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Phosphorus acquisition and competitive abilities of two herbivorous zooplankton, *Daphnia pulex* and *Ceriodaphnia quadrangula*

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Abstract Contrary to an expectation from the size-efficiency hypothesis, small herbivore zooplankton such as *Ceriodaphnia* often competitively predominate against large species such as *Daphnia*. However, little is known about critical feeding conditions favoring *Ceriodaphnia* over *Daphnia*. To elucidate these conditions, a series of growth experiments was performed with various types of foods in terms of phosphorus (P) contents and composition (algae and bacteria). An experiment with P-rich algae showed that the threshold food level, at which an individual's growth rate equals zero, was not significantly different between the two species. However, the food P:C ratio, at which the growth rate becomes zero, was lower for *Daphnia* than for *Ceriodaphnia*, suggesting that the latter species is rather disfavored by P-poor algae. *Ceriodaphnia* showed a higher growth rate than *Daphnia* only when a substantial amount of bacteria was supplied together with a low amount of P-poor algae as food. These results suggest that an abundance of bacteria relative to algae plays a crucial role in favoring *Ceriodaphnia* over *Daphnia* because these are an important food resource for the former species but not for the latter.

Keywords Competition · Growth rate hypothesis · Zooplankton · P:C stoichiometry · Size-efficiency hypothesis

Introduction

Because the food resources of herbivorous zooplankton species highly overlap, the competitive ability of these species is one of the crucial factors determining their spatial and seasonal distribution and relative abundance

(e.g., DeMott 1989). According to the size-efficiency hypothesis (SEH), a classical theory proposed by Brooks and Dodson (1965), larger herbivorous zooplankton species are viewed as superior competitors because the rate of energy gain by ingestion and assimilation likely increases with body size more rapidly than the rate of energy loss due to respiration. These allometric relations of feeding and metabolic rates to body size are partly consistent across a wide range of zooplankton with various body sizes (e.g., Hall et al. 1976). In addition, some studies have experimentally shown that the threshold food level, above which the growth rate is positive, is lower for larger zooplankton species (Gliwicz and Pijanowska 1989; Gliwicz 1990). Furthermore, larger zooplankton species have been found to be able to survive for a longer period under starvation conditions (Threlkeld 1976; Tessier and Goulden 1982). These lines of evidence indicate that large zooplankton species can maintain their population density at low food supplies, whereas smaller species cannot.

However, there is also evidence contrary to the SEH as to the competitive ability of zooplankton species. *Daphnia* and *Ceriodaphnia* are herbivorous filter-feeding zooplankton common in ponds and lakes, and they have been frequently used for studying competitive interactions (Neill 1975; Lynch 1978; Smith and Cooper 1982; Romanovsky and Feniova 1985; Hall et al. 2004). Both in laboratory experiments by Neill (1975) and field experiments by Lynch (1978), *Ceriodaphnia* were often found to predominate and exclude *Daphnia* in zooplankton assemblages without any predator species such as planktivorous fish, although the former is smaller than the latter in body size. Smith and Cooper (1982) showed that *Daphnia* excluded *Ceriodaphnia* in situ in some cases. Apparently, the consequence of competitive interactions varied between these two species. These authors suggested that the competitive abilities of herbivorous zooplankton change depending on the age structure and temporal variations in food supply rates. This suggestion was supported experimentally and theoretically by Romanovsky and Feniova (1985) who

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showed that *Ceriodaphnia* depressed *Daphnia* abundance in low-productivity environments, presumably because the threshold food level was rather lower for the former species. However, the inverse relationship occurred in highly productive environments, presumably due to a large oscillation in food abundance, which inevitably resulted in such a very low food period that only adult individuals of the latter species could survive due to their greater ability to resist starvation. However, a recent study has shown that *Ceriodaphnia* can predominate over *Daphnia* even in highly productive environments (Hall et al. 2004).

Other than food quantity, recently advancing theories of ecological stoichiometry suggest that food quality, notably the phosphorus (P) content of food relative to carbon, also plays a role in competitive interactions among zooplankton (Sterner and Elser 2002; Urabe et al. 2002; Hall 2004). Surprisingly, however, few studies have examined how P content in algal food affects competitive abilities of herbivorous zooplankton. According to the growth rate hypothesis (Elser et al. 2000), animals with higher P contents can grow at higher rates when fed with P-rich food but are more vulnerable to growth depression under P-poor food conditions because of their higher P demand. If this is the case, it is probable that herbivorous zooplankton with high P contents are superior to those with low P contents when P-rich algae are supplied as food, but not when P-poor algae are supplied. Hall et al. (2004) have shown that the P content in body tissue is much higher in *Ceriodaphnia* than in *Daphnia*. Thus, *Ceriodaphnia* may be inferior to *Daphnia* in P-poor food conditions even if they are superior competitors in P-rich food habitats.

Paradoxically, however, *Ceriodaphnia* dominated against *Daphnia pulex* in environments with P-poor algae in a predator-free mesocosm experiment conducted by Hall et al. (2004), although the former contained much more P in the body tissue compared to the latter. Hall et al. (2004) proposed two possible explanations to resolve this paradoxical result. First, it is likely that species traits such as P assimilation efficiency and metabolic loss rate differ between the species. Due to such a species-specific difference in traits, *Ceriodaphnia* may have grown even in P-poor environments where *Daphnia* could not maintain their population. The minimum P contents of food necessary for somatic growth at a certain food level would then be lower for *Ceriodaphnia* than for *Daphnia*. Second, a subtle difference in food composition may favor *Ceriodaphnia* in P-poor environments (Hessen and Andersen 1990). Previous studies have shown that *Ceriodaphnia* can ingest natural bacteria as efficiently as algae but that *Daphnia* cannot (Porter et al. 1983; Hart and Jarvis 1993). In general, mass- or carbon-specific P content (P:C ratio) is higher in bacteria than in algae (Hessen and Andersen 1990; Sterner and Elser 2002). Therefore, *Ceriodaphnia* may acquire a sufficient amount of P from both bacteria and algae in poor food environments where *Daphnia* cannot

acquire the necessary amount of P from algal food alone.

In this study, we conducted the three sets of experiments to test whether predominance of *Ceriodaphnia* over *Daphnia* in certain habitats can be explained by food conditions. Namely, we measured the growth rates of *Ceriodaphnia* and *Daphnia* under various food conditions in terms of quantity and P content to estimate the threshold food levels (Lampert 1977) and minimum food P:C ratio above which individual growth rates were positive. In addition, we examined the growth response of these species to the addition of bacteria to P-poor algae as a food resource. With this series of the experiments, we examined food conditions where *Ceriodaphnia* were competitively superior to *Daphnia*.

Materials and methods

Animals and algal foods

We used a strain of *Daphnia pulex* that was collected at a small farm pond in Japan and kept in the laboratory for more than 2 years. *Ceriodaphnia quadrangula* were collected from Takibuchi Zutsumi Pond in Miyagi, Japan in September 2004 and reared in the laboratory for 2 months before the experiments. To prepare neonates of these species with a constant quality, 10–20 individuals of *Daphnia* and 30–50 individuals of *Ceriodaphnia* were respectively reared for several generations in 1-l bottles with 1.0 mgC l^{-1} of *Scenedesmus obliquus* (Turpin) Kürzing. The medium and food were changed every other day. The algae were cultured in COMBO (Kilham et al. 1998), an artificial inorganic medium with 1 mM NaNO_3 and $50 \text{ }\mu\text{M K}_2\text{HPO}_4$, using a chemostat at 20°C with a light intensity of $300 \text{ }\mu\text{E m}^{-2} \text{ s}^{-1}$ and a dilution rate of 0.6 day^{-1} . After they had attained an equilibrium cell density, the algae were harvested, washed with distilled water, and used as food. Since the algae were grown in a medium with high P content, they are denoted by HIP algae hereafter. Both in stock cultures and experiments, cladocerans were reared in 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 both species, neonates from the third clutch of each generation were used to establish the next generation. The medium and food for the parents of the experimental neonates were changed every day.

Experiments

In experiment 1, the neonates of both species were reared with HIP algae over a gradient of several food abundances to estimate the threshold food level. The food abundance ranged from 0.027 to 2.0 mgC l^{-1} , covering below critical and over saturation levels (0.027 ,

0.035, 0.063, 0.071, 0.11, 0.188, 0.2, 0.35, 0.5, 0.63, 1.1, and 2.0 mgC l⁻¹ for *Daphnia*, and 0.027, 0.071, 0.188, 0.35, 0.5, 0.63, 1.1, and 2.0 mgC l⁻¹ for *Ceriodaphnia*). To initiate the experiments, we collected neonates born within 24 h for both species. Then, either three neonates of *Daphnia* or ten neonates of *Ceriodaphnia* were introduced into a 50-ml glass-stoppered bottle filled with appropriate feeding suspensions. We used these numbers of individuals to achieve a similar total grazing rate in the bottles between *Daphnia* and *Ceriodaphnia* based on their initial body mass (4.2 and 0.39 µg/ind., respectively; Burns 1969; Knoechel and Holtby 1986). Three to seven replicates (bottles) were made for each food abundance. Three subsamples of either five *Daphnia* or 15 *Ceriodaphnia* neonates were collected to determine the initial body mass of each species. To maintain homogeneity of the algal food particles in the feeding suspension, the bottles with zooplankton were secured to a wheel (RCC-100, IWAKI, Tokyo, Japan) that was rotated at a speed of 1 rpm. The neonates were manually transferred to new bottles filled with fresh feeding suspensions every day. During the experiments, aliquots of the feeding suspension in the bottles were filtered onto pre-combusted GF/F glass-fiber filters (Whatman; ca. 0.7 µm pore size) for estimating actual C and P concentrations of algal food that cladocerans received. The experimental run lasted for 5 days and 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 4 to 5). At the end of the experiments, all individuals in each bottle were pooled, placed on a small aluminum boat, and dried at 60°C for 24 h. The animals for the initial body mass were also dried in the same way. These samples were then weighed with a Mettler Toledo UMX2 microbalance (Mettler Toledo, Columbus, OH, USA) to determine the initial and final body masses. The somatic growth rates (μ : in unit of day⁻¹) of animals were calculated as

$$\mu = \frac{\ln(W_f/W_i)}{5} \quad (1)$$

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

In experiment 2, we grew both species using different algal food in terms of P:C ratio to evaluate the growth responses to the changes in food P content and to estimate the minimal P requirements. In this experiment, other than HIP algae, we prepared *S. obliquus* grown in medium with low P content (LOP algae). The LOP algae were obtained by culturing *S. obliquus* in COMBO with 0.5 mM NaNO₃ and 10 µM K₂HPO₄ using a chemostat with dilution rate of 0.2 day⁻¹. To initiate the experiment, we created six different feeding suspensions in terms of P:C ratios by mixing HIP and LOP algae in different proportions (Table 1) as in Acharya et al. (2004). Neonates of *Daphnia* and *Ceriodaphnia* born within 24 h were then grown in the same method as experiment 1 except that each treatment was conducted

Table 1 Mixture proportions of HIP and LOP *Scenedesmus*, and measured P:C ratios of the algae supplied to *Daphnia* and *Ceriodaphnia* in experiment 2

Mixture proportion		Measured P:C ratio (µgP mgC ⁻¹)	
HIP	LOP	<i>Daphnia</i>	<i>Ceriodaphnia</i>
1.00	0	16.80	19.63
0.94	0.06	4.05	—
0.84	0.16	2.78	3.89
0.68	0.32	2.33	2.60
0.42	0.58	2.12	2.17
0	1.00	2.01	1.96

The mixture proportion was estimated from the number of cells added to the feeding suspensions to achieve an appropriate P:C ratio

in triplicate. The experiment was done at two food levels (2.0 and 0.63 mgC l⁻¹). These two levels were chosen to examine if food abundance would affect on their performance under different food P:C ratios.

Experiment 3 was designed to evaluate the relative importance of bacteria as a food resource. For this experiment, bacteria were collected from an indoor aquarium. Thus, they were not necessarily a monospecies. To prepare algal and bacterial food, *S. obliquus* and bacteria were respectively grown in semi-batch cultures, in which 35% of each culture suspension was manually diluted with appropriate culture medium every day. The cultures contained COMBO with 0.5 mM NaNO₃ and 10 µM K₂HPO₄, and 270 mg l⁻¹ glucose was also added to the bacterial cultures as a carbon source. Temperature and light conditions for algal cultures were the same as in experiment 1. Bacterial cultures were kept in the dark at the same temperature as the algal cultures (20°C). These bacteria were cocci and solitary cells according to microscopic observation. Abundances of these algae and bacteria reached saturation levels in 7–10 days after inoculations. Before use, these suspensions were centrifuged and washed with distilled water. Aliquots of algae and bacteria were then respectively concentrated on to GF/F filters and analyzed to estimate C and P concentrations as below. Microscopic observation with the acridine orange direct count method (Hobbie et al. 1977) for a GF/F filtrate of the bacteria samples showed that 95% of bacterial cells were retained on the GF/F filters. Three different food treatments were employed: algae alone, bacteria alone, and a 1:1 mixture of algae and bacteria. The 1:1 mixture contained the same carbon concentrations of algae and bacteria that were calculated based on their cell-specific C contents. *Daphnia* and *Ceriodaphnia* were then grown with each food treatment. To initiate the experiment, three neonates of *Daphnia* or ten neonates of *Ceriodaphnia* were introduced into a 50-ml glass-stoppered bottle. The experiment was made at two food levels, 0.2 and 2.0 mgC l⁻¹ in total to examine if effects of food treatments on their performance differed between high and low food abundance. Each treatment was done in triplicate. The feeding suspension

was changed daily and the experiment lasted for 5 days as in experiment 1.

C and P contents of food and animals

Algae and bacteria collected onto GF/F filters were analyzed for the P contents by spectrophotometric methods after oxidation with persulfate (APHA 1998) and for the C contents using a CHN analyzer (Perkin-Elmer model 2400; Perkin Elmer Inc., Wellesley, MA, USA).

Apart from animals used in growth experiments, ca. 100 neonates of *Daphnia* and 500 neonates of *Ceriodaphnia* were grown with 2.0 mgC l^{-1} of HIP algae in several 1-l bottles. In each species, the animals were collected after 5 days, pooled randomly into 13 groups as replicate samples, placed on a small P-free aluminum boat, and dried at 60°C for 24 h. After weighing the dry weight, ten samples were digested in 10 ml of persulfate solution. These were used to estimate the P content of animals by spectrophotometric methods according to Shimizu and Urabe (2008). The remaining three samples were used to estimate the carbon contents of animals, measurement being done with the above-mentioned CHN analyzer.

Data analyses

In experiment 1, the threshold food level (TFL) of HIP algae was estimated from the regression line of the growth rate against the log-transformed food abundance as in Gliwicz (1990). It was calculated as the food abundance yielding a zero growth rate on the regression line. In general, the feeding rate and thus the growth rate of animals such as cladocerans increase with food abundance and level off at and above a certain food abundance, termed “incipient limiting level” (ILL; Peters 1984). Thus, to estimate TFL by a simple linear regression line, growth rates above the ILL should be excluded from the analysis. For this, the data were first analyzed by the hockey stick regression method (Yanagimoto and Yamamoto 1979) to determine the food abundances above the ILL where the growth rates leveled off. The bootstrap method for the simple regression model with 2000 replications (Crawley 2002) was then applied to data below the ILL to estimate the mean and standard deviation of the TFL. Statistical significance of difference in the TFL between *Daphnia* and *Ceriodaphnia* was tested by a *t*-test using standard deviation from the bootstrap. A *t*-test was also used to test statistical significance of the difference in the growth rate between these two species above the ILL where we used the growth rates at the food abundances above the ILL as replicate data.

The hockey stick regression method was also applied to data of experiment 2 to estimate a range of algal P:C ratios affecting the growth rates of animals. Using

growth data from this range, an algal P:C ratio at which animals did not grow at all was estimated. For each species at each food level, the mean and standard deviation of this “zero-growth elemental ratio (ZGER)” were calculated by the bootstrap method for regression line of growth rate against log-transformed algal P:C ratios (2000 replications). Significant differences in the ZGER between the species and between the food abundances were examined by pairwise multiple comparisons using a *t*-test with the significant level adjusted by controlling the false discovery rate (FDR; Benjamini and Hochberg 1995). This adjustment of significant level is designed to control the proportions of falsely rejected hypotheses out of all the rejected hypotheses and now being increasingly used in ecological and evolutionary studies (e.g., Higgie and Blows 2008). Further details on the FDR adjustment for ecological studies can be found elsewhere (Verhoeven et al. 2005; Waite and Campbell 2006).

In experiment 3, significant differences in the growth rate between the two species and among food treatments were examined for each food abundance by two-way ANOVA. Pairwise multiple comparisons using a *t*-test with FDR correction were conducted to examine significant differences between the species and among the food treatments.

Results

Experiment 1

The P content of HIP relative to C was $19.63 \text{ } \mu\text{gP mgC}^{-1}$ on average. For both *Daphnia* and *Ceriodaphnia*, somatic growth rates increased with algal food abundance (Fig. 1). However, the hockey stick regression showed that the growth rate reached a maximum and then leveled off at an algal abundance of 0.5 mgC l^{-1} for *Daphnia* and at 0.35 mgC l^{-1} for *Ceriodaphnia* (Fig. 1). Thus, the ILL was slightly lower for *Ceriodaphnia*. Below the ILL, regression lines of the growth rates were well explained by the food abundance in both species ($r^2 > 0.92$) and provided estimations of the TFL. The estimated TFL did not differ between the two species and was around 0.04 mgC l^{-1} (Fig. 2a: $t = 0.065$, $p = 0.95$). However, the growth rate at and above the ILL was significantly higher for *Daphnia* than for *Ceriodaphnia* (Fig. 2b: $t = 8.544$, $p < 0.0001$).

Experiment 2

The P content of LOP algae relative to C was $1.96 \text{ } \mu\text{gP mgC}^{-1}$. Thus, by mixing HIP and LOP algae in different proportions, we created the algal foods with P contents from 1.96 to $19.63 \text{ } \mu\text{gP mgC}^{-1}$. The hockey stick regression showed that the growth rates of *Daphnia* and *Ceriodaphnia* were not affected by algal P content above $\sim 4.0 \text{ } \mu\text{gP mgC}^{-1}$ when algal abundance

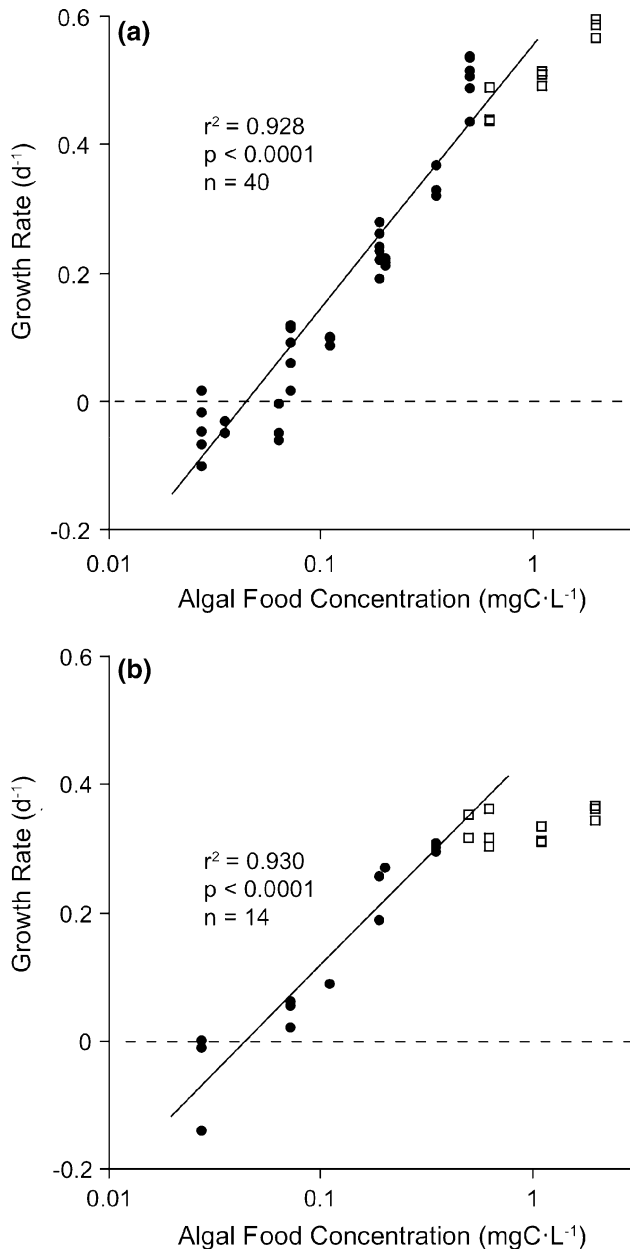


Fig. 1 Body growth rate of **a** *Daphnia* and **b** *Ceriodaphnia* fed various amounts of P-rich algae. The growth rates above the ILL determined by the hockey stick regression model are denoted by open squares and those at and below the ILL are denoted by solid circles. The solid lines are simple linear regressions fitted to data at and below ILL

was 0.63 mgC l^{-1} and $\sim 3.0 \text{ } \mu\text{gP mgC}^{-1}$ when it was 2.0 mgC l^{-1} (Fig. 3). At and below these P contents, the growth rate decreased with decreasing algal P content and reached zero, indicating that there was a food P:C ratio at which animals can no longer grow. Multiple comparison tests showed that this ZGER was significantly higher for *Ceriodaphnia* than for *Daphnia* at both food abundances (Fig. 4; $p < 0.05$). A significant difference in ZGER was also detected between the two food abundances for *Ceriodaphnia*.

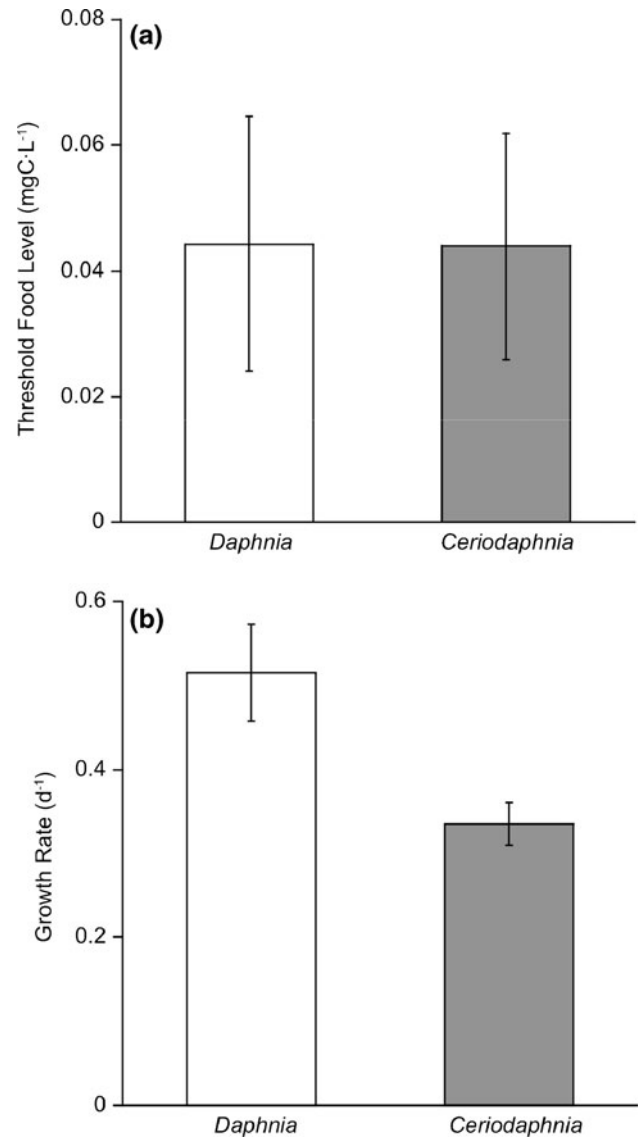


Fig. 2 **a** Threshold food levels of *Daphnia* and *Ceriodaphnia* for P-rich algae estimated by the bootstrap method, and **b** mean body growth rates above the ILL measured in experiment 1. The error bars represent the standard deviation of the mean

Experiment 3

The P contents of algae and bacteria used in this experiment were 4.6 and $7.1 \text{ } \mu\text{gP mgC}^{-1}$, respectively. Thus, the relative P content of food with a 1:1 mixture of algae and bacteria was $5.9 \text{ } \mu\text{gP mgC}^{-1}$. According to ANOVA, the growth rates differed significantly between the two species and among the three different food treatments at a food abundance of 0.2 mgC l^{-1} . In addition, the interaction effects of species and food treatment were significant, indicating that the differences in growth rates between the species varied depending on the food treatments (Table 2). The growth rates of both *Daphnia* and *Ceriodaphnia* were lower when feeding on bacteria alone compared with when feeding on algae

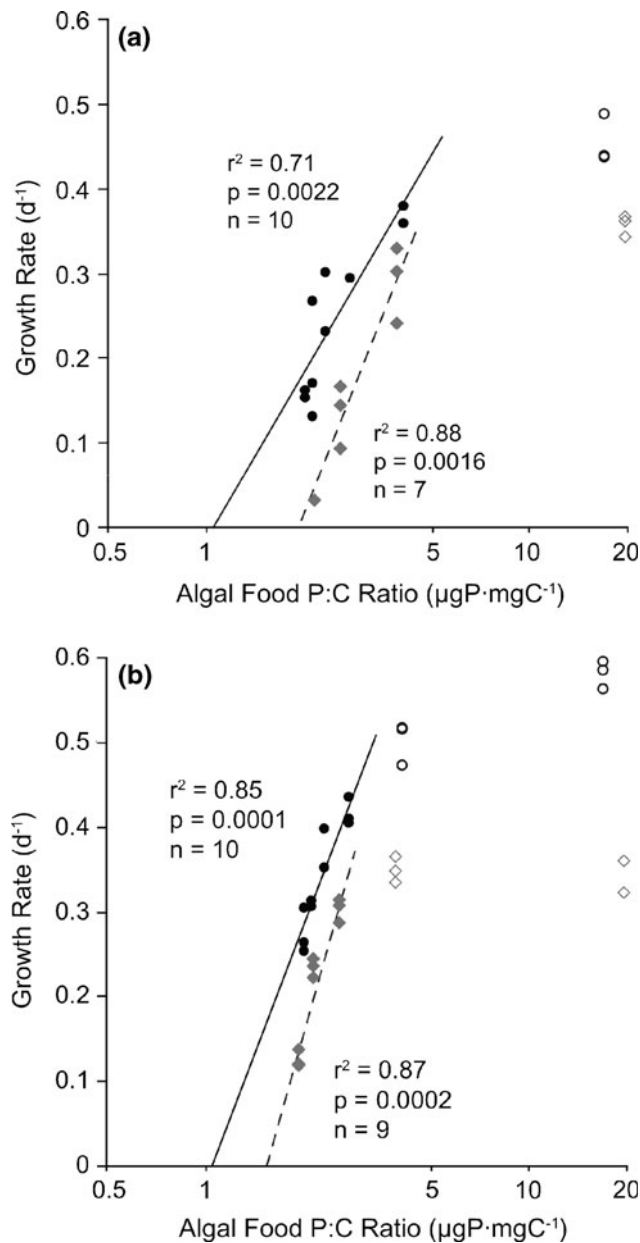


Fig. 3 Body growth rate of *Daphnia* (circles) and *Ceriodaphnia* (diamonds) fed algae with various P:C ratios at food levels of **a** 0.63 and **b** 2.0 mgC l⁻¹. The growth rates affected by algal P:C ratios, determined by the hockey stick regression model, are denoted by solid symbols and those not affected by algal P:C ratios are denoted by open symbols. Solid and dashed lines are simple regression lines for *Daphnia* and *Ceriodaphnia*, respectively, that are fitted for the growth rates affected by algal P:C ratios

alone (Fig. 5a). In these treatments, the growth rates were similar between the two species. However, when fed with a mixture of algae and bacteria, *Ceriodaphnia* maintained a growth rate as high as that when fed on algae alone, while *Daphnia* did not (Fig. 5a). At a food abundance of 2.0 mgC l⁻¹, the growth rate was higher for *Daphnia* than for *Ceriodaphnia* when fed with either algae alone or the mixture of algae and bacteria (Fig. 5b;

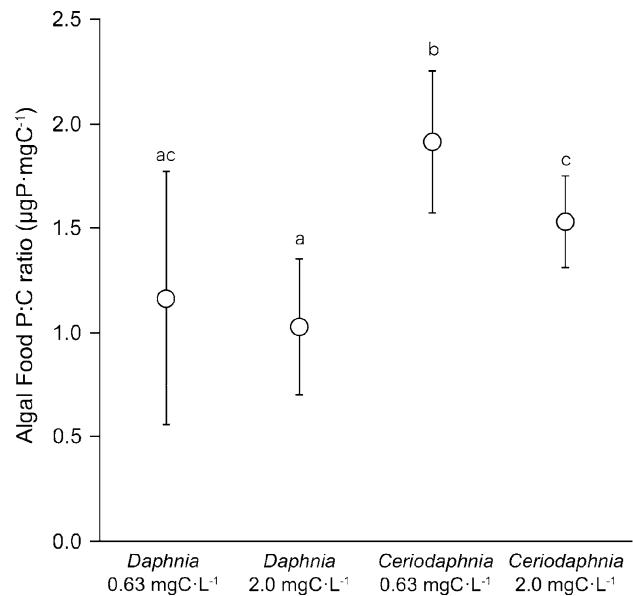


Fig. 4 Food P:C ratios at which body growth rates of *Daphnia* and *Ceriodaphnia* equal zero (zero-growth elemental ratio; ZGER) at food levels of 0.63 and 2.0 mgC l⁻¹. The ZGER significantly different from the others in multiple comparisons of *t*-test with FDR correction are denoted with the different letters on the bars. The error bars represent the standard deviations

Table 2 Results of two-way ANOVA for the effects of species and food type and their interaction on the growth rate at food levels of 0.2 and 2.0 mgC l⁻¹ in experiment 3

	<i>df</i>	MS	<i>F</i>	<i>p</i> -value
0.2 mgC l ⁻¹				
Species	1	0.0068	18.73	0.004
Food type	2	0.0149	41.15	<0.001
Species × food type	2	0.0033	9.182	0.002
Error	19	0.0003		
2.0 mgC l ⁻¹				
Species	1	0.0572	20.91	<0.001
Food type	2	0.0543	19.83	<0.001
Species × food type	2	0.0142	5.18	0.014
Error	22	0.0027		

Table 2). Within the species, the growth rates did not significantly differ between algae alone and the mixture treatments. In both species, the growth rates were equally lower when fed with bacteria alone.

Body P contents and P:C ratios of zooplankton

Body P contents (± 1 SE) of *Daphnia* and *Ceriodaphnia* were $1.27 \pm 0.10\%$ dry wt. and $1.20 \pm 0.07\%$ dry wt., respectively, and did not significantly differ from each other ($t = 1.903$, $p = 0.0732$). The average P contents of the two species relative to C were 26.1 and 24.6 μgP mgC⁻¹, respectively.

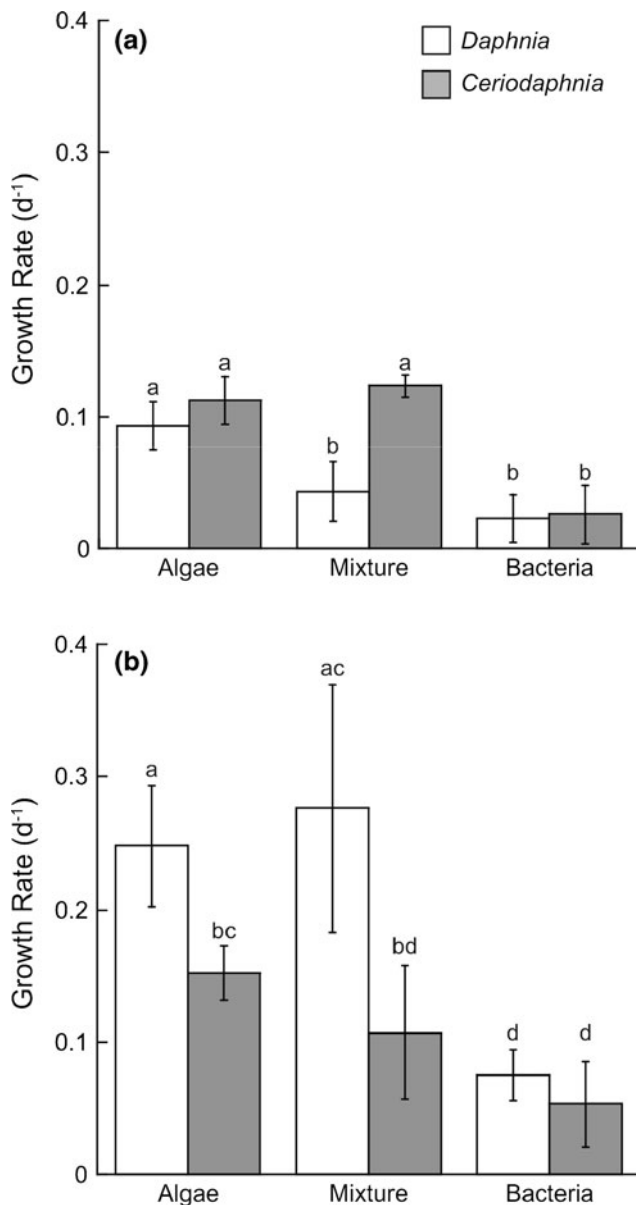


Fig. 5 Body growth rates of *Daphnia* and *Ceriodaphnia* fed algae alone, bacteria alone and a 1:1 mixture of algae and bacteria at total food levels of **a** 0.2 and **b** 2.0 mgC l⁻¹. The growth rates significantly different from the others in multiple comparisons of *t*-test with FDR correction are denoted with the different letters on the bars. The error bars represent the standard deviations

Discussion

This study showed that the individual growth rate was generally lower for *Ceriodaphnia quadrangula* than *Daphnia pulex* when algae were the sole food resource. However, the threshold food levels (TFL), at which the growth rate equals zero, did not significantly differ between these two species. The results do not accord with the SEH, which states that small herbivorous zooplankton such as *Ceriodaphnia* are competitively inferior for food resources to large *Daphnia* species (Brooks and

Dodson 1965). The equivalency in the TFL supports the view that the consequence of competitive interactions between *Ceriodaphnia* and *Daphnia* is not necessarily fixed, but likely changes depending on subtle differences in the competitive conditions. Such a view is consistent with the previous findings that competitive superiority between these species changes according to temporal patterns of food supply rates and their population structures (Neill 1975; Lynch 1978; Smith and Cooper 1982; Romanovsky and Feniova 1985). However, these previous studies did not consider how food quality and food composition play roles in the competitive interactions between the herbivorous zooplankton. Experimental results of this study suggest that a consequence of competitive interactions between herbivorous zooplankton also depends on algal quality in terms of cellular P content and relative abundance of other food resources such as bacteria.

The present study provides evidence partly untangling the seemingly paradoxical situations between the body phosphorus content and the growth performances reported on the previous studies (Elser et al. 2003; Hall et al. 2004). In this study, when fed with P-rich algae, the P content of *Ceriodaphnia* was ~1.2% and did not largely differ from that of *Daphnia pulex* (~1.3%). Similarly, Ferrão-Filho et al. (2007) showed that the P contents of *Ceriodaphnia* were 1.3–1.4% at various amounts of algal food with different P contents. Thus, the P content of *Ceriodaphnia* seems to be at the same level as that of *Daphnia*. It is not clear why the P content of *Ceriodaphnia* in Hall et al. (2004) was much higher than that used in this study. Since Hall et al. (2004) measured specimens collected in mesocosms where various organisms propagated, *Ceriodaphnia* samples might have been contaminated with epibionts. The similarity in the body P contents between *Ceriodaphnia* and *Daphnia* is, however, not a sufficient explanation for the predominance of *Ceriodaphnia* in P-poor environments. Hall et al. (2004) proposed that some specific traits may have enabled *Ceriodaphnia* to efficiently acquire P from algal food and thus to predominate over *Daphnia* under P-poor conditions. If this were the case, the growth rate of *Ceriodaphnia* should have been higher than that of *Daphnia* when they fed on P-poor algae. However, it was consistently lower than the growth rate of *Daphnia* in a wide range of food P:C ratios when algae were the sole food. These results indicate that body P content cannot explain neither the difference in growth rates of the two species in our experiment nor the predominance of *Ceriodaphnia* in P-poor conditions as reported by Hall et al. (2004).

It should be noted that for both *Daphnia* and *Ceriodaphnia*, the growth rate decreased with decreasing food P:C ratio and tended to reach zero at certain low food P:C ratios. Such food P:C ratios (ZGER) imply that there is a maintenance expenditure for P as is the case for C (i.e., respiration). Initial theories of ecological stoichiometry assumed that there was no maintenance cost for P and thus that animals can retain all of the

assimilated P when feeding on P-deficient food (Hessen 1992; Urabe and Watanabe 1992; Sterner and Elser 2002). However, observations and theoretical considerations suggest that animals such as cladocerans pay maintenance costs not only for carbon (energy) but also for other elements such as P (DeMott et al. 1998; He and Wang 2007; Shimizu and Urabe 2008). The existence of ZGER for P indicates that cladocerans expend a certain fraction of assimilated P on maintenance. Interestingly, the estimated ZGER was significantly higher for *Ceriodaphnia* than *Daphnia* regardless of food abundance in terms of carbon. This result implies that compared to *Daphnia*, *Ceriodaphnia* acquire P less efficiently, expend a higher fraction of assimilated P on maintenance, or both. Ferrão-Filho et al. (2007) showed that *Ceriodaphnia* have a slightly higher assimilation efficiency for P than *Daphnia* when fed on P-deficient green algae. Thus, it is unlikely that *Ceriodaphnia* have less ability to acquire P from algal food than *Daphnia*. Alternatively, the maintenance cost for P may be greater for *Ceriodaphnia*. Recently, He and Wang (2007) showed that loss of P through the molting cast is a major component of P expenditure for animals such as cladocerans. Since smaller species have a higher surface-to-volume ratio, *Ceriodaphnia* may suffer more from P loss due to molting when fed P-deficient food compared with *Daphnia*. Lynch et al. (1986) indicated that the weight-specific molting cast was larger for smaller cladoceran individuals. The SEH predicts a competitive inferiority of smaller zooplankton species because of the larger mass-specific respiration rate. Similar to such an argument, ZGER may be higher for *Ceriodaphnia* due to the greater mass-specific maintenance cost for P. This inference implies that smaller cladocerans are vulnerable to P-poor feeding conditions because of their greater maintenance (molting) cost for P.

However, *Ceriodaphnia* are not always competitively inferior to *Daphnia*. The present study shows that *Ceriodaphnia* were able to grow faster than *Daphnia* in a particular food condition containing bacteria, supporting the speculations by Hall et al. (2004). In general, the cellular P:C ratio of bacteria is higher than that of algae under the same nutrient conditions (Vadstein et al. 1988; Hessen and Andersen 1990; Makino et al. 2003). Indeed, the P:C ratio of bacteria in this study was $7.1 \mu\text{gP mgC}^{-1}$, which was much higher than that of algae grown under the same P-limited nutrient condition. However, the growth rates were highly limited in both *Daphnia* and *Ceriodaphnia* when bacteria were the sole food resource, as was the case in other studies (Pace et al. 1983; Goulden and Henry 1984). The low growth rates were apparently due to a lack or deficiency of some nutritional substance in bacteria such as poly-unsaturated fatty acids and sterols that are essential to animal growth (Goulden and Henry 1984; von Elert et al. 2003; Ravet and Brett 2006). However, when a low amount of equally mixed bacteria and P-deficient algae was supplied as food, *Ceriodaphnia* maintained a growth rate as high as those fed on the same amount of P-deficient

algae alone, while *Daphnia* did not, resulting in that the former had a higher growth rate than the latter (Fig. 5a). The result implies that *Ceriodaphnia* can efficiently use bacteria as energy and material sources but that *Daphnia* cannot. Since we used cultured bacteria, they were probably much larger in size than natural bacteria (Peterson et al. 1978). Thus, it is probable that *Daphnia* in our experiment could have ingested bacteria as efficiently as *Ceriodaphnia*. *Ceriodaphnia*, however, assimilate bacteria more efficiently than *Daphnia* (Pace et al. 1983). It is, therefore, most likely that *Ceriodaphnia* can acquire energy and P from bacteria more efficiently than *Daphnia*. Hessen and Andersen (1990) argued that bacteria could be an important source of P for some zooplankton species. The present study supports their notion and suggests that bacteria may be a key food resource determining the consequence of competitive interactions between *Daphnia* and *Ceriodaphnia*, especially when food abundance is low and algal P content is limited. Note that in nature, most solitary bacteria are too small in cell size for *Daphnia* species to graze as efficiently as *Ceriodaphnia* (Peterson et al. 1978; Porter et al. 1983). The fact suggests that bacterial food plays more important roles in the competitive interactions between *Daphnia* and *Ceriodaphnia* than assessed in this study.

In conclusion, the growth rate of *Ceriodaphnia* was generally lower than that of *Daphnia* when algae were the sole food resource. Therefore, the predominance of *Ceriodaphnia* in nature is, if any, likely occurs under existence of planktivorous fish that selectively prey on large *Daphnia* species (Brooks and Dodson 1965). Even if there is no fish, however, *Ceriodaphnia* can predominate over *Daphnia* when the nutrient supply is low and a large proportion of food is consisted of bacteria. Most of the previous studies on competitive interaction and growth of cladocerans have focused on and used algae as a food resource. However, experiments with algal food alone are apparently not sufficient to clarify the competitive interactions between herbivorous zooplankton in a real ecosystem (Pace et al. 1983; Hessen et al. 1990). To deepen our understanding of the competition among zooplankton, nutritional quality and availability of not only algae but also other potential food resources such as bacteria should be considered.

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