 mm GF/F glass fibre filters
146 under low vacuum (0.2 μm PC membrane filters were used at stations in Hong Kong waters).
147 All filters were stored at -80°C until further analysis. Following the protocol of Joint Global
148 Ocean Flux Study, the filters were extracted in 90% acetone at -20°C or 4°C in the darkness
149 and the Chl *a* concentrations were measured within 24 h using a Turner Designs fluorometer
150 with a non-acidification module (Model No. Trilogy 040) (Welschmeyer 1994).
151 To collect the microzooplankton samples, seawater (100 mL to 500 mL) was gently
152 siphoned from carboys into plastic amber bottles containing acidic Lugol's solution with a
153 final concentration of 5%. The samples were stored in the dark at room temperature until
154 further analysis. Aliquots of 10-100 mL of each sample were settled for 24 h using Utermöhl
155 chambers (the samples with large volumes (500 ml) were processed by a two-stage settling
156 process) and observed with an inverted microscope (Olympus IX51 or Leica Dmirb) at 200×
157 magnification. We primarily focused on phagotrophic protists larger than 20 μm, including
158 ciliate and heterotrophic dinoflagellates, which mainly consume phytoplankton and are the
159 major contributors to the grazing rate estimated by dilution technique (Calbet 2008). The
160 general categories, including ciliates (aloricate ciliates and Tintinnids), dinoflagellates, and
161 copepod nauplii (< 200 μm) were enumerated (Elangovan and Padmavati 2017). As the
162 copepod nauplii cannot be spotted in most samples and their abundance cannot be accurately
163 estimated through this method, we excluded this category when calculating the total
164 abundance and biomass of microzooplankton. For each sample, at least 50 cells were
165 counted.
166 We estimated microzooplankton biomass by summing up the biovolumes of all cells
167 counted under the microscope. To estimate the cell volume, each cell was assigned to a
168 geometrical shape, and their width and length were measured using the software SPOT

169 (version3.5) or Simple PCI6. The carbon content of each microzooplankton was estimated
170 based on the corresponding empirical equations for acidic Lugol's fixed cells. For ciliates, a
171 conversion factor of 0.19 pg C μm^{-3} was used on the aloricate ciliate (Putt and Stoecker
172 1989), and the equation of pg C $cell^{-1}$ = 444.5 + 0.053 × biovolume was used on Tintinnids
173 (Verity and Lagdon 1984). The biovolume of dinoflagellate was converted to cell carbon
174 based on the equation of pg C $cell^{-1}$ = 0.76 × $biovolume^{0.819}$ (Menden-Deuer and Lessard
175 2000). The average size of ciliates or dinoflagellates (pg C $cell^{-1}$) was calculated by dividing
176 their total biomass (pg C L^{-1}) by the corresponding total abundance (cells L^{-1}) in a sample.
177 We also assembled the data of mesozooplankton dry weight measured at the same
178 time with our experiments at some stations (Chen et al. 2011; 2015; and unpublished data). In
179 brief, a total of 58 mesozooplankton samples were towed vertically from 200 m (or near the
180 bottom for shallow water stations) to the surface using a plankton net (200 μm mesh size)
181 attached by a digital flowmeter. The samples were filtered onto pre-weighted PC filters (20
182 μm, 47 mm) to measure mesozooplankton dry weight after oven-drying (24h, 60°C). The
183 carbon biomass of mesozooplankton was estimated by assuming carbon weight = 0.4 × dry
184 weight (Steinberg et al. 2000).
185 *Microzooplankton herbivory*
186 Microzooplankton herbivory rates were estimated via the dilution technique, which is
187 the standard method to estimate the microzooplankton herbivory rate on phytoplankton
188 (Landry and Hassett 1982). Although the group of microzooplankton may include
189 multicellular grazers such as copepod nauplii based on its technical definition (20-200 μm
190 organisms), the grazing rate measured through this method was principally due to protistan
191 grazers including ciliates and heterotrophic dinoflagellates (Calbet and Landry 2004; Calbet
192 2008). Dilution experiments were conducted at every sampling station (Fig.1). The measured
193 volume of particle-free seawater, which was obtained by filtering the seawater through a 0.2

μm filter capsule (Pall Corporation) by gravity, was added into the 1.2 L polycarbonate bottles. The bottles were then filled with the unfiltered seawater to the full capacity to get a mixture of certain percentages of unfiltered seawater with particle-free seawater. Five dilution treatments with unfiltered seawater percentages of 15, 27, 50, 73, and 100%, respectively, were established in all experiments during most cruises. In monthly observations in Hong Kong waters (2016/2017) and Xiamen Bay, two dilution treatments (15% / 25% and 100%) were used, which has been proven as accurate as five dilution treatments (Chen 2015). Inorganic nutrients were added into all bottles to ensure constant phytoplankton growth. The final nutrient concentration in the bottles was 0.5 μmol L^{-1} NH_4Cl, 0.03 μmol L^{-1} KH_2PO_4, 1 nmol L^{-1} $FeCl_3$, and 0.1 nmol L^{-1} $MnCl_2$ for the oceanic stations, and 10 μmol L^{-1} $NaNO_3$ and 1 μmol L^{-1} KH_2PO_4 for coastal stations. Two bottles filled with unfiltered seawater without nutrient addition were prepared for no-nutrient-addition controls. All bottles were tightly capped, incubated for 24 h in a deck incubator covered with neutral screens and cooled by running seawater during all cruises. All experimental carboys, filter capsules, bottles, and tubing were washed with 10% HCl and rinsed with distilled water and in situ seawater prior to each experiment. After incubation, Chl *a* samples were taken from each experimental bottle and analysed using the method mentioned above.

The microzooplankton grazing rate was estimated following Landry et al. (2008). Assuming exponential growth for phytoplankton in each bottle, the net growth rate of phytoplankton (g, d^{-1}) was calculated as $g = (1/t)\ln(P_t/DP_0)$, where P_0 and P_t were the phytoplankton biomass represented by Chl *a* concentration before and after incubation. D is the dilution factor (the percentages of unfiltered seawater) of each bottle. The mortality grazing rate due to microzooplankton (m, d^{-1}) was estimated as the slope of the linear regression of the net growth rate against the dilution factor. At coastal stations, saturated

grazing was observed occasionally. When it occurred, the grazing rate was estimated as the slope of the regression curve within the non-saturation range (Gallegos 1989). The grazing rate was removed when the slope of the regression was positive.

Theory

Effects of prey concentration on microzooplankton biomass: Predator-prey power law

The relationship between predator and prey biomass has been described by a power-law relationship and further transformed into the linear regression model after logarithmic transformation (Hatton et al. 2015):

$$B_Z = cP^{\gamma}$$

$$\log B_Z = \gamma \log P + \log c \qquad (1)$$

where c is the coefficient and γ is the dimensionless scaling exponent; B_z (μg C L^{-1}) is the carbon biomass of total microzooplankton; P (μg C L^{-1}) is the phytoplankton carbon biomass converted by multiplying the Chl *a* concentration by the model-derived carbon-to-chlorophyll ratio (C:Chl, gC $gChl^{-1}$). The C:Chl ratio was estimated using a Boosted Regression Trees model (Elith and Leathwick 2017) which was constructed based on a published dataset consisted of C:Chl ratio, light, temperature, and dissolved inorganic nitrogen (Chen and Liu 2010). In this model, the C:Chl ratio is a function of temperature, nutrient, and light (Supplementary Information). The exponent $\gamma < 1$ means the trophic pyramid is more bottom-heavy at higher biomass and indicates bottom-up control of prey on the predator (Hatton et al. 2015). Ordinary least squares (OLS) was used to fit the log-transformed data following Hatton et al. (2015), which is commonly used in fitting bivariate power laws (Del Giorgio and Gasol 1995). We also tried to fit a second-order term in the linear regression to test whether curvilinear can fit the data better than linear:

$$\log B_Z = \gamma_1 \log P + \gamma_2 \log P^2 + \log c \qquad (2)$$

where both γ_1 and γ_2 are regression coefficients.

Effects of temperature on microzooplankton biomass

We explored the theoretical prediction of how temperature affects the ratio of microzooplankton to phytoplankton biomass. We assumed that at steady state, microzooplankton production (*MP*) is a constant fraction of primary production ($P\mu$) (Landry and Calbet 2004). This fraction is the gross growth efficiency (*GGE*).

$$MP = B_Z \times G_z = GGE \times P \times \mu$$

$$B_z = P \times GGE \times \frac{\mu}{G_z} \qquad (3)$$

where G_z and μ are the growth rates (d^{-1}) of microzooplankton and phytoplankton, respectively. The growth rate of both microzooplankton and phytoplankton are affected by temperature, which can be described by Boltzmann-Arrhenius equation in the Metabolic Theory of Ecology (Brown et al. 2004):

$$\frac{\mu}{G_z} = \frac{\mu_0 e^{E_P(\frac{1}{kT_0} - \frac{1}{kT})}}{G_{z0} e^{E_{zg}(\frac{1}{kT_0} - \frac{1}{kT})}} \qquad (4)$$

in which G_{z0} and μ_0 are normalization constants for the growth rates of microzooplankton and phytoplankton, respectively. E_p and E_{zg} are the activation energies (eV) of the growth rates of phytoplankton and microzooplankton, respectively, which describes the temperature sensitivity of rates, k is the Boltzmann's constant (8.62×10^{-5} eV K^{-1}), T is temperature (K), T_0 is the reference temperature (288 K). As the activation energy of microzooplankton growth rate (E_{zg}) is around twice of that of phytoplankton (E_p) (Chen et al. 2012), we should have:

$$\frac{B_z}{P} \propto e^{\frac{E_{zg} - E_p}{kT}} \qquad (5)$$

which means that the biomass ratio of microzooplankton to phytoplankton should decrease with increasing temperature if we assume that the gross growth efficiency does not vary with temperature (Chen et al. 2012).

Modelling microzooplankton grazing rate based on metabolic theory of ecology and functional response of grazing

The microzooplankton grazing rate (m, d^{-1}) estimated by the dilution approach is the microzooplankton community clearance rate, which is the sum of clearance rate of each grazer in a unit volume of water parcel:

$$m = \sum_{i=1}^{N} F_i = \sum_{i=1}^{N} \frac{I_i}{P_i} \qquad (6)$$

where F_i (L grazer^{-1} d^{-1}) is the clearance rate of the i^{th} grazer. N is the abundance of grazers in the community. The clearance rate can be calculated by dividing the ingestion rate I_i (μg C grazer^{-1} d^{-1}) by the mean food concentration P_i (μg C L^{-1}) based on their definitions (Båmstedt et al. 2000). Although the grazing activities of each grazer could be affected by many factors such as the grazing pressure by their consumers and predator-prey size ratio, we consider an ideal situation where the ingestion rate of each grazer is only dependent on prey concentration and can be described by the maximum ingestion rate and functional response as in many biogeochemical models (Hansen et al. 1997; Laufkotter et al. 2016):

$$m = \sum_{i=1}^{N} \frac{I_{m,i} f(P_i)}{P_i} \qquad (7)$$

where $I_{m,i}$ (μg C grazer^{-1} d^{-1}) is the maximum ingestion rate of the i^{th} grazer, and $f(P_i)$ is the functional response of each grazer describing the relationship of ingestion rate and food concentration. According to the metabolic theory of ecology, the maximum ingestion rate of each grazer can be calculated by a function of body size and temperature, thus *Eq. 7* becomes:

$$m = \sum_{i=1}^{N} I_0 e^{E_z(\frac{1}{kT_0} - \frac{1}{kT})} M_i^{\alpha} f(P_i) / P_i$$

$$= I_0 e^{E_z(\frac{1}{kT_0} - \frac{1}{kT})} \sum_{i=1}^{N} M_i^{\alpha} f(P_i)/P_i \qquad (8)$$

where I_0 (d^{-1}) is a normalization constant; $e^{E_z(\frac{1}{kT_0}-\frac{1}{kT})}$ describes the effect of temperature on the maximum ingestion rates in which E_z is the activation energy of microzooplankton grazing rate and other symbols are the same with *Eq. 4*; M_i (μg C $grazer^{-1}$) is the body mass of the i^{th} grazer and α is the allometric exponent for the body mass. When the M_i is in the unit of carbon content, α could approximatively equal to 1. Then the sum of the α-scaled body mass ($\sum_{i=1}^{N} M_i^{\alpha}$) could approximatively equal to the total biomass of the grazers (B_z, unit: μg C L^{-1}) (Hansen et al. 1997; Chen et al. 2012). To explore the effect of prey concentration on the community grazing rate of microzooplankton, we assumed a system-level functional response of ingestion rate vs. the prey concentration ($f(P) = \sum_{i=1}^{N}(f(P_i)/P_i)$), which is the aggregate behaviour of different grazers and preys under different environmental conditions ranging from eutrophic coastal water to the oligotrophic offshore waters. Then the *Eq. 8* becomes:

$$m = I_0 e^{E_z(\frac{1}{kT_0}-\frac{1}{kT})} B_z f(P) \qquad (9)$$

Thus the biomass-specific grazing rate (m/B_z, unit: d^{-1} $(\mu g\ C)^{-1}$ L) should be determined by a function of temperature and prey concentration:

$$\frac{m}{B_z} = I_0 e^{E_z(\frac{1}{kT_0}-\frac{1}{kT})} f(P) \qquad (10)$$

By taking logarithms on both sides, we obtain:

$$\ln(\frac{m}{B_z}) = \ln(I_0) + \ln\big(f(P)\big) + E_z(\frac{1}{kT_0} - \frac{1}{kT}) \qquad (11)$$

There are several mathematical formulations for the functional response of grazers $f(P)$. In the present study, we examined the three most common nonlinear formulations (Gentleman et al. 2003; Sandhu et al. 2019) to find the most suitable one for our dataset:

$$\text{Ivlev:} \quad \frac{1-e^{-P/K_{p1}}}{P} \qquad (12)$$

311 Holling II: $\frac{1}{K_{p2}+P}$ (13)

312 Holling III: $\frac{P}{K_{p3}^2+P^2}$ (14)

313 in which K_{p1}, K_{p2}, and K_{p3} are constants (unit: μg C L^{-1}). K_{p2} and K_{p3} are half-saturation
314 constants. *P* is the food concentration. As phytoplankton is the major food source of
315 microzooplankton (Calbet and Landry 2004), and the grazing rate estimated through dilution
316 approach is the phytoplankton mortality grazed by microzooplankton, the food concentration
317 here is the phytoplankton concentration in carbon unit (μg C L^{-1}). The fitting of nonlinear
318 formulation combining *Eq. 11* and *12*, *13*, or *14* to data was implemented using the R
319 function "*nls*" and package "*nlstools*" in the software R 3.4.3 (Florent et al. 2015).
320 We also used Generealized Additive Models (GAMs) to describe the variation in
321 ln(m/B_z) with the log-transformed phytoplankton carbon biomass as a smoother term to
322 demonstrate the patterns without parametric constraints (Woods 2006). The GAMs analysis
323 was implemented using the function "*gam*" in the R package "*mgcv*" (Woods 2006).
324
325 **Results**
326 *Environmental controls on microzooplankton biomass*--We find that
327 microzooplankton biomass is significantly correlated with phytoplankton biomass (Spearman
328 $r = 0.61$, $p < 0.001$; Fig. 2; Detailed results for each experiment are summarized in the
329 Appendix). The relationship between microzooplankton and phytoplankton biomass can be
330 described by a linear regression model on a log-log scale (*Eq. 1;* Fig. 3a, adjusted $R^2 = 0.36$,
331 n = 223, $p < 0.001$). The scaling exponent γ is 0.77 ± 0.07, similar to the scaling exponent
332 (near 3/4) found in the universal relationship between predator and prey biomass (Hatton et
333 al. 2015). A second-order term is insignificant in the regression (*Eq. 2*, ANOVA, $p > 0.05$).
334 The biomass of major groups of microzooplankton also increase with increasing

phytoplankton biomass (Fig. 3b). The scaling exponents for the relationship between ciliates and heterotrophic dinoflagellates vs. phytoplankton biomass are 0.73 ± 0.07 and 0.83 ± 0.11, respectively.

Microzooplankton biomass is also significantly correlated with mesozooplankton biomass (Spearman $r = 0.53$, n = 58, $p < 0.001$; Fig. 2). As the predator of microzooplankton and phytoplankton, mesozooplankton biomass increases with microzooplankton and phytoplankton (Fig. 3c). The slope of mesozooplankton biomass vs. microzooplankton biomass, phytoplankton biomass, and total prey biomass (microzooplankton + phytoplankton) on a log-log scale is 0.47 ± 0.09, 0.64 ± 0.12, and 0.67 ± 0.12, respectively.

No correlation between temperature and microzooplankton biomass is observed (Spearman $r = -0.06$, n = 223, $p > 0.05$; Fig. 2). The biomass ratio of microzooplankton to phytoplankton does not correlate with temperature either (Fig. S1).

In summary, we find that the relationship between microzooplankton biomass and phytoplankton biomass is well consistent with the universal 3/4 scaling law (Hatton et al. 2015) and does not show any curvilinearity as shown by Irigoien et al. (2004) and Yuan and Pollard (2018). Inconsistent with the theoretical prediction, we cannot find any temperature effect on microzooplankton biomass.

Environmental controls on microzooplankton grazing rate-- As predicted by *Eq. 11*, temperature, the total biomass of microzooplankton and phytoplankton all play important roles in affecting the microzooplankton community grazing rate derived from the dilution experiments. The community microzooplankton grazing rate strongly increases with temperature (Spearman $r = 0.31$, $p < 0.001$), phytoplankton biomass (Spearman $r = 0.29$, $p < 0.001$), and microzooplankton biomass (Spearman $r = 0.35$, $p < 0.001$), but not correlated with microzooplankton community composition which is roughly represented by fraction of heterotrophic dinoflagellates (HDF.ratio, Spearman $r = 0.07$, $p > 0.05$; Fig. 2). When

phytoplankton biomass is low (0 ~ about 25 μg C L^{-1}), the biomass-specific grazing rate (m/B_z) increases with phytoplankton biomass (Fig. 4a, Fig. S2). Above 25 μg C L^{-1}, m/B_z decreases with increasing phytoplankton biomass. m/B_z also increases with temperature (Fig. 4b, Fig. S2).

To investigate which form of functional response performs the best, we further fit the experimental data to *Eq. 11* with three types of functional responses (*Eqs. 12*, *13*, *14*; Table 1). All parameter estimates are significant (the corresponding *p* values are shown in the table). The model with Holling III function explains 27% variation of biomass-specific grazing rate measurements, which is better than those with Ivlev and Holling II functions (differences of AIC are about 4, Table 1). The nonparametric GAMs fit with the log-transformed phytoplankton biomass as a smoother term also shows similar patterns of ln(m/B_z) variation (R^2 = 30%, n = 208; Fig. S2). Thus, the model with Holling III function was used to predicted m/B_z and community grazing rate (Figs. 4, 5) and it estimates an E_z value of 0.51 eV (95% CI = 0.23 - 0.79 eV) and a maximum ingestion rate of 4.76 d^{-1} (95%CI = 2.99 – 6.52 d^{-1}) at 15 °C and a half-saturation constant of 0.27 μM N (95%CI = 0.18 - 0.35 μM N).

Changes of microzooplankton community composition—We examined whether the biomass ratio of heterotrophic dinoflagellates to total microzooplankton changed with trophic status (Sherr and Sherr 2007). We found that although both the biomass of ciliates and heterotrophic dinoflagellates increased with increasing phytoplankton biomass (Fig. 3b), there was no correlation between the fraction of heterotrophic dinoflagellates and phytoplankton biomass (Fig. 2). The fraction of heterotrophic dinoflagellates is significantly higher at the Coastal stations than in other regions because the heterotrophic dinoflagellates are very abundant in the eutrophic waters (Fig. 6a). While in the mesotrophic and oligotrophic waters, the fraction of heterotrophic dinoflagellates is relatively low and shows

385 no correlation with Chl *a* concentration (Spearman r = -0.09, n = 123, $p > 0.05$; Fig. 6a). In
386 these regions, ciliates are the major consumers of phytoplankton. The average size of ciliates
387 is positively correlated with Chl *a* concentration in the regions of the South China Sea, the
388 East China Sea, and the Yellow Sea (Spearman r = 0.3, n = 151, $p < 0.001$; Fig 6b).
389
390 **Discussion**
391 One major mission in ecology study is to seek for general laws underlying patterns
392 across scales and ecosystems (Lawton 1999). It is particularly striking that some general
393 patterns and regularities can be found in the ecosystems despite significant environmental
394 and measurement noises. In the course of pursuing the quantitative insight into what controls
395 microzooplankton biomass and herbivory, we have found supporting evidence for three
396 remarkable general patterns across ecosystems which could benefit a better understanding of
397 the role of microzooplankton in the marine food web and for parameterizing and validating
398 biogeochemical models.
399 We have found that the biomass relationship between microzooplankton and
400 phytoplankton follows a simple power-law function with a scaling exponent of 0.77 ± 0.07
401 close to 3/4 (Fig. 3), which is highly consistent with the universal scaling law of predator and
402 prey biomass in diverse terrestrial and aquatic systems (Hatton et al. 2015). Similar exponent
403 is also obtained for the relationship between mesozooplankton biomass and their total prey
404 biomass (0.67 ± 0.12, Fig. 3c), serving as evidence for the predator-prey power law again. It
405 has been found surprisingly that from small zooplankton to large mammals, as long as they
406 are predators, their biomass exhibits a robust near 3/4 scaling with their total prey biomass.
407 Furthermore, the universal scaling exponents of these large-scale patterns are very similar to
408 the body mass allometries supported by theoretical explanations based on general features of
409 metabolic networks (Brown et al. 2004). However, the potential mechanisms linking large-

410 scale ecosystem patterns with finer-grain processes remain elusive in ecology (Hatton et al.
411 2015). Although our study cannot provide any direct clues for the underlying mechanisms,
412 the striking pattern observed in our dataset serves as strong supports for the predator-prey 3/4
413 power law.

414 In the increasing pattern between logarithmic microzooplankton and phytoplankton
415 biomass, no saturation at higher biomass was observed, which indicates that the
416 microzooplankton biomass is mainly determined by the bottom-up control even in the
417 eutrophic coastal waters in China seas. The negligible microzooplankton biomass increase at
418 higher phytoplankton biomass, which was found in the previous studies (Irigoien et al. 2004;
419 Yuan and Pollard 2018), should only occur in the extremely eutrophic environments
420 particularly in the lakes (Yuan and Pollard 2018) or occur during phytoplankton blooms
421 when the enlarged size and worsened quality of phytoplankton help them escape from the
422 grazing of microzooplankton (Irigoien et al. 2005). In general, the microzooplankton biomass
423 is mostly determined by phytoplankton biomass in the ocean, although it is also affected by
424 the top-down control from mesozooplankton.

425 Mesozooplankton not only feeds on microzooplankton but also competes with them
426 for the phytoplankton food. In the current study, we found that the mesozooplankton biomass
427 was significantly correlated with microzooplankton biomass and grazing rate (Fig. 2),
428 suggesting their influence on microzooplankton. Nevertheless, we also found the
429 mesozooplankton biomass increased with phytoplankton biomass with a larger scaling
430 exponent (Fig. 3c), which indicates that mesozooplankton may feed on more phytoplankton
431 than microzooplankton. Under such circumstances, microzooplankton will suffer less grazing
432 pressure from mesozooplankton but compete with them. Thus, the bottom-up control by
433 phytoplankton should be prevalent than top-down controls. In fact, it is difficult to quantify
434 the top-down control on microzooplankton because it is highly dynamic and depends on the

435 biological conditions in the study area including the community compositions of
436 mesozooplankton and the characteristics of other preys such as phytoplankton (Broglio et al.
437 2004). For instance, more grazing pressure on microzooplankton is found in the oligotrophic
438 ocean, because the small-sized phytoplankton is dominated and rarely consumed by copepods
439 (Calbet 2008). While the mesozooplankton is also found to shift their predation from
440 phytoplankton to microzooplankton over the course of blooms as the quality of
441 phytoplankton food decreases, which leads to a strong top-down control on
442 microzooplankton (Löder et al. 2011). As such, more investigations are highly necessary to
443 well define the top-down control by mesozooplankton (Calbet 2008).
444 For the effect of temperature on microzooplankton biomass, we cannot find the
445 biomass ratio of microzooplankton to phytoplankton decrease with increasing temperature as
446 in previous studies (Chen et al. 2012). Conversely, temperature shows little effect on the
447 microzooplankton biomass (Fig. 2, S1). According to *Eq. 3* and *5*, the expected effect of
448 temperature on microzooplankton biomass is based on one assumption that the gross growth
449 efficiency of microzooplankton is temperature insensitive. However, the gross growth
450 efficiency of some protists can increase with increasing temperature (Mayes et al.1997),
451 which would counteract the downward trend between biomass ratio and temperature.
452 Nevertheless, how temperature affects the gross growth efficiency of protist is still
453 controversial as decreased and constant gross growth efficiency with increasing temperature
454 had also been reported for protists (Rose et al. 2009). Thus, studying the temperature
455 sensitivity of gross growth efficiency of microzooplankton could be a prerequisite for
456 understanding how temperature affects the microzooplankton biomass.
457 We have also found that microzooplankton herbivory is highly regulated by
458 temperature and prey concentration (Fig. 4, 5), which can be well quantified following the
459 remarkable regularities in ecology, i.e., the metabolic theory of ecology and the functional

460 response of grazing. The metabolic theory of ecology reveals a regularity in scaling the
461 metabolic rates of organisms with their body size and temperature. Since all organisms share
462 the same fundamental metabolic reactions for energy transformation and biosynthesis, this
463 regularity has been scaled up from the individual to population, community, and the
464 ecosystem properties such as nutrient flux (Enquist et al. 2015). Despite disputes on the
465 adequacy of mechanistic basis (O'Connor et al. 2007), the metabolic theory of ecology
466 provides a quantitative framework allowing us to study how temperature affects the
467 microzooplankton grazing rate. The activation energy of microzooplankton biomass-specific
468 grazing rate estimated by the model with Holling III function is 0.51 eV (Table 1, 95% CI =
469 0.23 – 0.79 eV), which is not significantly different from the predicted value by the metabolic
470 theory of ecology (0.65 eV). Our estimate was consistent with the results of previous regional
471 (Chen and Liu 2015; Liu et al. 2019) and global studies (Chen et al. 2012), explicitly
472 elucidating the general pattern that microzooplankton grazing rate increase with increasing
473 temperature with average activation energy predicted by the metabolic theory of ecology
474 (Figs. 4, 5).

475 Functional response of grazing is another recognized regularity in ecology, albeit
476 several different mathematical formulations had been proposed (Gentleman et al. 2003). In
477 our study, although the natural microzooplankton communities in various locations are highly
478 diverse and involved in complicated trophic interactions, the bulk biomass-specific
479 microzooplankton grazing rate varies with phytoplankton concentration following the
480 generally observed functional response (Gentleman et al. 2003; Sandhu et al. 2019). The
481 biomass-specific grazing rate increases with increasing phytoplankton concentration (0 ~
482 about 25 μg C L^{-1}) and started to decrease above 25 μg C L^{-1} (Fig. 4, S2). This pattern can be
483 well depicted by the Holling III function (Figs. 4, 5). Compared with the Holling II response,
484 the Holling III response has been less used in the models in previous studies, which would

485 overlook the response of grazing at very low food concentrations (Sarnelle and Wilson,
486 2008). Our results suggested that Holling III response is more accurate to depict the
487 functional response of microzooplankton grazing in nature as the prey concentration varies in
488 a broad range among different ecosystems.

489 The accurate description of grazing functional response is crucial for modelling the
490 dynamics of planktonic ecosystems. As conceptual modelling approaches usually put
491 microzooplankton and their prey in "boxes" in the model structure, an overall functional
492 response describing how the bulk microzooplankton grazing rate varies with prey
493 concentration is more applicable for food web models, which entails estimation using the
494 natural microzooplankton assemblages (Sandhu et al. 2019). Due to the scarcity of field rate
495 measurements, the parameters used in many biogeochemical models were usually from the
496 laboratory experiments on single species grazing. Recently, some studies have parameterized
497 the functional responses using field datasets of dilution experiments (Buitentuis et al. 2011).
498 By contrast, our study not only used the natural microzooplankton communities but also
499 incorporated the effect of temperature into the grazing functions since temperature also exerts
500 a profound influence on microzooplankton grazing rate (Hansen et al. 1997, Chen et al.
501 2012). The parameters in our models, therefore, could be closer to natural situations. The
502 maximum ingestion rate (I_0) in our models are all within the range of maximum ingestion rate
503 of individual ciliates species (2.4 - 11.5 d^{-1} at 20°C), but a bit higher than that of individual
504 heterotrophic dinoflagellates (0.26 - 4.08 d^{-1} at 20°C; Hansen et al. 1997). The high ingestion
505 rate could be due to the fact that ciliates are dominant in the microzooplankton community in
506 the study area as we found the fraction of heterotrophic dinoflagellates in all four regions is
507 relatively low (Fig. 6a). To compare with the grazing rate derived from dilution approach, we
508 converted the maximum ingestion rate to the clearance rate of microzooplankton community
509 based on their definitions (Båmstedt et al. 2000) using the mean carbon biomass of

510 phytoplankton and microzooplankton (121.75 and 13.47 μg C L^{-1}, respectively). For such
511 community, the grazing rate is 0.53 d^{-1} at 15°C (Holling III) which is close to the average
512 grazing rate estimated from dilution experiments in the coastal and estuarine systems (Calbet
513 and Landry 2004). The half-saturation constants (K_p) were converted to μM N equivalents
514 assuming a Redfield ratio of carbon to nitrogen (C:N) being 6.625 (Table 1). The estimates
515 are all within the ranges of K_p for individual species obtained from laboratory experiments
516 (ciliates: K_{p2} = 0.06 - 2.46 μM N; heterotrophic dinoflagellates: K_{p2} = 0.2 - 11 μM N at 20 °C;
517 Hansen et al. 1997). Whereas our results suggested a lower K_p (0.27 μM N) for Holling III
518 function than that used in some ecosystem models, which were usually 0.5 μM N (Chen and
519 Smith 2018).
520 Built on the metabolic theory of ecology and the functional response of grazing, our
521 model illustrates the effect of temperature and phytoplankton biomass on the biomass-
522 specific grazing rate quantitatively, although the variation of biomass-specific grazing rate
523 the model can explain is relatively low (Table 1). The explanatory power of the GAMs
524 without parametric constraints is also limited (30%; Fig. S2). The explanatory power of the
525 model could increase if more relevant factors such as the prey size and the potential effects of
526 mesozooplankton were incorporated. However, these factors are hard to be quantified in the
527 field experiments and usually tangling together in nature. In addition, potential sources of
528 errors would undermine the accuracy of parameter estimates and model performance. One
529 potential problem lies in the estimates of grazer biomass. The small metazoan such as
530 copepod nauplii which are potentially important contributors to microzooplankton biomass in
531 the coastal waters were not included in the grazer biomass because the sampling method
532 hinders an exact estimate for their abundance. Similarly, due to the methodological issue, we
533 included some mixotrophic flagellates and dinoflagellates in the total grazer biomass as they
534 cannot be unequivocally distinguished from obligate heterotrophs based on the analysis of

Lugol's-preserved samples. The mixotrophs are vague components of microzooplankton of which the contributions to the community grazing rate are difficult to be gauged. In addition, the nanoflagellates less than 20 μm were also excluded from the grazer biomass in our study. Although this grazer group does not belong to microzooplankton according to the technique definitions, they could be the major consumers of picophytoplankton and significantly contribute to the grazing mortality assessed by dilution experiments in the offshore waters. However, the percentage they account for in the total microzooplankton biomass should be small due to their small size. The other potential issue existed in the estimates of phytoplankton biomass. Due to the lack of the data of phytoplankton biomass, we used model-derived C:Chl ratio which is a function of temperature, nutrient, and light (Supplementary information). Although this approach will introduce errors into the model, it can reflect the variation of the phytoplankton biomass better than a constant C:Chl ratio. Furthermore, a common issue in estimating microzooplankton community grazing rate is that not all preys are equally accessible to all microzooplankton involved in the estimates. The model, therefore, suffers from the above methodological limitations and could be improved with more accurate estimates of grazer and prey biomass from more detailed information related to plankton community compositions.

Despite the highly diverse environmental conditions and potential measurement bias, our dataset reveals certain patterns which are consistent with the regularities in ecology including the predator-prey power law, the metabolic theory of ecology, and the functional response of grazing. These patterns and regularities provide useful avenues for studying and predicting the dynamics of marine microbial food web and how it will respond to climate changes. They also pose significant challenges for modelling the functioning of ecosystems. Ecosystem model outputs should be consistent with the general patterns observed from empirical studies. For instance, the global biogeochemical model outputs should be able to

560 reproduce the power-law relationships between predator and prey biomass. In addition, the
561 regularities in ecology offer frameworks for quantifying the important compartments and
562 processes in ecosystems. Based on the metabolic theory of ecology, the functional response
563 of grazers, and the predator-prey power laws, we studied the primary factors determining the
564 microzooplankton grazing rate and biomass in China seas and constructed models to well
565 quantify them (Figs. 4, 5). Thus, our models supported by the regularities in ecology can be
566 used for validating regional biogeochemical models.
567 In addition to the factors examined under the framework of certain regularities in
568 ecology, attempts had also been made to explore the effects of microzooplankton community
569 composition on their biomass and herbivory. The microzooplankton community composition
570 was found to be uncorrelated with microzooplankton grazing rate (Fig. 2). It is probably
571 because that the fraction of heterotrophic dinoflagellates is a rough index of
572 microzooplankton community composition without detailed information at the species level.
573 Hence, we did not incorporate it in the above model. The fraction of heterotrophic
574 dinoflagellates increased with increasing Chl *a* concentration, which is consistent with
575 previous studies (Sherr and Sherr 2007) and implies a shift in microzooplankton community
576 composition from ciliates to heterotrophic dinoflagellates across the gradients of trophic
577 status. In the mesotrophic and oligotrophic regions (the South China Sea, the East China Sea,
578 and the Yellow Sea), ciliates were the dominant groups of the microzooplankton community
579 (Fig. 6). The community structure of ciliates in these regions also responds to the change of
580 trophic status as their average size increased with Chl *a* concentration (Fig. 6b). There could
581 be a shift in community structure of ciliates from small aloricate ciliates to large loricate
582 ciliates (i.e. Tintinnids) with increasing Chl *a* concentration.
583
584 **Conclusion**

585 Our results revealed some general patterns that are consistent with the metabolic
586 theory of ecology, the functional response of grazing, and the predator-prey power law,
587 despite the geographical distance and highly dynamic environments, as well as
588 methodological limitations. These general patterns provide us with common conceptual
589 frameworks to understand the interactions between phytoplankton and their grazers and
590 predict how the plankton ecosystem will respond to climate changes. Through this study, we
591 gained comprehensive and quantitative insights into what factors control microzooplankton
592 biomass and herbivory across China seas. The model derived from field measurements can be
593 used to predict the microzooplankton biomass and grazing rates in the ocean and for
594 parameterizing and validating ocean ecosystem models, particularly in China seas.
595

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760 **Figure legends**

761 Fig. 1 Map of sampling stations in the marginal seas of China. (HK: Hong Kong coastal
762 waters; XM: Xiamen coastal waters; SCS: South China Sea; ECS: East China Sea; YS:
763 Yellow Sea)

764

765 Fig. 2 The correlations of temperature, *ln phytoplankton biomass* (Phytoplankton), *ln*
766 *microzooplankton biomass* (Microzoo), *ln grazing rate* (Grazing.rate), *ln mesozooplankton*
767 *biomass* (Mesozoo) (n = 58), and microzooplankton community composition represented by
768 fraction of heterotrophic dinoflagellates (HDF.ratio). The lower-off diagonal shows the
769 scatter plots between every two factors with correlation ellipses and lowess regression lines
770 (red lines), the red dots are the points of the mean of x and y; the diagonal shows the
771 histograms of every factor; the upper-off diagonal reports the Spearman correlations with
772 significant levels (*$p < 0.5$; **$p < 0.1$; ***$p < 0.001$). Noting that the HDF.ratio is arcsine
773 square-root transformed ratio (arcsin$\sqrt{HDF-ratio}$).

774

775 Fig. 3 (a) Relationship between phytoplankton biomass and total microzooplankton biomass.
776 The solid line is the linear regression model, dashed lines denote 95% confidential intervals.
777 (b) Relationship between phytoplankton biomass and the biomass of ciliates and
778 heterotrophic dinoflagellates. (c) Relationship between the biomass of microzooplankton /
779 phytoplankton / total prey (microzooplankton + phytoplankton) and mesozooplankton. All
780 solid lines are fitted by the OLS linear regression. γ is the slope of the regression model (the
781 scaling exponent in *Eq. 1*). n is the number of data used in the corresponding regression
782 models.

783

784 Fig. 4 (a) Effect of phytoplankton biomass on the biomass-specific microzooplankton grazing
785 rate (m/B_z). Contour plots show the density distribution of original observations; the solid
786 line, dashed line and dotted lines are the model (Holling III) results predicting the
787 relationship of m/B_z and phytoplankton biomass when temperatures are 15°C, 25°C, and
788 35°C, respectively. (b) Effect of temperature on the log-transformed biomass-specific
789 microzooplankton grazing rate (ln (m/B_z)). Contour plots show the density distribution of
790 original observations; the solid line, dashed line and dotted lines are the model (Holling III)
791 results predicting the relationship of ln (m/B_z) and temperature at three levels of
792 phytoplankton concentration: 5, 50, 250 μg C L^{-1}, respectively.
793
794 Fig. 5 (a) Predicted log-transformed biomass-specific microzooplankton grazing rates (ln
795 (m/B_z)) based on the model with Holling III function ($R^2 = 0.27$). (b) Experimental
796 microzooplankton community grazing rates compared to the corresponding modelled rates
797 (model with Holling III function). Dashed line: 1:1 relationships.
798
799 Fig. 6 (a) Boxplot of the fraction of heterotrophic dinoflagellates (HDF ratio) in the four
800 regions. (b) Relationship of Chl *a* concentration and average size of ciliates. The dashed line
801 is the linear regression on the data excluding Coastal stations (grey dots). (Coastal stations:
802 stations in Hong Kong and Xiamen coastal waters; SCS: South China Sea; ECS: East China
803 Sea; YS: Yellow Sea)