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Responses of alkaline phosphatase activity to phosphorus stress in *Daphnia magna*

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SUMMARY

We examined how alkaline phosphatase (AP) activity within the bodies and in the materials released by the crustacean *Daphnia magna* responds to variable algal food phosphorus (P)-content. We found that *Daphnia* eating P-poor food (C:P~700) had significantly higher AP activity in their bodies on a mass-specific basis compared with individuals eating P-rich food (C:P~100). This dietary P effect on AP activity was not altered by *Daphnia* starvation but was partially related to differences in the P concentration of animal body homogenates. By contrast, poor P-nutrition of *Daphnia* lowered AP activity in released materials compared with that measured from their P-sufficient conspecifics. Moreover, AP activity in *Daphnia* release was lowest in animals consuming P-poor food for longer time periods. Our results support the hypothesis that AP activity increases inside P-limited *Daphnia* as a mechanism to increase P-acquisition and retention from ingested algae in these nutritionally stressed animals. The lower level of AP activity present in the water of P-deprived animals could reflect a change from largely free to membrane-bound AP isotypes in the digestive tracts of P-starved animals or a decrease in the shedding of membrane-anchored AP from their intestinal lining. These results supplement accumulating evidence that P-poor algal food reduces the dietary mineral P available to *Daphnia*. In addition, animal body AP activity measurements, with some refinement, may prove useful as an *in situ* indicator of P-stress in aquatic consumers.

Key words: ecological stoichiometry, enzyme activity, nutrition, freshwater zooplankton, cladoceran.

INTRODUCTION

The phosphorus (P) content of algal food can strongly affect the growth and reproduction of aquatic consumers (Sterner and Elser, 2002; Frost et al., 2005). While the effects of consumer P-limitation on growth and reproduction have received considerable attention (e.g. Sterner and Hessen, 1994; Gulati et al., 1997; Sterner and Schulz, 1998; Sterner and Elser, 2002), dietary P-stress also affects other aspects of animal metabolism (e.g. Balseiro et al., 2008). In particular, physiological processes involved in the disposal of excess carbon and the acquisition/retention of P can be especially sensitive to the P content of food (DeMott et al., 1998; Darchambeau et al., 2003; Jensen and Hessen, 2007). Given these physiological responses to P-deficient foods, we hypothesize that the activity of enzymes involved in P-metabolism is also affected by this consumer's dietary P intake. We further predict that this may especially be the case in P-rich invertebrate taxa such as the crustacean *Daphnia*, which is known to respond strongly to changes in the P content of its food (e.g. Urabe et al., 1997; Elser et al., 2001).

One biochemical mechanism involved in the acquisition of dietary P in crustacean zooplankton is the production of alkaline phosphatase (AP), an enzyme that hydrolyzes phosphate monoesters and which exhibits low substrate specificity (Boavida and Heath, 1984). Examples of this enzyme class are found in organisms ranging from bacteria to mammals. Animals express a variety of isotypes, including an intestinal isotype in mammals (Yeh et al., 1994) and a midgut isotype in insects (Azuma et al., 1991). Additionally, histological methods have located phosphatase activity in the midgut of the crustacean copepod *Centropages typicus* (Arnaud et al., 1984). Previous work has also shown *Daphnia magna* can produce soluble phosphatases that are distinct from, and in larger quantities than, those produced by their algal food sources (Boavida and Heath, 1984). In addition, AP activity has been found in the dissolved release from axenic *Daphnia*, which is further evidence that the soluble enzyme is not produced by associated gut flora (Rigler, 1961; Wynne and Gophen, 1981). Whether the activity of AP within the body or in their release from the body is altered by the P-content of the zooplankter's diet remains untested.

Most work examining AP in freshwater zooplankton has focused on the activity of the enzyme released by the crustacean *Daphnia* (e.g. Boavida and Heath, 1984). The measurement of significant AP activity in *Daphnia* release may reflect AP being flushed out of the gut along with waste products. Given its established role in digestion in other animals, the location of AP in the gut or its lining in *Daphnia* would not be surprising. Seven AP genes have been identified in the genome of *Daphnia pulex*; two of these genes appear to encode soluble AP and three other genes match types known to be membrane anchored (http://genome.jgi-psf.org/Dappu1/Dappu1.home.html). It is likely that one or more of these AP have roles in dietary P-acquisition and P-recycling within the *Daphnia* body and would be altered by dietary P-intake by this animal.

In this study, we examined whether AP activity in *Daphnia* bodies and their release would change as a function of algal food P content. We grew *Daphnia* on algal diets that varied in P content and measured the AP activity in their bodies at different ages. We considered the possibility that algal food and phosphate inhibition were potential regulators of AP activity in *Daphnia* body homogenates and designed additional experiments to examine these potential mechanisms. AP activity was also measured in the water containing the differentially P-nourished *Daphnia*. This was performed with animals raised entirely on a single P-diet and with animals that were shifted from P-rich to P-poor algae. We predicted that the AP activity would increase in the bodies of P-stressed *Daphnia* as P-acquisition would be relatively more important in P-

Table 1. Elemental composition (C:N, N:P and C:P ratios) of *Scenedesmus acutus* cultured with different KH_2PO_4-P concentrations and dilution rates

Media [P] (umol l^{-1})	Dilution (day^{-1})*	C:N	N:P	C:P
34	0.4	5.89	16.1	95
28	0.4	7.43	26.0	193
22	0.4	8.05	45.2	364
17	0.2	6.68	64.2	429
15	0.2	7.75	73.7	571
7.0	0.2	9.59	82.4	790
5.6	0.1	7.67	111	850
5.6	0.1	8.78	141	1239
4.2	0.1	9.57	181	1734

Note: this is representative data of the algae cultures throughout the experiments.
*The dilution rate was the proportion of the total volume of freshwater culture medium (COMBO) replaced daily.

limited animals. In addition, we expected that the AP activity in the materials released from *Daphnia* would increase with increasing food carbon:phosphorus (C:P) ratios. Our results demonstrate that AP activity is sensitive to P-limitation in the diets of this important aquatic consumer with effects that differ between animal body tissues and released materials.

MATERIALS AND METHODS

Daphnia and algae cultures

Laboratory-grown *Daphnia magna* Straus were fed algae of different C:P ratios (100–700) throughout these experiments, which is approximately the range of algal C:P ratios found in natural lakes (Guildford and Hecky, 2000). For all of the experiments, neonates (<1-day-old) were taken from second-generation monoclonal *Daphnia* fed P-rich food (algae C:P ratios of 60–100). Only neonates born after the first brood were used for experiments. In general, experimental *Daphnia* were raised in groups of 10 in replicate jars containing 200 ml (days 1–4), 300 ml (days 5–8) and 400 ml (days 9–15) of P-free freshwater culture medium (COMBO) (Kilham et al., 1998). Throughout, we provided *Daphnia* high concentrations of algal food (2–4 mg C l^{-1}) by replenishing food every other day over the course of each experiment.

The algal food, *Scenedesmus acutus* (University of Toronto Culture Collection 10), was grown in semi-continuous cultures that were diluted daily with COMBO (Kilham et al., 1998). A range in algae C:P ratios was created by changing the media's concentration of P (KH_2PO_4) and the dilution rate (Table 1). The cultures were grown with a 16 h:8 h light:dark photoperiod at 20°C with two 400 W Lumalux bulbs (LU400/ECO, Sylvania Ltd, Mississauga, ON, Canada). Harvested algae were centrifuged for 20 min at 4000 ***g*** and resuspended in P-free COMBO. We estimated the C:P ratios of this concentrated algae, which allowed us to mix the algae to create targeted nominal food C:P ratios fed to the *Daphnia*. Algal %P was measured on dried and digested samples by the molybdate-blue ascorbic acid colormetric assay (APHA, 1992). The %C and %N of dried algae was determined on additional samples by a CN Elemental Analyzer (Vario EL III, Elementar Incorporated, Mt Laurel, NJ, USA) and was used to calculate more precisely nominal C:P ratios of algal food.

AP activity in *Daphnia* bodies

We measured the AP activity in the bodies of *Daphnia* fed algae at one of seven C:P ratios (100–700) and at three different ages (5-, 10- and 15-day-old *Daphnia*). For this experiment, *Daphnia* neonates were collected and randomly assigned to one of seven algal food C:P ratios (3–10 replicate jars per food level with 10 animals per jar). On the specified day (5, 10 or 15), AP activity was measured on 10 groups of *Daphnia* collected from replicate jars within each food level (Table 2). Because smaller *Daphnia* produce less AP, different numbers of *Daphnia* were grouped for AP activity measurements depending on the sampling day. *Daphnia* that were not used for body AP activity measurements were dried and weighed with an ultra-microbalance (MX5, Mettler-Toledo Inc., Columbus, OH, USA). With these dried *Daphnia*, %P was measured by the molybdate-blue ascorbic assay and their %C and %N was determined with a CN Elemental Analyzer as described above.

Daphnia were prepared for the body AP activity assay by washing them twice in P-free COMBO for 3–5 min to remove any AP in their holding water. Each group of *Daphnia* was placed in a 1.5 ml plastic ultracentrifuge tube and was homogenized in 0.75 ml of P-free COMBO. The volume was then brought up to 1.5 ml, and two 600 µl aliquots were removed for duplicate measurements during the enzyme assay. AP activity was measured in real-time by monitoring the hydrolysis of *p*-nitrophenyl phosphate (pNPP) into *p*-nitrophenol (pNP) and phosphate by detecting the absorbance of pNP formation at 400 nm. This colormetric assay was modified from the methods described by Boavida and Heath (Boavida and Heath, 1984). In 1 cm plastic cuvettes, the enzyme assay mixture contained: 600 µl of sample or P-free COMBO blank, 300 µl of 0.1 mol l^{-1} Tris-HCl buffer (pH 8.0) and 100 µl of 0.5 mmol l^{-1} pNPP (Bioshop Canada Inc., Burlington, ON, Canada). The assay was run within 2–5 h after the *Daphnia* had been homogenized and was performed at room temperature. The absorbance of pNP formation was measured by a UV-visible recording spectrophotometer (Shimadzu UV-160 U, Columbia, MD, USA). Readings were taken for 40 min at 10 min intervals, starting on the tenth minute. Phosphatase

Table 2. Grouping of *Daphnia* for body and released alkaline phosphatase (AP) activity analysis on different sampling days

Experiment	Sampling day (days after birth)	Number of replicate jars	Number of groups per jar	Animals per group
Body AP activity	5	10	1	5
	10	4	5	2
	15	2	5	1
Released AP activity	15	10	1	8–10
Diet-shifted *Daphnia* released AP activity	15	10	1	8–10

In all cases, 10 *Daphnia* were grown in each replicate jar during each experiment.

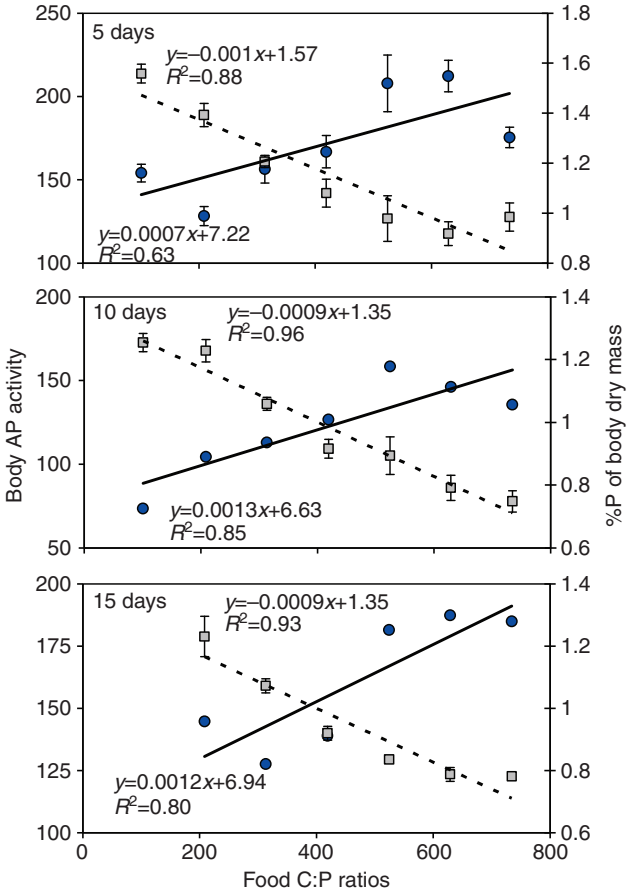

Fig. 1. Alkaline phosphatase (AP) activity (body AP activity; μmol pNP mg^{-1} dry mass h^{-1}; blue circles) of body homogenates and %P of body dry mass (gray squares) in different aged Daphnia magna consuming a range of food C:P ratios. In all panels, simple linear regressions were fit to treatment means (solid and broken lines). Note that for the day 15 analysis, food C:P ratio 100 was dropped from the dataset due to abnormally low growth rates of these animals.

activity was measured as the moles of pNP produced per unit of *Daphnia* mass per unit of time (μmol pNP mg^{-1} *Daphnia* h^{-1}). We used a literature value for the extinction coefficient of pNP in our calculations (0.0173 μmol l^{-1} cm^{-1}) (Bosron et al., 1975).

A primary product of AP-catalyzed reactions is phosphate, which is known to inhibit AP enzyme activity. Product inhibition potentially reduces AP activity in the body homogenates of P-rich animals due to their greater P-content. To address the potential that phosphate inhibition affected AP activity in *Daphnia* tissue homogenates, we designed two experiments where we altered the phosphate found in homogenized *Daphnia* bodies and measured the change in the corresponding AP activity. In both experiments, *Daphnia* were either fed a P-rich diet (algae C:P ratio ~150) or a P-poor diet (algae C:P ratio ~525) for 5 days. For the first experiment, we made the soluble phosphate concentration equivalently high in tissue homogenates from animals consuming both food types. After measuring soluble phosphate on the homogenates of animals, we spiked homogenates from the *Daphnia* eating the P-poor diet with KH_2PO_4 to ensure that the P concentration in these samples was equivalent to that in the samples from the *Daphnia* eating the P-rich diet. AP activity in the homogenates was then measured as described above.

In the second experiment, we used gel filtration chromatography to reduce the soluble phosphate concentration of samples of 5-day-old *Daphnia* consuming P-rich and P-poor food to nearly undetectable levels. Groups of five animals were homogenized in 400 μl of P-free COMBO. 15 μl of protease inhibitor cocktail and 15 μl of Triton X-100 were added to each cell suspension and left to sit for 20 min. Cellular debris was removed by centrifuging samples at 12,000 ***g*** for 20 min. 300 μl of each supernatant was added to 10 ml spin columns containing 2.0 ml of desalting gel resin (P-6 DG, Bio-Gel, Hercules, CA, USA). Samples were centrifuged at 3000 ***g*** for 2 min, and 250 μl of flow through was assayed either for AP activity or soluble phosphate concentration.

We also designed two experiments to assess whether algal-associated AP contributes to *Daphnia* body AP activity. The first experiment involved feeding *Daphnia* heat-treated algal food. Heating algae can denature algae AP and reduce its contribution to the AP activity found in the animal's body. After heating algal food at 97°C for 10 min, we measured the AP activity and C:P ratios of saved subsamples of the food as previously described. After we found that algae AP activity was reduced by 95% using this method, we fed *Daphnia* a diet of heat-treated (C:P ratios 510 or 1480) or non-heat-treated algae (C:P ratios 180, 510 or 1640) for 5 days. Body AP activity was then measured on *Daphnia* homogenates as previously described. In the second experiment, we included a longer pre-measurement incubation period where *Daphnia* were held for 2 h without food to reduce the algal influence on AP activity. For this experiment, *Daphnia* were grown for 5 days under a P-sufficient (C:P ratio 170) or P-deficient (C:P ratio 550) algae food diet. Body AP was also measured on these animals as described above.

AP activity in released enzymes

We also examined the AP activity on the materials released from *Daphnia* (age 15 days) fed algae of different C:P ratios (100–700). At the start of this experiment, we placed *Daphnia* neonates into one of the seven algae C:P food levels (100–700) with 10 replicate jars per food level and 10 animals per jar (Table 2). On the specified day, AP activity was measured in P-free COMBO water containing all of the *Daphnia* from each replicate jar within each food level. After a 60 min incubation, these animals were saved and analyzed for their dry mass, %C, %N and %P as described above.

In preparation for measuring AP activity of *Daphnia* release, animals were washed for 3–5 min in P-free COMBO to remove any AP in the water. After two rinses with fresh P-free COMBO, *Daphnia* from each jar were incubated together in 1.5 ml of P-free COMBO for 1 h. Subsequently, two 600 μl aliquots of media were removed for duplicate measurements in the enzyme assay. Phosphatase activity was monitored as the absorbance of pNP formation during 120 min with readings taken every 30 min. Because AP activity measurements on released dissolved enzymes required the crowding of animals to detect appreciable activity, we performed an initial trial to assess whether crowding affects *Daphnia*'s release of AP. *Daphnia* were grown for 15 days on a low C:P algae diet (~150) in this experiment. AP activity did not differ in the release from *Daphnia* that were incubated in groups of 1, 5 or 10 individuals in 1.5 ml of P-free COMBO for the duration of 60 min (data not shown).

We also measured the AP activity in the released enzymes of adult *Daphnia* that experienced a dietary shift from high quality (C:P ratio 100) to low quality (C:P 700) algal food. We collected *Daphnia* neonates and fed them low C:P algae (100) for 10 days. Starting on day 11, one-half of the individuals was switched to higher algal C:P ratios (300, 500 or 700) with 13 replicate jars for each

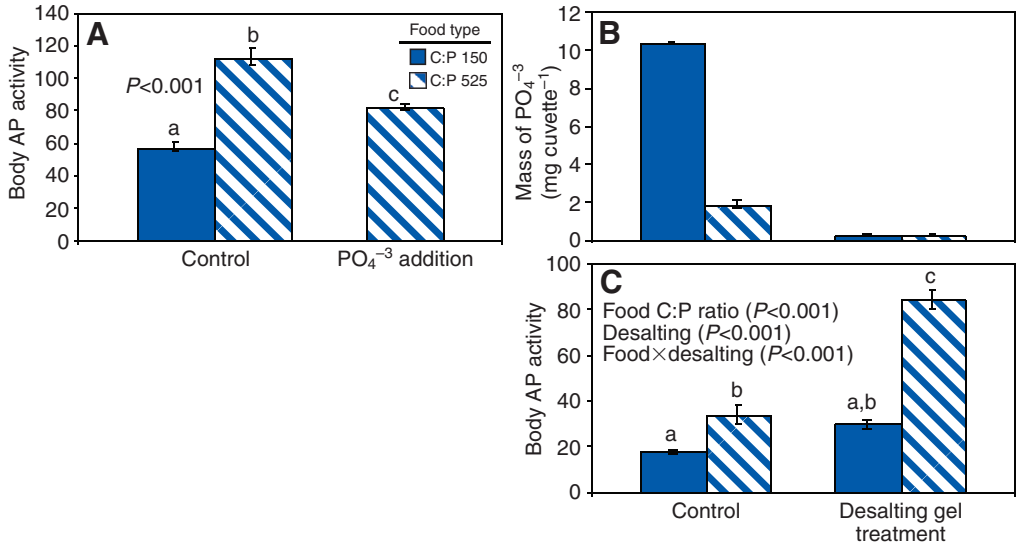

Fig. 2. (A) Alkaline phosphatase (AP) activity (mass-specific AP activity; μmol pNP mg^{-1} dry mass h^{-1}) of *Daphnia* (age 5 days) consuming P-rich food, P-poor food and P-poor food after P was added to body homogenates. Shown are the means and standard errors of 8–9 replicate jars. Treatment (food C:P ratio and day) and interactive effects were assessed on body AP activity with two-way ANOVA analysis on SAS (version 9). Bars with different letters are significantly different from each other as determined with *post-hoc* Tukey's HSD tests. (B) Phosphate in homogenates (mg P $cuvette^{-1}$) derived from animals (age 5 days) consuming P-rich and P-poor algal food before and after desalting gel treatment. (C) AP activity (mass-specific AP activity; μmol pNP mg^{-1} dry mass h^{-1}) of body homogenates before and after desalting gel treatment from animals consuming P-rich and P-poor food. Shown are means and standard errors of five replicate jars. Significance of treatment and interactive effects was assessed with two-way ANOVA using SAS (version 9). Bars with different letters are significantly different from each other as determined with *post-hoc* Dunn–Sidak contrasts.

food level. These *Daphnia* were fed P-deficient algae for four days. Starting on day 13, another portion of the remaining *Daphnia* were switched to higher algal C:P ratios (300, 500 or 700) with 13 jars per food C:P level. These *Daphnia* were fed P-deficient algae for two days. The remaining *Daphnia* were fed C:P 100 algae for the entire duration of the experiment and served as a control group (N=13 jars). On day 15, AP activity was measured on the water containing *Daphnia* incubated together for 60 min in 1.5 ml of P-free COMBO. *Daphnia* dry mass, %C, %N and %P were measured as described above.

RESULTS AND DISCUSSION

We found that P-nutrition of *Daphnia* can significantly affect the AP activity in their bodies. For all three ages of *Daphnia* in this study, AP activity increased in the bodies of animals consuming higher food C:P ratios (Fig. 1). Proximally, the increase in mass-specific body AP activity reflected both an increase in AP activity per animal and the smaller mass of P-limited *Daphnia* bodies (data not shown). The greater AP activity in *Daphnia* bodies may be attributed to an increased expression of AP, increased activity of existing AP pools or a combination of the two. Our results also indicate that *Daphnia* under P-stress maintain or increase AP activity despite having less total protein in their bodies (as evidenced by a concurrent reduction in body %N content, data not shown).

To assess the extent to which phosphate inhibition of AP accounts for the lower AP activity found in P-rich *Daphnia* homogenates, we measured the AP activity in P-spiked and P-free extracts of homogenized bodies of *Daphnia*. We found that increasing the $[PO_4^{3-}]$ in homogenates of P-poor *Daphnia* slightly reduced the AP activity of these samples but this reduction could not account for the differences seen with P-rich animals (Fig. 2A). In addition, we found the removal of endogenous phosphate in *Daphnia* homogenates by gel filtration chromatography (Fig. 2B) did not eliminate the differences in AP activity produced by P-limitation in *Daphnia*. Rather, this treatment enhanced the observed differences in AP activity between samples derived from animals fed different P-quality food (Fig. 2C). Together, these results provide strong evidence that P-limitation elevates the quantity and activity of AP in the bodies of *D. magna*.

Another potential explanation for the differences in AP activity seen in the bodies of differentially P-nourished *Daphnia* is that consumed algae are a significant source of AP activity in animal body homogenates. Initial trials demonstrated that relatively elevated temperatures (>90°C) were required to significantly reduce the AP activity of algal food. This heating treatment increased the C:P ratios of both of our food types (P-rich from ~150 to ~500 and P-deficient from ~500 to ~1500), perhaps due to cell lysis and loss of internal pools of P. Nonetheless, animals fed on these relatively AP-free (but P-deficient) foods retained elevated body AP activity relative to animals consuming P-rich food (Fig. 3A). We also measured the AP activity of differentially nourished *Daphnia* after a short (5 min) and a long (2 h) period of starvation. While gut residence time of algal material can be as long as 12–24 h in starving animals (e.g. Gillis et al., 2005), even short periods of starvation should nonetheless reduce the quantity of algal material in *Daphnia* due to both egestion and digestion. We found no difference in AP activity of either P-rich or P-poor *Daphnia* bodies when these animals were starved for 5 min or 2 h (Fig. 3B). Moreover, by directly measuring AP activity on algae samples, we estimated that algae could only contribute up to 8% of *Daphnia* body AP activity, if we conservatively assume that 10% of a daphnid's dry mass is derived from live algal cells in the gut. These results, coupled with those from the heat-killed algal experiment, suggest that algal associated AP is not the primary source of AP activity within *Daphnia* bodies and does not likely account for the increased AP activity that we observe in animals fed P-poor food.

The AP activity in the bodies of *Daphnia* also varied with their age. Body AP activity was highest in the 5-day-old *Daphnia* and

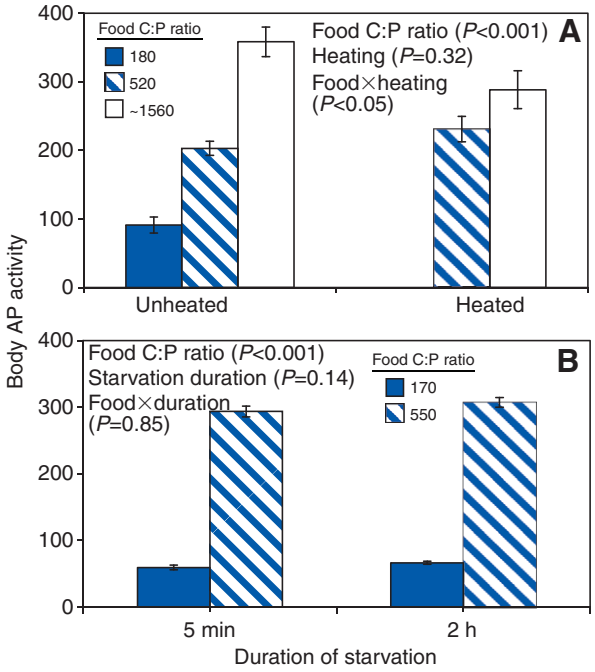

Fig. 3. (A) Alkaline phosphatase (AP) activity (mass-specific AP activity; μmol pNP mg^{-1} dry $mass^{-1}$ h) of body homogenates from *Daphnia* (age 5 days) consuming different C:P ratios with and without food heating treatment. Food heating was performed to substantially reduce the AP enzyme activity of the algal food. Shown are the means and standard errors of 8–9 replicate jars. Treatment (food C:P ratios and food heating) and interactive effects were assessed with two-way ANOVA using SAS (version 9). (B) AP activity (mass-specific AP activity; μmol pNP mg^{-1} dry mass h^{-1}) of body homogenates from *Daphnia* (age 5 days) consuming different food C:P ratios starved for 5 min and 2 h. Shown are the means and standard errors of 8–9 replicate jars. Treatment (food C:P ratios and starvation) and interactive effects were assessed with two-way ANOVA using SAS (version 9).

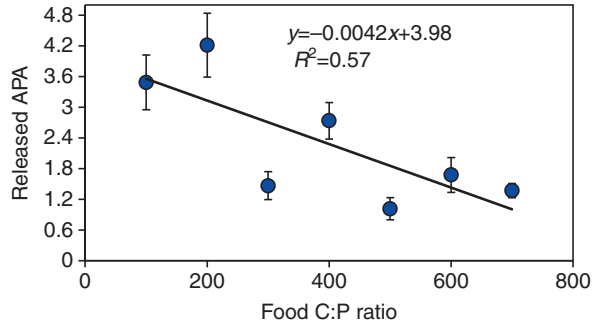

Fig. 4. Alkaline phosphatase (AP) activity (APA) (AP activity; μmol pNP mg^{-1} dry $mass^{-1}$ h) in materials released from *Daphnia magna* fed different algal C:P ratios. Shown are the means and standard error of released AP activity of 10 replicate *Daphnia*. Regression statistics were derived from a simple linear regression fit to the averaged data (solid line).

lowest for the other two ages tested, particularly for animals consuming P-rich food. While this increased AP activity may reflect the higher P demands of the faster-growing, juvenile *Daphnia* (Main et al., 1997), animals of different ages having the same body C:P ratios varied considerably in their mass-specific body AP activity (Fig. 1). One possible explanation for this ontogenetic disconnection between body C:P ratios and AP activity in *Daphnia* bodies is that juveniles allocate more proteins into digestive activities, including AP activity. By contrast, adult *Daphnia* may reallocate protein resources from digestion to reproduction and/or maintenance. In any case, age-related changes to AP activity in *Daphnia* potentially confounds simple interpretation of variable AP activity in these animals as it relates to food C:P ratios in natural environments.

P-nutrition also controls the amount of AP activity that is released into the water with lower levels of AP released from P-starved animals (Fig. 4). *Daphnia* may produce a greater quantity of membrane-anchored AP under P-starvation and reduce their production of secreted AP that moves into, through and out of the gut. Alternatively, release of membrane-anchored AP into the gut may be restricted under P-starved conditions. Although intestinal AP in animals is attached to the cell membrane by a glycosylphosphatidylinositol anchor at the carboxy-terminus of the protein chain, membrane-anchored AP can be released in mammals by the action of phospholipases or by solubilization with endogenous surfactants in response to an increase in dietary fat (Deng et al., 1992). The responses of *Daphnia* that we observe are broadly consistent with this mechanism, in that AP released to the water is higher in well-fed *Daphnia* and is lower in P-starved animals. From a materials conservation standpoint, this reduced AP loss would be advantageous for P-limited *Daphnia,* given the strong constraints on their protein production imposed by P-constraints on ribosomal RNA content (Elser et al., 2003).

We also examined AP activity in the materials released from P-sufficient *Daphnia* in response to short-term changes in food C:P ratios. Body P content showed a consistent decrease with increasing duration of exposure to P-poor food in *Daphnia* consuming foods of all three ratios but this decrease was relatively greater for animals consuming food C:P 700 (Fig. 5A). Longer exposure to P-limited food also reduced the AP activity in *Daphnia* release, but in contrast

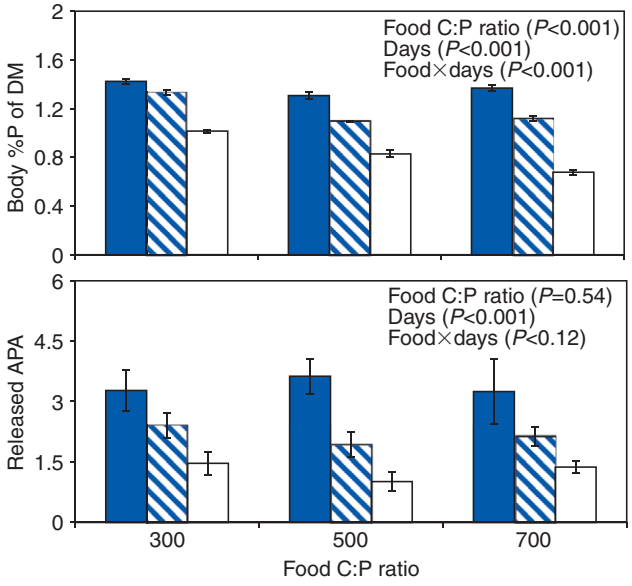

Fig. 5. Body %P and alkaline phosphatase (AP) activity (APA) (AP activity; μmol pNP mg^{-1} dry mass h^{-1}) in the release from 15-day-old *Daphnia* fed P-poor algae for 2 (solid blue bars), 4 (hatched bars) or 15 (open bars) days. Treatment (food C:P ratio and day) and interactive effects were assessed on body %P and AP activity with two-way ANOVA analysis on SAS (version 9). DM, dry mass.

to their body P-content, these changes in released AP activity did not differ among animals consuming different food C:P ratios (Fig. 5B). The reduced AP activity in released materials with increasing duration of P-poor exposure matches the result of lower AP activity in the release from P-stressed *Daphnia* observed in the previous experiment (Fig. 4). These results are further evidence of the P-sensitivity of *Daphnia* to even short periods of nutritional deprivation, which can be detected with analysis of AP activity in their tissues and/or released materials.

The responses of AP activity in the bodies of *Daphnia* to variable P-nutrition could lead to the development and refinement of biochemical indicators of zooplankton nutrition. Such bioreporters are being developed for phytoplankton (e.g. McKay et al., 2005) and could prove useful for the study of zooplankton nutrition. Elemental bioreporters would allow for immediate and relatively rapid assessment of the *in situ* nutritional state of an animal. However, there are a range of potential complications to the development and use of metabolic indicators of nutrition including: (1) many digestive enzymes are used for the acquisition of multiple elements from diverse natural substrates and thus lack element-specificity, (2) enzyme activities may vary within a taxa due to ontogeny or other environmental variables [as we found in this study; also see Balseiro et al. (Balseiro et al., 2008)], and (3) detailed knowledge of many target enzymes and their molecular regulation remains lacking. Consequently, there is a need to further study the regulation of metabolic enzymes, such as exploring the gene expression of nutritionally-relevant proteins in animals consuming foods of contrasting elemental quality under controlled laboratory and more realistic field conditions. Ultimately, elemental bioreporters, developed and refined, would provide an important and needed tool to assess and understand the nutrition of animals in nature.

LIST OF SYMBOLS AND ABBREVIATIONS

AP	alkaline phosphatase
C	carbon
COMBO	freshwater culture medium
C:P	elemental carbon to phosphorus molar ratio
N	nitrogen
pNP	*para*-nitrophenol
pNPP	*para*-nitrophenyl phosphate
P	phosphorus
PO_4^{3-}	phosphate
UV	ultraviolet

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