Food quality and food threshold: implications of food stoichiometry to competitive ability of herbivore plankton

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Abstract. Exploitative competition for food resources is considered to be an important factor determining the dominant species in communities. In such a competition, a threshold food concentration (TFC), where the growth rate becomes zero, is crucial in determination of competitive outcomes. In addition, recent studies on ecological stoichiometry have suggested that the TFC of a species would differ due to changes in food elements that limit growth. If the magnitude of change in TFC differs between species, competitive superiority among species may also change. The experiments showed that the rank order of the species in TFC changed according to the P content of food, suggesting that competitively superior species can vary with elemental composition of food. These results imply that as well as quantity, the elemental composition of food is important in exploitative competition, and the changes in elemental composition of food may be critical to shape an entire community structure.

Key words: algae; competition; Daphnia; food resource; P:C stoichiometry; resource ratio theory; threshold food level; zooplankton.

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INTRODUCTION

According to the competition theory by Tilman (1982), the outcome of exploitative competition is determined by the minimal resource level necessary to sustain a population ($R^*$). A species with lower $R^*$ maintain its growth at such a low resource level that another species with higher $R^*$ cannot survive. Organisms would compete for virtually any kind of resources that are necessary to sustain their own lives and population. For consumer animals, $R^*$ is considered to be comparable to threshold food concentration (TFC), at which the metabolic expenditure is balanced with net food intake or simply at which the net growth rate is zero (Lampert 1977, Lampert and Schober 1980). A number of studies support the predictability of competitive outcome by TFC among herbivore consumers such as zooplankton (see review by Grover 1997). However, these studies have assessed food quantity in terms of carbon (C) or mass, but not considered effects of food quality on TFC and thus competitive outcomes among consumers.

Both in terrestrial and aquatic ecosystems, the primary food for herbivorous consumers such as foliage and algae changes its elemental contents such as nitrogen (N) and phosphorus (P) relative to C depending on supply rates of these nutrients (Sterner and Elser 2002, Elser et al. 2007). The fact implies that these consumers often have to cope with stoichiometrically unbalanced food (Urabe and Sterner 1996, Sterner et al. 1997, Denno and Fagan 2003, Schade et al. 2003). Among nutrient elements, P is closely associated with the growth of heterotrophic organisms (Elser et al. 2000b)
and often limited nutrients in aquatic ecosystems (Sterner and Elser 2002, Elser et al. 2007). Consumer species with higher body P content would therefore grow faster when the P content of food is high (Main et al. 1997, Elser et al. 2000b), but they are likely to be more vulnerable to P deficiency in the food especially in aquatic habitats (Sterner and Hessen 1994, Sterner and Elser 2002). Indeed, Seidendorf et al. (2010) showed that among Daphnia, key herbivore consumers in lakes and ponds, the species with higher growth rates under favorable food conditions decreased their growth rates by greater degrees when fed low P food. However, a species with greater sensitivity to change in P content of food does not necessarily mean that it is an inferior competitor under conditions of low P food, because the growth rate of the species can be still higher than that of a slow growing species. Boersma and Kreutzer (2002) showed that a cladoceran species Daphnia magna changed its TFC according to the P content of food. It is not clear, however, if the magnitudes of changes in TFC differ among consumer species with different traits such as body size and P content. In addition, it is not clear how the magnitude is linked with metabolic expenditure and feeding properties of these species, which determines TFC. If the rank order of species in TFC changes depending on relative nutrient contents of food, it is likely that competitively superior species also change even if these exploit the same food item. Several studies have reported that dominant herbivorous plankton in nature seasonally changed in parallel with changes in abundance and composition of algae that should differ in P content and digestibility (DeMott 1983, Hoenicke and Goldman 1987, Hu and Tessier 1995). These seasonal changes may be attributed to changes in competitive superiority among herbivore species due to difference in food qualities in terms of elemental stoichiometry. However, due to scarcity of knowledge on stoichiometric effects of food quality on TFC, it is not clear if changes in food nutrient contents have an impact on competitive outcomes.

The aims of this study was to clarify (1) how TFC of herbivore species varies according to changes in P content of food relative to C, and (2) how the rank order of the species in TFC would change according to P content of food. To tackle these subjects systematically, we first theoretically examined how TFC of consumer species is affected by food P contents through the physiological (metabolic costs) and behavioral processes (feeding response). Then, to examine consistency of the theoretical consideration and species-specific difference in the response of TFC to changes in food, we conducted experiments using seven Daphnia species with different body size and body P content, which have been often proposed to be related with competitive superiority of herbivorous plankton (Brooks and Dodson 1965, Sterner and Elser 2002).

**Theoretical considerations**

In this section, we theoretically examine how changes in the P content of food influence competitive superiority among species through the difference in species-specific sensitivity of the somatic growth rate as a difference between net gain and metabolic expenditures, and hence TFC. For simplicity, we set species A to be superior competitor to species B at food with optimal quality (Fig. 1a). Thus, under conditions of high P food, we assume that species A has higher growth rate at any food abundance and a lower TFC. According to the expectation of ecological stoichiometry (Sterner and Elser 2002), we also assume that species A is more vulnerable to a decrease in food P content and thus drastically decreases its growth performance under conditions of low P food, while species B is less sensitive to change in the food quality and its growth performance hardly changes due to the P content of food.

When the P content of food declines, there are three possible patterns of decrease in the growth rate, depending on physiological responses in P retention within the body and feeding rate. The first pattern is a decrease in the slope of a growth line against food abundance without changing the x-intercept (TFC) (Fig. 1b). This response would occur if P is efficiently recycled within the body of an animal when food is scarce. Under this assumption, animals are not limited by P when the growth rate is zero, as they do not need to allocate P to new biomass (Sterner and Robinson 1994, Sterner 1997). In this pattern, TFC does not change according to P content of food. Thus, although the increase rate of the
population may be lower for species A than for species B at onset of competitive interactions when food is abundant, the former species would accordingly maintain a high population density when food becomes scarce while the latter would decrease its population density. As such, competitive superiority between the species would not affected by the P content of food. 

The second pattern is an increase in the x-intercept of a growth line (Fig. 1c). Boersma and Kreutzer (2002) showed that when fed P-deficient food the TFC of *Daphnia magna* was higher than when fed P-sufficient food. One reason for this response is compensatory feeding. When animals encounter food with low P content, they may increase the carbon expenditure to consume

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Fig. 1. Patterns showing growth response to changes in food quality (P content of food). (a) Species A is higher in growth rate at any food abundance under conditions of high quality food. (b) Pattern that the slope of the growth curve for species A is lowered when encountering low quality food, while that of species B merely changes. The x-intercept, however, does not change. (c) Pattern that the x-intercept of the growth curve of species A greatly increases and exceeds that of species. (d) Pattern that both a decrease in the slope and an increase in the x-intercept occur on the growth curve of species A. In this case, species A is now competitively inferior to species B.
more food. With this behavioral response, it can improve the relative acquisition of P against C to counteract P-limitation (Plath and Boersma 2001, Boersma and Kreutzer 2002). In this case, the slope changes little because compensatory feeding functions at higher food abundance, as well. Thus, if species A has a greater ability of compensatory feeding compared to species B, TFC of the former would increase at a greater magnitude and surpass that of the latter when P content of food decreases (Fig. 1c). Ironically, although compensatory feeding functions to maximize the growth rate under low P content food (Suzuki-Ohno et al. 2012), it may make the consumer species inferior when there is a competitor.

The third pattern stems from metabolic loss for P, which also gives rise to an increase in TFC when relative P content in the food decreases. Even under P-limitation, animals may lose P through metabolism (DeMott et al. 1998, He and Wang 2007). Unlike the previous pattern with compensatory feeding, the slope decreases as food P content decreases due to a decrease in net P gain. In this last pattern, if both an increase in TFC and a decrease in slope occur more intensely for species A than for species B, the former species would have lower growth rate regardless of food abundance. Accordingly, species A will turn to be a consistently inferior competitor when P content of food is low (Fig. 1d).

Effects of changes in the elemental composition of food on competition are thus expected to change depending on which of the three above-mentioned response patterns animals would show. If the first pattern is true, changes would exhibit only in slopes but not in TFC. If species differ only in response of the x-intercept (TFC) (the second pattern) or in response of both the slopes and the x-intercept (the last pattern), competitively superior species may change depending on the elemental composition of food. To examine which pattern is most likely, we performed experiments to measure the growth rates of seven Daphnia species under various food abundances using algal food with high and low P contents.

**METHODS**

**Algal food and animals**

*Scenedesmus obliquus* (Turpin) Kürzing was used as algal food for *Daphnia*. Algae with P:C ratios of $9.3 \times 10^{-3}$ (high P algae, HIP) and $2.0 \times 10^{-3}$ (low P algae, LOP) were obtained from the chemostat cultures at $20^\circ C$ under a light intensity of $200 \mu E m^{-2} s^{-1}$ with COMBO, an artificial inorganic medium (Kilham et al. 1998). The P content of these two algae was manipulated by the P concentration in the medium (100 $\mu$M $K_2HPO_4$ for HIP and 25 $\mu$M $K_2HPO_4$ for LOP) and the dilution rate (0.5 per day for HIP and 0.2 per day for LOP). After they had attained an equilibrium cell density, the algae were harvested, washed with distilled water and used as food.

We used seven differently sized *Daphnia* species: *D. dentifera*, *D. galeata*, *D. magna*, *D. pulex*, *D. pulicaria*, *D. similis*, and *D. tanakai*. *D. dentifera* was collected in 2004 at Shikibu Pond in Hokkaido, Japan. *D. galeata* was collected in 2006 at an irrigation pond in Yamaguchi, Japan. *D. magna* was a strain maintained for 10 years at the University of Shiga Prefecture. *D. pulex* has been kept in our lab for more than nine years and is the same strain as “clone 2” in Weider et al. (2005). *D. pulicaria* was collected in 2004 at Shikibu Pond in Hokkaido, Japan. *D. galeata* was collected in 2006 at an irrigation pond in Yamaguchi, Japan. *D. magna* was a strain maintained for >10 years at the University of Shiga Prefecture. *D. pulex* has been kept in our lab for more than nine years and is the same strain as “clone 2” in Weider et al. (2005). *D. pulicaria* was collected at Lake Biwa and has also been kept in our lab for more than nine years (Urabe et al. 2003). *D. similis* was collected in 2007 at the Second Fukurokura Dam in Chiba, Japan. *D. tanakai* was collected in 2005 at a marsh in Mt. Zao, Miyagi, Japan. All seven species were maintained at $20^\circ C$ with algal food for several months before the experiments. To prepare neonates of each species with a constant quality, 10–20 individuals were reared for several generations in 1 L bottles with 1.0 mg C/L of HIP algae. For these *Daphnia* cultures, we used 40% strength of N- and P-free “basal” COMBO, which was made by diluting with distilled water. Before use, we adjusted the pH of the basal COMBO to 7.0–7.2. In all seven species, neonates from the third clutch of each generation were used to establish the next generation. The medium and algal food were changed every other day, except for the cultures of the mothers of neonates used in the experiments, whose medium and algal food were changed every day.
Growth experiment

To estimate growth responses of seven *Daphnia* species against food abundance with high and low P contents, neonates of each species were reared with either HIP or LOP algae over a gradient of food abundances from 0.02 to 0.5 mg C/L. The highest food abundance of our experiment (0.5 mg C/L) was above the incipient limiting levels for *Daphnia* species (Lampert 1987). To initiate the experiments, we collected neonates born within 24 hours for each species. One neonate of each species was then introduced into a 50-ml glass-stoppered bottle filled with appropriate feeding suspensions. A single experimental run consisted of four to seven food abundances (bottles) for a single species. For both HIP and LOP algae, three to five experimental runs were made for each *Daphnia* species as replicates. Apart from individuals used for experimental runs, at least three subsamples of five neonates were collected for the initial body masses for each run. To maintain homogeneous feeding suspensions, the bottles with *Daphnia* were installed on a wheel (RCC-100, IWAKI, Tokyo, Japan) that was rotated at a speed of 1 rpm. The individuals were manually transferred to new bottles filled with fresh feeding suspensions every day. During the experiments, aliquots of the feeding suspension were filtered onto pre-combusted glass fiber filters (GF/F, Whatman; ca. 0.7 μm pore size) for estimating actual C and P concentrations of algal food that cladocerans received. Each experimental run lasted for five days. The temperature was maintained at 20°C during the experiments. Cell counting under a microscope showed that the algal depletion due to the grazing was less than 20% between the last water exchanges (day four to five). At the end of the experiments, the individual in each bottle was placed on a P-free small aluminum boat and dried at 60°C for 24 hours. A few *Daphnia* individuals produced eggs in the brood pouch, but never released neonates during five days of the experimental runs. The neonates for the initial body mass were also dried for later measurements. These samples were then weighed three times with a microbalance (UMX2, Mettler Toledo, Columbus, USA) and mean values of these three measurements were used to determine the initial and final body masses. The somatic growth rates ($\mu$ in unit of d$^{-1}$) of animals were calculated as

$$\mu = \frac{\ln(W_f/W_i)}{5}$$

where $W_i$ and $W_f$ are the initial and final body masses, respectively.

C and P contents of food and animals

Algae collected on GF/F filters were analyzed using a CHN analyzer (Perkin–Elmer model 2400; Perkin Elmer, Wellesley, MA, USA) for the C contents and by spectrophotometric methods after oxidation with persulfate (APHA 1998) for the P contents. For each *Daphnia* species, we measured the P contents of individuals grown at 0.5 mg C/L of HIP algae. In this measurement, three to five replicated samples were prepared for each species by placing one individual on each P-free aluminum boat. After weighing the dry weight, we digested the samples in 10 ml of persulfate solution and estimated the P contents by the spectrophotometric method according to Shimiizu and Urabe (2008).

Data analyses

For each run we related growth rates of *Daphnia* with log-transformed food abundance by linear regression analysis. TFC was calculated as a food abundance yielding zero growth rate on the regression line. The slope of the regression line was used to measure the magnitude of the growth response to change in food abundance. Because we performed 3–5 experimental runs for each species and each algal food, TFC and slope were obtained with 3–5 replicates. Significant differences in TFC and slope between seven species were tested by two-way ANOVA followed by a post hoc Tukey–Kramer multiple comparison test on species at each food. A one-tailed $t$ test was performed to test if TFC was higher and slope was lower when animals were fed with LOP algae than when they were fed with HIP algae. Because the same data set was used to test significant difference within and between species, we adjusted the significant level by Bonferroni correction.

To examine effects of body size on TFC when animals were fed HIP algae, linear regression analysis was conducted between TFC and dry weight of seven *Daphnia* species grown at
saturated food level (0.5 mg C/L) for five days \((n = 7)\). The relationship between body P content and TFC of individuals fed LOP algae was also examined with linear regression analysis to test if TFC was determined by the body P contents under P-deficient conditions.

We also estimated the threshold food concentrations in terms of P (TFP) for Daphnia fed LOP algae. Since Daphnia growth is likely limited by P in the food when fed LOP algae (Urabe et al. 1997), TFP should correspond to \( R^* \) for P. To estimate TFP, food P concentration was calculated using the abundances and P:C ratio of algal food for each experimental run. Then the food P concentration that yields zero somatic growth was estimated from the linear regression analysis on the growth rates against log-transformed food P concentration. TFP was estimated only for LOP algae because HIP food contained a sufficient amount of P for Daphnia growth (Sterner et al. 1993, Urabe et al. 1997). One-way ANOVA was conducted to test the significant difference in TFP between species, followed by a post hoc Tukey-Kramer multiple comparison test. Linear regression analysis was also conducted between TFP and body P content to test if body P content is an important factor determining TFP.

All statistical analyses were performed using GraphPad Prism 4.0c (GraphPad Software, San Diego, CA, USA).

RESULTS

In all species, the growth rates of individuals fed HIP and LOP algae increased with food abundance and were well fit by the regression lines (Appendix: Fig. A1, Table A1). TFC under conditions of LOP food were generally higher than that under conditions of HIP food, but the magnitudes of the changes were different between species as evidenced by the significant interaction effect in ANOVA (Figs. 2 and 3a). In all species, the slopes of regression lines for growth rate against food abundance were significantly smaller for LOP algae according to ANOVA (Figs. 2 and 3b). In contrast to TFC, however, the interaction effect of food quality (algal P:C ratio) and species was not significant for the slopes. Within each species, both TFC and slope were significantly different between HIP and LOP algae for D. galeata, D. magna, D. pulicaria, D. pulex, and D. tanakai, but not for D. dentifera and D. similis (Table 1).

The post hoc multiple comparison test showed that the rank order of the species in TFC were different between HIP and LOP algae (Fig. 3). For example, when fed HIP algae, D. dentifera, D. tanakai, and D. pulicaria had significantly lower TFC than D. similis, D. galeata, and D. magna. When fed LOP algae, D. dentifera and D. pulicaria were significantly lower in TFC than D. galeata, D. tanakai, and D. magna. Thus, the species with the lower TFC were different between HIP and LOP algae.

TFC of the seven Daphnia species fed HIP algae were not significantly correlated with their dry weight \((n = 7, r^2 = 0.004, p = 0.89)\). TFC of Daphnia fed LOP algae were not significantly correlated with their body P contents either \((n = 7, r^2 = 0.001, p = 0.95)\). Similarly, there was no significant association between slope when fed high P food and body size \((n = 7, r^2 = 0.002, p = 0.93)\) nor between slopes when fed low P food and body P content \((n = 7, r^2 = 0.26, p = 0.25)\).

Finally, we estimated the threshold food concentration in terms of P (TFP) for Daphnia fed LOP algae. It was significantly different between species (Fig. 4), but again not correlated with the body P content \((n = 7, r^2 = 0.02, p = 0.76)\).

DISCUSSION

Growth response to changes in quantity and elemental composition of food

Because TFC reflects the competitive ability, a number of studies have estimated TFC of planktonic consumers using qualitatively optimal algal food. These studies showed that TFC of planktonic consumers such as Daphnia species ranged from ~0.02 to ~0.95 mg C/L (Duncan et al. 1985, Lampert 1994, Kreutzer and Lampert 1999). TFC of Daphnia species fed high P food in our experiments were comparable to those values. However, no studies have examined TFC with low quality food. The exception was the study by Boersma and Kreutzer (2002), who showed that D. magna increased the TFC when fed lower P food. This study showed such an increase of TFC for seven Daphnia species. Note that the TFC of D. magna fed low P food in our experiments (0.12 mg C/L) was somewhat lower than that in Boersma and Kreutzer (2002: 0.13–
Fig. 2. Regression lines of growth rates against food abundance for seven *Daphnia* species fed HIP or LOP algae. Each line represents one experimental replicate.
0.31 mg C/L). The difference may have been due to difference in food P contents, because the P:C ratio of algal food in our study (2.0 × 10^{-3}/C0.3) was higher than that in their study (0.8 × 10^{-3}).

In addition to the increase of TFC, Daphnia species in this study showed lower slope of growth regression against food abundance when fed algae with low P content (Fig. 2). This result is coincident with the last pattern in our theoretical expectation (Fig. 1d), suggesting that metabolic P loss was substantial in any Daphnia species. Indeed, several studies have suggested...
that even when not growing, animals likely lose P and need to obtain it from food (DeMott et al. 1998, Acharya et al. 2004, He and Wang 2007). Note, however, that metabolic P loss and compensatory feeding (Fig. 1c) are not mutually exclusive, and both may have concurrently contributed to the growth response against changes in food P contents. The slopes of these Daphnia species might have been even lower if they had not exhibited compensatory feeding. Although knowledge is still limited on compensatory feeding (Suzuki-Ohno et al. 2012) and metabolic P loss (He and Wang 2007) of consumer animals, our study clearly showed that the TFC and growth response to food abundance were both affected by food P content through feeding performance for P acquirement and metabolic loss processes for maintenance.

Gliwicz (1990) suggested that differences in TFC among species were related to body size, and there was a negative correlation between TFC and body size for Daphnia. Contrary to that,

**Table 1.** Within-species comparison for TFC and slope between HIP and LOP algae. Significant differences are denoted with an asterisk.

| Species      | df | TFC $t$ | df | Slope $t$ |
|--------------|----|---------|----|-----------|
| D. dentifera | 6  | 1.71    | 6  | 1.71      |
| D. galeata   | 6  | 7.49*   | 6  | 5.17*     |
| D. magna     | 6  | 4.21*   | 6  | 2.98*     |
| D. pulicaria | 4  | 4.11*   | 4  | 3.20*     |
| D. pulex     | 6  | 3.38*   | 6  | 3.44*     |
| D. similis   | 4  | 2.26    | 4  | 1.32      |
| D. tanakai   | 7  | 8.62*   | 7  | 3.57*     |

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**Fig. 4.** TFP of seven Daphnia species fed LOP algae. The species are in increasing order in body dry weight from the left as in Fig. 3. The error bars represent the standard deviations. Species that significantly differed from the others by Tukey-Kramer multiple comparison are denoted by different letters.
we found no significant correlation between TFC and body size. Notably, the largest species (*D. magna*) had the highest TFC in this study. Such a result was also observed by Lampert (1994), who suspected that the result of Gliwicz (1990) for this species might have been an underestimate because *D. magna* is not a pure filter-feeder and has the ability to feed on settled algae on the bottom of the feeding vessels. In addition to Lampert (1994), Tessier et al. (2000) and Tessier and Woodruff (2002) failed to detect such a relationship. Together with these results, this study suggests that consumer species with larger body size are not necessarily superior competitors even under qualitatively favorable food conditions.

The relative elemental content of the body has often been assumed to represent vulnerability to deficiency of food elements (Sterner and Elser 2002). To examine this possibility, we measured the body P contents and TFP for seven daphniid species. If vulnerability to P deficiency food is higher for species with high body P content, TFP should be higher for species with high P content relative to C. However, TFP was not correlated with body P content. This fact suggests that factors other than body P content play a role in vulnerability of consumer species to P deficient food. For example, even if the body P content is high, consumers can maintain the vitality under low P food if they have abilities to efficiently ingest and assimilate P from the food, and/or to efficiently recycle P acquired within the body for metabolisms as theoretically suggested by Hall (2004) and Loladze et al. (2004). Recent studies have shown that food quality for herbivorous consumers in terms of nutritional content is not necessarily optimal in a variety of ecosystems including aquatic and terrestrial habitats (Elser et al. 2000a, Fagan and Denno 2004, Raubenheimer et al. 2009). This study suggests that although assimilation and metabolic expenditures for multiple elements have received less attention (Kooijman et al. 2004, Andersen et al. 2007), species-specific traits of these physiological properties should be taken into consideration for clarifying mechanisms determining competitive and exploitative abilities of consumers under qualitatively suboptimal food conditions.

**Competition and food P content**

In this section we discuss implication of the present results in context of competitive interactions among the same set of consumer species. When fed high P food, *D. tanakai*, *D. dentifera*, and *D. pulicaria* had lower TFC and thus are likely to be superior competitors to the other species (Fig. 3a). The result was consistent with the previous studies. For example, it has been reported that when fed high P food, *D. pulicaria* had a lower TFC than *D. galeata* (Gliwicz 1990, Achenbach and Lampert 1997, Kreutzer and Lampert 1999). Among these studies, Kreutzer and Lampert (1999) showed that the former excluded the latter in the competition experiment. Likewise, Urabe et al. (2002) showed that *D. pulicaria* excluded *D. magna* under the condition where food abundance was stable at near their TFC (~0.05 mg C/L) and the food P:C ratio was around 5.0 × 10⁻³.

This study also showed that the effects of food P content on TFC were different between species. For example, the TFC of *D. magna* and *D. similis* remained among the highest for both HIP and LOP algae. By contrast, although *D. tanakai* was one of the species showing the lowest TFC when fed HIP algae, it had the highest TFC when fed LOP algae (Fig. 3a). Thus, the rank order of the species in TFC changed with the P content of food. It suggests that the competitive superiority of *Daphnia* species changes with the elemental composition of food.

The TFC under conditions of LOP food can be viewed as the reflectors of R* for P (i.e., TFP), because *Daphnia* were limited by P. We thus should consider the rank order of the species in TFP for the competition for low P food. Then, if we take TFC under conditions of HIP food and TFP under conditions of LOP food into account, changes in competitive superiority among the species mediated by P:C ratio of food can be predicted based on a graphical presentation (Fig. 5) as in the competition theory by Tilman (1982). The figure shows x-y plot of TFC when the animals were fed HIP algae and TFP when they were fed LOP algae. If one extends the lines along the x-axis and along the y-axis from the cross-points of the TFP and TFC rightward and upward, respectively, they can be viewed as zero net growth isoclines (ZNGI) (Tilman 1982). In this figure, a line drawn across the origin
represents the trail of changes in food abundance, and its slope represents the P:C ratio of food. As the food resource decreases due to consumption, it eventually reaches the ZNGI, in the order of the highest to the lowest among the species. However, this order is different depending on how steep the slope is (i.e., whether it reaches TFC or TFP). Thus, it can be predicted that competitive superiority among the species should vary by the elemental composition of food. In the companion study, we experimentally confirmed that this x-y plot diagram could indeed predict the competitive superiority among consumer species (Iwabuchi and Urabe 2012).

In this study, we examined aquatic herbivore consumers with focusing on phosphorus as deficient nutrient in algal food. In terrestrial ecosystems, changes in nutrient contents of foliage are also not unusual (Cotrufo et al. 1998, Güsewell 2004) and the growth of consumers is often limited by the nutrient contents of the food (Sterner and Elser 2002, Denno and Fagan 2003, Schade et al. 2003). Thus, nutritional composition of food may be also important in exploitative competition among terrestrial consumers.

**Conclusion**

Our experiments showed that the decrease in relative contents of nutrient elements such as P in...
food could increase TFC and decrease magnitudes of growth response to changes in food abundances (slope). These results agree with the theoretical expectation that metabolic P loss accompanied by body maintenance (Anderson et al. 2005) plays a crucial role in the growth response of consumer species to changes in relative elemental content of food. In addition, the effect of changes in the elemental composition of food on the TFC quantitatively differed between consumer species regardless of the body size and P content. On the one hand, these results suggest that competitive superiority among consumer species sharing the same food resource can change depending on the elemental content of food resource (see also Iwabuchi and Urabe 2012). The possibility implies that nutritional or elemental contents of food resource can generate a stoichiometric niche space (Elser and Hessen 2005) and may promote coexistence of potentially competitive species under a single food resource if the food quality changes spatially or temporally. On the other hand, the results of this study showed that to understand mechanisms determining competitive superiority at given feeding conditions and thus “habitats” of stoichiometric niche space, knowledge on species-specific properties of assimilation and retention efficiencies for multiple elements was needed. It will serve to appropriately link physiological traits of consumer species to organizations of community assemblages under a given nutrient environment (Hall 2004).

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Fig. A1. Growth rates of seven *Daphnia* species under HIP and LOP food. Different symbols represent different experimental runs.
Fig. A1. (continued).
Table A1. Correlation coefficients and $p$-values of growth regression against food abundance for seven *Daphnia* species.

| Species    | HIP run | n | $r^2$  | p    | LOP run | n | $r^2$  | p    |
|------------|---------|---|--------|------|---------|---|--------|------|
| *D. similis* | 1       | 5 | 0.947  | 0.015 | 1       | 6 | 0.869  | 0.025 |
|            | 2       | 6 | 0.907  | 0.013 | 2       | 5 | 0.830  | 0.082 |
|            | 3       | 6 | 0.922  | 0.001 | 3       | 5 | 0.940  | 0.017 |
| *D. galeata* | 1       | 4 | 0.948  | 0.052 | 1       | 5 | 0.875  | 0.052 |
|            | 2       | 4 | 0.995  | 0.005 | 2       | 6 | 0.923  | 0.009 |
|            | 3       | 4 | 0.992  | 0.008 | 3       | 6 | 0.885  | 0.019 |
| *D. dentifera* | 1       | 6 | 0.966  | 0.002 | 1       | 5 | 0.882  | 0.048 |
|            | 2       | 6 | 0.908  | 0.012 | 2       | 4 | 0.970  | 0.030 |
|            | 3       | 5 | 0.915  | 0.029 | 3       | 6 | 0.941  | 0.005 |
| *D. tanakai* | 1       | 6 | 0.983  | 0.000 | 1       | 5 | 0.901  | 0.037 |
|            | 2       | 6 | 0.977  | 0.001 | 2       | 5 | 0.939  | 0.018 |
|            | 3       | 6 | 0.973  | 0.001 | 3       | 9 | 0.863  | 0.003 |
| *D. pulicaria* | 1       | 7 | 0.922  | 0.003 | 1       | 7 | 0.969  | 0.000 |
|            | 2       | 5 | 0.924  | 0.025 | 2       | 7 | 0.935  | 0.002 |
|            | 3       | 6 | 0.936  | 0.006 | 3       | 6 | 0.910  | 0.012 |
| *D. pulex* | 1       | 6 | 0.970  | 0.001 | 1       | 6 | 0.913  | 0.011 |
|            | 2       | 5 | 0.995  | 0.000 | 2       | 5 | 0.910  | 0.032 |
|            | 3       | 5 | 0.968  | 0.007 | 3       | 8 | 0.888  | 0.005 |
| *D. magna* | 1       | 5 | 0.968  | 0.007 | 1       | 9 | 0.898  | 0.001 |
|            | 2       | 5 | 0.993  | 0.001 | 2       | 6 | 0.928  | 0.008 |
|            | 3       | 7 | 0.932  | 0.002 | 3       | 5 | 0.932  | 0.021 |
|            | 4       | 5 | 0.923  | 0.025 | 4       | 5 | 0.879  | 0.050 |