A unified framework for understanding biomass ratio of herbivores to producers with a field test of plankton

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Abstract

The biomass ratio of herbivores to producers reflects the structure of a community. Four primary factors have been proposed to affect this ratio, including production rate, defense traits, and nutrient contents of producers as well as predation by carnivores. However, the relative importance of these factors across natural communities is elusive, in part because of the lack of a framework for quantitatively comparing their effect sizes. Here, we develop a framework based on Lotka-Volterra equations for examining the relative importance among these factors in determining the biomass ratio. We further utilize it to analyze plankton communities in experimental ponds with different carnivore (fish) abundance and light input. We found that all four factors contributed significantly to the biomass ratio, but carnivore abundance had the largest effect size, followed by the stoichiometric nutrient content. The present framework is useful for quantifying relative roles of these factors shaping terrestrial and aquatic communities.

Introduction

The biomass ratio of herbivores (H) to primary producers (P) reflects the structure of a community. Because of its fundamental importance, a large number of studies have empirically and theoretically examined the H/P biomass ratio and showed that factors related with either bottom-up or top-down forces play crucial roles in determining it. These factors are production rate (Coe et al. 1976; Power 1992; Word et al. 2015), defense traits (Coley et al. 1985; Wolfe et al. 1997; Poelman et al. 2008; Moony et al. 2010) and nutrient contents of producers (Sterner & Elser 2002; Cebrian et al. 2009), and predation rate by carnivores including food-chain length (Hairston et al. 1960; Hanley & La Pierre, 2015; Carpenter et al. 1985). However, the relative importance of these factors across natural communities is elusive, since few studies have considered a theoretical framework for explicitly examining the relative importance of these effects on community structure.

The top-down forces are determined by the feeding rate and the abundance of consumers at higher trophic levels (Shurin et al. 2002; Vanni et al. 1990), while the bottom-up forces are determined by the primary production rate regulated by supplies of nutrients (Schindler 1974; Smith & Schindler 2009) and light (Karlsson et al. 2009). In addition, both in terrestrial and aquatic producers including vascular plants and algae, chemical and physical defense traits are well documented as factors limiting herbivory (Wolfe et al. 1997, Agrawal

& Fishbein 2006; Pančić & Kiørboe 2018), indicating that edibility or defense traits of primary producers to consumers are crucial factors in determining the biomass ratio (Coley et al. 1985; Moony et al. 2010). The nutrient content of producers is also viewed as a prime factor in regulatingH/P mass ratio (Cebrian 1999; Sterner & Elser 2002; Cebrian et al. 2009; Konno 2016). Depending on supply rates of light and nutrients, contents of biologically important elements such as nitrogen and phosphorus relative to carbon vary widely among primary producers (Sterner & Elser 2002). Since herbivore growth depends highly on the elemental content of primary producers (Frost et al. 2006; Urabe et al. 2018), the stoichiometric mismatch in carbon to phosphorus or nitrogen ratios between primary producers and herbivores likely results in decreasing the H/P ratio (Cebrian 1999; Sterner & Elser, 2002Cebrian 2009). However, no study has yet formulated how these different factors affect simultaneously the H/P biomass ratio.

In this study, therefore, we constructed a framework for assessing the relative importance of primary production rate, defense traits and nutrient contents of producers, and predation rate on the H/Pratio in natural communities based on the classic Lotka-Volterra equations. Then, by fitting biomass data from planktonic producers and herbivores in experimental ponds to the model, we tested a hypothesis if these factors simultaneously affect the H/P ratio and examined the relative contributions of these factors to the biomass ratio. In experimental ponds, we manipulated light input by shading, and top-down force was assessed by regularly monitoring abundance of planktivorous fishes. We show that top-down forces and stoichiometry of primary producers played pivotal roles in determining the H/P ratio, followed by defense traits and primary production in the plankton communities.

Theoretical framework

A unified framework model of H/P ratio

Based on the Lotka-Volterra equations, biomass dynamics of primary producers (P) and herbivores (H) are described as follows:

$dP/dt = g(P)P - xP - f(P)PH, dH/dt = kf(P)PH - mH,$ (1)

where $g(P)$ is the per capita rate of biomass-specific primary production and may be a function of P due to the density-dependent growth, $f(P)$ is the per capita grazing rate of herbivores and also may be function of P depending on the functional response, x is the biomass-specific loss rate of primary producers other than grazing loss, k is the conversion efficiency of herbivores that is a fraction of ingested food converted into the herbivore biomass, and m is the per capita mortality rate of herbivores due to predation and other factors. If we assume both$g(P)$ and $f(P)$ are constant values, eq. (1) is basically the Lotka-Volterra model, while it is an expansion of the Rosenzweig-MacArthur model if we assume the logistic growth for$g(P)$ and the Michaelis-Menten equation (Holling type II functional responses) for $f(P)$. At the equilibrium state, i.e. $dP/dt = 0$ and $dH/dt = 0$, abundance of producers (P) and consumers (H) can be represented as:

$H = [g(P) - x](k/m)P.$ (2)

Thus, the relationship between H and P is not affected by types of the functional response in herbivores ($f(P)$). If we set g(P) as the biomass-specific primary production rate at the equilibrium state as in simple Lotka-Volterra equations (i.e.$g(P) = g$), then H/P ratio can be expressed with log transformation as:

$\log(H/P) = \log(k) + \log(g - x) - \log(m).$ (3)

At the equilibrium state, $(g - x)P$ is the amount of primary production that herbivores consumed ($f(P)PH$). Thus, if we define $\beta = f(P)PH/(Pg)$ $(= 1 - x/g)$ as the grazed fraction of primary production that herbivores consume, i.e. edible fraction of the producers or inefficiency of the producers' defensive traits, the H/P ratio can be expressed as:

$\log(H/P) = \log(k) + \log(\beta) + \log(g) - \log(m).$ (4)

This equation implies that the H/P biomass ratio on a log scale is affected additively by the specific primary production rate ($\log(g)$), the grazable fraction of primary production ($\log(\beta)$), the conversion efficiency (log(k)), and mortality rate of herbivores (log(m)). According to this equation, communities having few amounts of carnivores (with small$m$) will exhibit high herbivore biomass relative to producer biomass (H/P) while those with low primary production (with small$g$) due to, for example, low light supply will show low H/Pratio. Increase in defended producers such as armored plants or decrease in edible producers will decrease β by increasing the loss rate$x$ due to the cost of defense), and will result in decreasing$H$ $/P$ ratio. Finally, when nutritional values of producers decrease, the conversion efficiency of herbivores (k) should be low, which in turn decrease the H/P biomass ratio.

Test of the model with the plankton community

To apply eq. (4) to a natural community, some modifications are necessary. Here, we consider a plankton community composed of algae and zooplankton. Since carbon content of food relative to nutrient contents such as nitrogen and phosphorus, e.g. carbon to phosphorus ratio, is one of the most important food properties affecting growth efficiency in herbivore plankton ((Frost et al. 2006; Urabe et al. 2018), k can be expressed as:

$k = q_1 \times a_{\text{nut}}^{\varepsilon 1}$, (5)

where a_{nut} is carbon content relative to nutrient content of primary producers and q_1 is the conversion factor for adjusting to biomass unit. In this study, we applied a power function with coefficient of ε_1 as a first order approximation since effects of this factor on H/Pbiomass ratio may not be linearly related to plant nutrient content. For example, if ε_1 is much smaller than zero, it means that negative effects of carbon to phosphorus ratio of algal food on herbivore's k are more remarkable when carbon to phosphorus ratio levels are high comparing to the case when carbon to phosphorus ratios are low. However, if this factor does not affect the H/P biomass ratio, $\varepsilon_1 = 0$ and k is constant.

Since herbivore plankton cannot efficiently graze on larger phytoplankton due to a gape limitation (Lampert and Sommer 2007), the feeding efficiency of herbivores or defense efficiency of producers' resistance traits, β , would be related to the fraction of edible algae in terms of size as follows:

$\beta = q_2 \times a_{\text{edi}}^{\varepsilon 2}$, (6)

where a_{edi} is a trait of primary producers determining edibility, q_2 is a factor for converting the traits to edible efficiency, and ε_2 is how effective the trait is in defending against grazing. We expect $\varepsilon_2 = 0$ if this factor does not matter in regulating theH/P biomass ratio but $\varepsilon_2 > 0$ if it plays a role. Similarly, g can be described as

$g = q_3 \times \mu^{\varepsilon 3}$, (7)

where μ is the specific growth rate of producers,q_3 is a conversion factor, and $\varepsilon_3$is the effects of μ on growth rate. Again, we expect that ε_3 [?] 0 if g plays a role in determining theH/P ratio. Finally, assuming Holling type I functional response of carnivores, mortality rate of herbivores, m , is expressed as:

$m = q_4 \times \vartheta^{\varepsilon 4}$, (8)

where ϑ is abundance of carnivores, q_4 is specific predation rate, and ε_4 is the effect size of carnivore abundance on m .

By inserting eq. (5) - (8) to eq. (4), effects of factors on theH/P biomass ratio is formulated as:

$\log(H/P) = \varepsilon_1 \log(a_{\text{nut}}) + \varepsilon_2 \log(a_{\text{edi}}) + \varepsilon_3 \log(\mu) - \varepsilon_4 \log(\vartheta) + \gamma$, (9)

where γ is $\log(q_1) + \log(q_2) + \log(q_3) - \log(q_4)$. If differences in the H/P ratio among communities are regulated by growth rate (μ), edibility (a_{edi}), and nutrient contents (a_{nut}) of producers as well as predation by carnivores (ϑ), we expected non-zero values for ε_1 to ε_4. Thus, eq. (9) can be used to test the hypotheses if all of these factors simultaneously affect the$H$ $/P$ ratio and to examine the relative importance among these factors in determining the ratio in given communities by directly fitting it to data in natural communities. These were substantiated using data from natural plankton communities in experimental ponds where primary production rate was manipulated with different abundance of carnivore fish.

Materials and methods.

Experimental design

The experiment was carried out at two ponds (pond ID 217 and 218) located at the Cornell University Experimental Ponds Facility in Ithaca, NY, USA during June 4 to August 28, 2016 (Fig. 1). Each pond has a 0.09 ha surface area (30 × 30 m) and 1.5 m deep. To initiate the experiment, we equally divide each of the two ponds into four sections using vinyl coated canvas curtains, and randomly assigned the four sections to either high shade (64% shading), mid shade (47% shading), low shade (33% shading), or no shade treatments (no shading). Shading in each treatment was made using opaque floating mats (6 m diameter as in Fig. 1 of Yamamichi et al., 2018). Details of the experimental design are described in supplementary methods.

Samplings

Sampling was performed biweekly during the experiment by collecting 11-L water from the bottom to surface with repeated deployment of a 2.2-L tube sampler. Duplicate water samples were taken in each treatment (section) of the two ponds, and used for measuring water chemistry and abundance of phytoplankton and small zooplankton (copepod nauplii and rotifers). Besides these samples, crustacean plankton were collected by filtering a total of ca. 30-L of vertically integrated water from three different sites in each section with a 100 µm mesh net, and fixed with 99% ethanol for enumeration. During sampling, we measured vertical profiles of water temperature, dissolved oxygen (DO) concentration, conductivity and pH using a multiparameter probe (600XLM, YSI) at each section of both ponds. We also measured photosynthetic active radiation (PAR) using a spherical quantum sensor (LI-193; LiCor, Inc.) at 10-cm intervals from the surface to bottom and calculated extinction coefficients of PAR in the water.

Chemical analyses and estimation of plankton biomass

In the laboratory, sestonic particles in the water were concentrated onto pre-combusted GF/F (0.7 µm mesh size) filters. Seston carbon and phosphorus were determined with a CN analyzer (model 2400; Perkin-Elmer, Inc.) and by the ascorbate-reduced molybdenum-blue method, respectively. Chlorophyll *a* was extracted by 90% ethanol for 24 hours in the dark and quantified using a fluorometer (TD-700; Turner Designs, Inc.). For measuring abundance of phytoplankton and small zooplankton, 250 to 500-mL of the collected water were fixed with dilute Lugol's solution and concentrated into 20 mL by gravity. For each phytoplankton taxa, number of cells in 0.2 to 1.0 mL of the concentrated sample were counted and size of 20 to 50 cells were measured for estimating cell biovolume. For small zooplankton, we counted all individuals in the concentrated samples according to taxa with measurements of body length and width. For crustacean zooplankton, we categorized size classes according to body length and counted individuals of each size category in 10% of the sample. Most zooplankton including small cladocerans and copepods are known to graze efficiently on algae smaller than 30 µm in size (Lampert and Sommer 2007). Therefore, we estimated the fraction of phytoplankton smaller than 30 µm for the major axis of the cell or colony in the total phytoplankton biomass as edible fraction (α_{edi}). Carbon biomass of phytoplankton was estimated using the cell abundance and biovolume (μm^3) and that of zooplankton using the individual abundance and length-weight relationships. Details of these methods were described in supplemental methods.

Primary production rate

In each section of the Pond 217 and 218, we routinely measured photosynthetic and community respiration rate by measuring changes in O_2 concentration rate in light and dark bottles (Wetzel and Likens 2010). Then, net-photosynthetic rate – irradiance curve (P-I curve) was estimated for each section of the both ponds. Using the P-I curve, extinction coefficient and daily changes in photosynthetically available radiation (PAR) in the water column, we estimated specific production rate (μ: µg O_2chl-*a* $^{-1}$ d^{-1}). To minimize specificity in light conditions at measured date, we calculated µ using temporal profile of PAR for three days before each sampling date. Then average value for these three days was used in analysis. Details of the method estimating primary production rate were described in supplemental methods.

Fish abundance

In each of the different treatment sections, fish were sampled using minnow traps with carp bait. The traps were placed for 3 to 7 days in each section of the ponds, and wet weight of the collected fish (g WW) was measured. Total weight of fish per trap per day was used as a relative measure of fish abundance (θ: catch per unit effort). When no fish were collected, we set $\theta = 1$. Note that shading may have affected on number of fish collected by the traps. In this case, CPUE can be viewed as a measure of fish activity and therefore an indication of predation rate (Hairston 1987).

Statistical analyses

We used mean values from June 10 to August 27 for all variables in the following statistical analyses (Table S1). Relationships among phytoplankton and zooplankton biomasses, specific production rate and fish abundance were examined by correlation analysis. To test differences in phytoplankton and zooplankton community composition among the treatments and between the two ponds, permutational multivariate analysis of variance (PERMANOVA) was performed by the adonis() function in R package "vegan" (Oksanen et al. 2018). In this test, we used 999 permutations and the Euclidean distance both for phytoplankton and zooplankton communities as an index of dissimilarity in the community.

We applied mean phytoplankton carbon biomass, zooplankton carbon biomass, fish abundance, specific production rate and fraction of edible phytoplankton for P , H , ϑ , μ , and$a_{\epsilon\delta\iota}$ in eq. (9), respectively. For$a_{\nu\upsilon\tau}$, we focused on phosphorus since freshwater limnetic ecosystems are primarily phosphorus limited (Schindler 1974; Smith a& Schindler 2009) and since growth of zooplankton is affected by relative phosphorus in algae (Frost et al. 2006, Urabe et al. 2018). Specifically, we used the carbon to phosphorus ratio of seston as a surrogate for $a_{\nu\upsilon\tau}$ because this ratio has been generally used in theories of ecological stoichiometry (Sterner & Elser 2002). Thus, we expected lower H/P ratios at larger values of seston carbon to phosphorus ratio. To examine effects of these explanatory variables on the H/P ratio, a simple regression analyses was performed. Then, after checking multicollinearity among the explanatory variables by variance inflation factors (Kennedy 2008), we fitted these data to eq. (9) using a lm function of R 3.2.1 (R core team, 2018) with the examination of Akaike's information criterion. In this analysis, 95% confidence intervals of the regression coefficients were estimated using bootstrapping with a residual resampling procedure (Moulton & Zeger 1991) and 1999 replicates. Since eq. (9) indicates an*a priori* effect direction of a given variable, we estimated upper or lower one-tailed 95% confidence intervals (100 and 5 percentiles) for the explanatory variables according to negative or positive effects predicted by eq. (9). Effect sizes of these explanatory variables were assessed using standardized regression coefficients of the multiple regression. Finally, to examine whether effects of explanatory variables on the H/P mass ratio were independent of each other and significant, we performed partial regression analysis with residual leverage plot according to Sall (1990) using leveragePlots() in R package "car" (Fox &bWeisberg 2011).

Results

Photosynthetic active radiation (PAR) in the water column was lower in the sections with larger shaded areas throughout the experiment (Fig. 2a). Water temperature varied from 18 to 25ºC during the experiment but showed no notable differences in mean values of the water columns or the vertical profiles among the four treatments of the two ponds regardless of degree of the shading (Fig. S1(a), S2). In all treatments, pH values gradually decreased towards the end of experiment and were higher in pond 218 (Fig. S1(b)). Dissolved oxygen (DO) concentration varied among the treatments and between the ponds, but were within the range of 5 to 12 mg L^{-1} (Fig. S1(c)).

Phytoplankton biomass (mg C L^{-1}) correlated significantly with chlorophyll a (µg L^{-1}) ($r = 0.702$, $p < 0.001$), varied temporally (Fig. S3), and was generally higher in the no shade treatments, followed by the low shade treatments in both Pond 217 and 218 (Fig. 2b). Zooplankton biomass also varied temporally (Fig. S4) and was generally lower in low shade treatments compared with other treatments (Fig. 2b). To remove effects of the initial conditions, we calculated mean phytoplankton (P) and zooplankton biomasses (H) in samples collected during the period from June 10 to August 28. Phytoplankton biomass was lower in Pond 218 regardless of the treatments, but such a notable difference between the ponds was not found in zooplankton biomass. Accordingly, no significant relationship was found between the mean values of phytoplankton and zooplankton biomass (Fig. 2a).

Both for zooplankton and phytoplankton, the community compositions were similar among the four treatments within the same pond (PERMANOVA,F =0.993, p =0.42 for algae; F =1.23, p =0.34 for zooplankton) but significantly differed between the two ponds (F =1.59, p =0.017 for algae; F =3.82, p =0.047 for zooplankton). In zooplankton communities, copepods predominated in pond 217, while large cladocerans including *Daphnia* occurred abundantly in pond 218 (Fig. S5). In phytoplankton communities, Euglenophyceae and Chrysophyceae occurred abundantly in pond 217, and Euglenophyceae and Dinoflagellata dominated in pond 218 (Fig. S6). In all treatments, cyanobacteria biomass was less than 10%. According to previous knowledge (Lampert and Sommer 2007), we defined that phytoplankton smaller than 30 µm for the major axis of the cell or colony were edible. Then, we estimated the ratio of edible phytoplankton biomass to total phytoplankton biomass as a fraction of the edible phytoplankton ($a_{\epsilon\delta\iota}$). It varied from near zero to almost one in all the treatments of both ponds (Fig. S3). Seston carbon to phosphorus ratio varied from 90 to 310 (Fig. S7) and was higher for treatments with less shade in pond 217, while in pond 218 seston carbon to phosphorus ratio did not vary among the treatments (Fig. 3b).

Chlorophyll *a* specific daily production rate estimated from the P-I curve (Fig. S8) varied temporally depending on weather conditions but was, in general, higher in treatments with less shade (Fig. 3c). Daily primary production rates also varied and were higher in treatments with less shade in pond 217, although among the treatments in pond 218 the levels were similar (Fig. S4).

Observations of fish abundance in each treatment section, determined using minnow traps, showed that banded killifish (*Fundulus diaphanus*) and fathead minnow (*Pimephales promelas*) were present (Fig. S9). Both fish species were collected on all sampling dates in pond 217 but were not caught after June 21 in pond 218 (Fig. S4). Thus, mean abundance of these fish species (ϑ) was higher in pond 217 than in pond 218 (Fig. 3d). In the former pond, fish abundance also varied among the treatments, and was greater in no shade treatments than in any of other treatments. Neither mean of zooplankton biomass (r = 0.310, p = 0.45) nor mean of specific production rate (μ) (r = 0.247, p = 0.56) were significantly related to mean fish abundance (ϑ).

Throughout the study period, the mass ratio of zooplankton and phytoplankton varied temporally (Fig. S4). Among the treatments, the temporal mean of this ratio (H/P ratio) was highest in the mid shade treatment and lowest in the low shade treatment in both ponds (Fig. 3). However, the H/P mass ratio was higher in pond 218 than in pond 217. A significant relationship was not detected between theH/P mass ratio and mean PAR in the water column (Fig. 2a;r = 0.155, p = 0.714), mean frequency of edible phytoplankton ($a_{\epsilon\delta\iota}$) (Fig. 3a; r = 0.241,p = 0.565), mean seston carbon to phosphorus ratio ($a_{\nu\upsilon\tau}$) (Fig. 3b; r = -0.265, p = 0.523), and mean specific production rate (μ) (Fig. 3c; r= 0.081, p = 0.849), whilst a significantly negative relationship was detected between the H/P mass ratio and mean fish abundance (ϑ) (Fig. 3d; r = -0.818, p = 0.013).

We fitted the H/P mass ratio by $a_{\epsilon\delta\iota}$,$a_{\nu\upsilon\tau}$, μ , and ϑ among treatments in the two ponds using a multiple regression linear model. The variance inflation factors (VIFs) for these explanatory variables ranged from 1.05 to 2.38, indicating a low probability of multicollinearity among explanatory variables. Moreover, an analysis with the generalized linear model showed that the model including all of these parameters had the lowest value of Akaike's Information criterion (Table S2), indicating that it was the best model. The multiple regression analysis revealed that all of these four variables were indeed significant, as evidenced by the 95% confidence intervals (CIs) that were smaller or larger than zero, and explained 94% of variance in the H/P ratio (Table 1). In addition, partial regression analysis showed that all the partial correlation coefficients of these factors were significant (Fig. 4), indicating that effects of these explanatory variables on the H/Pmass ratio were independent of each other. More importantly, the regression coefficient was significantly smaller than zero for seston carbon to phosphorus ratio ($a_{\nu\upsilon\tau}$) while it did not significantly differ from one for edible phytoplankton frequency ($a_{\epsilon\delta\iota}$) and specific production rate (μ), and was smaller than one but larger than zero for fish abundance (ϑ). To examine the effect sizes of these explanatory variables on H/P mass ratio, we estimated standardized regression coefficients. The absolute value of the coefficients showed that the effect size on the H/P mass ratio was highest for ϑ , followed by $a_{\nu\upsilon\tau}$ (Table 1).

Discussion

Since the green world hypothesis proposed by Hairston et al. (1960), a number of studies have examined effects of primary production and predation (Carpenter et al. 1985; Power 1992; Shurin et al. 2002; Hambright 1994; Hanley & La Pierre 2015; Ward et al. 2015;), and those of anti-herbivory defense or edibility of producers (Coley et al. 1985; Wolfe et al.1997; Agrawal & Fishbein 2006; Poelman et al. 2008; Mooney et al. 2010) on herbivore abundance relative to producer abundance (theH/P ratio). Although elemental stoichiometry of primary producers has often been considered as a factor that determines herbivore biomass (Ceberian 1999; Sterner & Elser 2002; Ceberian et al. 2009), few studies have experimentally examined whether nutrient contents can regulate herbivore relative to producer abundance at a community level (e.g., Urabe et al. 2002). Moreover, to the best of our knowledge, no studies have determined and compared the relative importance of these factors in natural communities, presumably because no theoretical framework has been developed for quantitatively examining their effects in a comparable way. This is the first study to examine simultaneously the effects of those four factors on the H/P mass ratio in a single natural community. By fitting our observed data to a modified Lotka-Volterra-based model, we have shown that in addition to primary production and predation, edibility and stoichiometry of primary producers plays pivotal roles in regulating the H/P mass ratio.

Our model, based on Lotka-Volterra equations, is derived from the equilibrium state of a system. In this study, both phytoplankton and zooplankton biomass dynamically changed in all the treatments throughout the experiment, indicating that no community in this study reached equilibrium. However, theoretically, temporal means of herbivore and producer abundances for at least one oscillation cycle should coincide with the equilibrium abundances in the Lotka-Volterra model (Haberman 1977). Theoretical and experimental studies have also shown that one oscillation cycle occurred within < 50 days in zooplankton-phytoplankton dynamics (McCauley & Murdoch, 1990). Thus, the present experimental run would be sufficiently long so that the temporal mean values among samples collected biweekly were close to equilibrium values in zooplankton-phytoplankton dynamics.

Among the factors we examined, fish abundance had the greatest effect on changes in the H/P mass ratio. This result might be caused by the fact that, in comparison with planktonic organisms, fish abundance was temporally more stable due to their longer life span. In all sections (treatments) of pond 217, where planktivorous fishes were abundant, the density of large cladocerans in the zooplankton community was low. The result was in accordance with the well-known fact that planktivorous fish prey selectively on larger zooplankton species (Carpenter et al. 1985; Hambright 1994; Lampert &Sommer 2007). Other than the direct effect of predation, several studies suggest that fish can indirectly affect zooplankton biomass by stimulating primary productivity through nutrient recycling (Williamson et al. 2018). However, in this study, the specific production rate (μ) was not related to fish abundance, suggesting that the net impact of fish abundance on the H/P mass ratio was largely attributable to a direct top-down force on zooplankton biomass rather than indirect bottom-up forcing through nutrient cycling.

A number of studies have argued that the H/P mass ratio is regulated by the efficacy of the producer's anti-predator defense (Coley et al. 1985; Poelman et al. 2008; Moony et al. 2010). In this study, occurrence of algae that might have contained some toxicities for zooplankton such as cyanobacteria (Lampert & Sommer 2007; Smith & Schindler 2009) was limited. Therefore, we focused physical defense of phytoplankton. Since most herbivore plankton cannot efficiently graze phytoplankton species with a cellular or colony size larger than 30 µm (Lampert and Sommer 2007), enlargement of cellular or colony size can be viewed as a defense trait against herbivory (Pančić & Kiørboe 2018). Therefore, we examined fraction of edible phytoplankton

($\alpha_{\epsilon\delta\iota}$) on H/P mass ratio. No significant relationship was detected between these in simple regression. However, if we considered only the treatments in pond 218 where fish abundance was limited, the H/P ratio tended to increase with this fraction. Indeed, $\alpha_{\epsilon\delta\iota}$ was significantly related with the mass ratio when other factors, such as fish abundance, were simultaneously considered in the multiple regression analysis. These results indicate that edibility or a defense trait such as enlargement of cellular or colony sizes indeed play a role in regulating H/Pmass ratio, even in aquatic communities.

Other than the defense trait, nutritional values or nutrient contents of producers have often been proposed as crucial factors determining the abundance of herbivores relative to that of producers (Cebrian 1999; Srerner & Elser 2002; Ceberian 2009; Konno, 2016). In this study cyanobacteria biomass was less than 10%, suggesting that deficiency of polyunsaturated fatty acids were not prime factors affecting quality of phytoplankton food for zooplankton in this study (Urabe et al. 2018). Cebrian (1999) argued that a lower H/P mass ratio in terrestrial communities compared with aquatic communities is attributable to lower nitrogen and phosphorus contents relative to carbon in terrestrial producers. In this study, we focused on phosphorus as the main nutrient since phosphorus limitation of herbivore growth at an individual level has been repeatedly pointed out. Indeed, this study successfully showed that seston carbon to phosphorus ratio was a significant factor affecting H/P mass ratio across communities with different taxonomic compositions of phytoplankton and zooplankton. The result supports a theory of ecological stoichiometry which states that the relative abundance of herbivores to producers changes depending on the stoichiometric mismatch between them (Sterner & Elser, 2002).

Conclusion

In this study, the effect size of fish abundance was greatest when our linear model was applied to the ponds, indicating that abundance of herbivores relative to that of primary producers depends highly on abundance of carnivores as classically and repeatedly pointed out since Hairston et al. (1960). We also found that stoichiometry, defense traits, and production rate of producers play substantial roles in regulating the H/P biomass ratio. Thus, this study revealed that herbivore biomass relative to producer biomass was regulated simultaneously by the abundance of carnivores, stoichiometric nutrient content and defense traits of producers, and primary production rate in natural ecosystems. In this study, we considered the size and phosphorus content of phytoplankton as the defense trait and nutritional quality of primary producers, respectively. However, it is also possible to consider effects of other chemical and physical defenses such as toxins and thorns, and nutritional substances such as protein and fatty acid contents in eq. (4). Since our theoretical framework (eq. (4) and (9)) can incorporates multiple factors, application of the model to various terrestrial and aquatic communities is possible and thus would serve to generalize the relative importance among production rate, defense traits and stoichiometric nutrient content of producers, and predation rate by carnivores in various ecosystems.

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Figure legends

Fig. 1 Two experimental ponds in CUEPF: Pond 217 (a) and Pond 218 (b) with vinyl-canvas curtains dividing ponds into four sections and floating mats used for partial shading. We set floating docks at the center of the ponds for sampling.

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Fig. 2. Biplots of mean algal biomass (mg C L^{-1}) and zooplankton biomass (µg C L^{-1}) (a) and mean photosynthetic active radiation (PAR, mol photon m^{-2} d^{-1}) in the water column and zooplankton/algae($\boldsymbol{H/P}$)mass ratio (b) during the experiment in no shade (blue), low shade (orange), mid shade (red), and

high shade (gray) treatments in pond 217 (circles) and 218 (squares). Bars denote standard errors on the means ($N = 7$).

Fig. 3. $H\ /P$ mass ratio plotted against mean values of edible phytoplankton fraction (a), seston carbon to phosphorus (C:P) ratio (b), specific production rate (c), and fish abundance (d) during the experiment in no shade (blue), low shade (orange), mid shade (red), and high shade (gray) treatments in pond 217 (circles) and 218 (squares). Bars denote standard errors on the means ($N = 7$).

Fig. 4. Partial regression leverage plots from multiple regression analysis showing effects of log(C:P ratio of seston) (a), log(Fraction of edible algae) (b), Log(Specific daily production) (c), and Log(Fish+1) (d) on H/P mass ratio. The vertical axis represents the partial residuals of H/P mass ratio and the horizontal axis represents the partial residual of the specific explanatory variable. Dashed and dotted lines in each panel represent the partial regression line and its 95% confidence curves. Partial correlation coefficients with p values are also inserted in each panel. Data from four different treatments of pond 217 (circles) and 218 (squares) are denoted by different colors.

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Table 1. Results of multiple regression analysis ($R^2 = 0.95$, $p < 0.05$) showing Regression coefficients with 95% confidence intervals estimated by bootstrapping procedure ($n = 1999$), and standardized regression coefficients for each parameter are shown.

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Supplementary Materials

Figure S1. Temporal changes in mean water temperature (a), pH (b), and dissolved oxygen (c) in the water column, and extinction coefficient (m^{-1}) (d) in the no shade (blue), low shade (orange), mid shade (red), and high shade sections (gray) of pond 217 (circle and sold line) and pond 218 (square and dashed line).

Figure S2. Vertical profiles of water temperature in the no shade (upper), low shade (mid-upper), mid shade (mid-lower), and high shade sections (lower) of pond 217 (left) and pond 218 (right).

Figure S3. Temporal changes in chlorophyll *a* (a), phytoplankton biomass (b), and fraction of edible phytoplankton (c) in the water column of no shade (blue), low shade (orange), mid shade (red), and high shade sections (gray) of pond 217 (circle and sold line) and pond 218 (square and dashed line).

Figure S4. Temporal changes in zooplankton biomass (a),H /P biomass ratio (b) daily production rate (c), and fish abundance (d) in the water column of no shade (blue), low shade (orange), mid shade (red), and high shade sections (gray) of pond 217 (circle and sold line) and pond 218 (square and dashed line).

Figure S5. Mean biomasses of zooplankton taxa during the experimental run in the no shade, low shade, mid shade, and high shade sections of pond 217 and pond 218.

Figure S6. Mean biomasses of phytoplankton taxa during the experimental run in the no shade, low shade, mid shade, and high shade sections of pond 217 and pond 218.

Figure S7. Temporal changes in total phosphorus concentration (a), sestonic carbon (b) seston C:P ratio (c) and seston N:P rations (d) in the water column of no shade (blue), low shade (orange), mid shade (red), and high shade sections (gray) of pond 217 (circle and sold line) and pond 218 (square and dashed line).

Figure S8. Chlorophyll-*a* specific photosynthetic rates plotted against the mean PAR in June 14 (blue), July 11 (red), and August 22 (gray) in the no shade (C), low shade (L), mid shade (M), and high shade sections (H) of pond 217 (upper) and 218 (lower). Each response curve was obtained by fitting data to non-rectangular hyperbola models.

Figure S9. Mean abundance of fish taxa during the experimental run in the no shade, low shade, mid shade, and high shade sections of pond 217 and pond 218. Vertical bars denote SE on the mean (N = 7).

Table S1. Temporal means and standard errors (parenthesis) of plankton biomasses, primary production rate, fish abundance, and sestonic elemental ratios in each treatment of the pond 217 and 218 during the experiment.

Table S2. Results of generalized linear models with the five lowest Akaike's information criterion values. Parameter are shown in Table. 1.