

Sex-specific plasticity in body phosphorus content of *Hyalella* amphipods

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Abstract Understanding the evolution of sexually dimorphic traits requires knowledge of the genetic and environmental sources of variation. However, we know surprisingly little about how the sexes differ in their responses to environmental nutrient supply. Here, we investigated how phosphorus (P) availability, a key metric of eutrophication, affects body composition in each sex of two *Hyalella* amphipod species. We also examined whether differences in food preference and acquisition are responsible for observed variation in body P. We discovered environmentally-driven changes in body P that were dependent on both species and sex. In both species, males contained less P when raised in low-P laboratory conditions compared to high-P field environments, while females exhibited no significant differences. Importantly, this difference was greater in the species that is known to

have larger sexual traits and higher growth rates. Variation in P content was not due to differences in acquisition of P because both sexes preferred high-P food and consumed it at a similar rate. Our study illuminates potentially important sex- and species-specific evolutionary consequences of rapid alterations to P availability due to cultural eutrophication.

Keywords Ecological stoichiometry · Food choice · Food quality · *Hyalella* · Phosphorus availability · Plasticity · Sexual dimorphism

Introduction

Human activities have strongly impacted many ecosystems throughout the world. Aquatic ecosystems have been particularly affected by cultural eutrophication caused by agricultural activity. The advent of artificial fertilizers has played a pivotal role in human population growth (Smil, 1999), leading to profound environmental alterations (Smil, 2000). Notably, synthetic phosphorus (P) fertilizer has been used to augment global agriculture for the past 150 years (Brown, 2000). Consequent runoff of P into lakes is a major cause of cultural eutrophication (Schindler et al., 2008). A common effect of excessive P loading is the increase in P content of seston (i.e., planktonic living and non-living matter; Hessen et al., 2002) which can alter the nutrition of aquatic consumers (Sterner & Elser, 2002). These changes in P content of

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consumer diet alter the development of traits, often with fitness consequences (Jeyasingh & Weider, 2005; Boersma & Elser, 2006; Jeyasingh & Weider, 2007; Bertram et al., 2006; Bertram et al., 2009; Jeyasingh et al., 2009; Cothran et al., 2012).

When examining the fitness consequences of nutrient supply, it is important to examine intraspecific differences in responses to the environment. Arguably the greatest source of intraspecific variation is sexual dimorphism. Sexual dimorphism allows the sexes to use different traits or enhancements of traits to employ different reproductive strategies or, less commonly, to decrease competition by exploiting different resources (Shine, 1989; Andersson, 1994). Sexual dimorphism implies differences in the material composition of the sexes, and thus they should have distinct nutritional demands (Morehouse et al., 2010). Numerous studies have documented striking differences in the material composition of the sexes (Tarnopolsky & Saris, 2001; Raymond & Himmelman, 2004) and such compositional differences are often reflected in food choice (Clarke et al., 1998; Ruckstuhl, 1998; Beck et al., 2007; Maklakov et al., 2008).

Because P supply is much lower than P demand in most biota (Westheimer, 1987), and P availability is positively related to the expression of sexually dimorphic traits (Bertram et al., 2006, 2009; Cothran et al., 2012), it is likely that P is a limiting resource that underlies honest signaling of male quality (Morehouse et al., 2010). Furthermore, previous studies have shown that P influences female oogenesis (Visanuvimol & Bertram, 2010), resulting in higher P content in females relative to males (Markow et al., 1999). Nevertheless, we know very little about sex-specific responses to P supply. The P content of the sexes could differ constitutively, plastically, or show no difference, and body P content should be driven by the ability of individuals to acquire and process P. Differences in body P content and P processing physiology should have dramatic effects on fitness optima of the sexes, causing one sex to be more dramatically affected by environmental heterogeneity than the other. Finally, while P is important for sexual traits, it also is important for other traits such as growth (Elser et al., 1996) that can be under different patterns of natural selection, especially in aquatic invertebrates (e.g., Wellborn et al., 1996). Thus, sexual differences in P content and responses to P supply should vary depending on the life history differences between closely related species.

Amphipods in the genus *Hyalella* are an ideal system to examine sex differences in P composition and sensitivity to P supply. Because of their vast geographic range, these amphipods inhabit most freshwater ecosystems, and experience a wide variety of nutritional environments (Bousfield, 1958). Laboratory experiments have shown that *Hyalella* obtain most of their nutrition by grazing on periphyton such as diatoms and bacteria, although much is still unknown about their diets in the wild (Hargrave, 1970). *Hyalella* amphipods in North America represent a complex of undescribed species that vary in morphology and life history (Wellborn et al., 2005; Witt et al., 2006; Wellborn & Broughton, 2008). The distribution of species in the complex is based primarily on the strength of fish predation (Wellborn, 1994a). Large ecomorph species live in habitats with little or no fish predation. Larval odonates are common predators in these habitats and these predators typically prefer smaller prey and thus select for higher growth rates (Wellborn, 1994a, b), which is a P-demanding trait (Elser et al., 2003). In contrast, small ecomorph species are found in habitats with fish, which prefer larger prey, and thus select for lower growth rates.

Large and small ecomorphs also differ in patterns of sexual selection. Although larger males that possess larger posterior gnathopods (PGs; large, claw-like appendages) are generally more successful in obtaining mates, this pattern is much stronger in the large ecomorph than in the small ecomorph (Wellborn, 1995, 2000; Wellborn & Bartholf, 2005). The PG is more sensitive to low P availability than other morphological traits (Cothran et al., 2012). Despite our knowledge of how P availability affects PG expression, it is unclear whether this translates into sex differences in P content.

In this study, we examined how each sex of *Hyalella* amphipods responds to changes in environmental P availability and whether food preferences and differences in acquisition underlie these responses. Specifically, we tested for sex-specific differences in body P content, with the prediction that males would be higher in P content than females because large PGs are likely large resource sinks like other sexual traits (Lincoln, 1992; Andersson, 1994; Emlen & Nijhout, 2000). Second, we tested whether environments with different concentrations of P induced sex-specific plasticity in body P content.

Because males were found to be more sensitive in their morphological response to changes in P supply (Cothran et al., 2012), we predicted that such sensitivity would be reflected in P content to a greater extent in males than females. If males are more sensitive to environmental P than females, then we predict that males will show greater preference for P-rich food or acquire P at a faster rate compared to females to optimize their resource intake. Our study tested these predictions in two amphipod species with different life histories, one with rapid growth and another with selection against rapid continual growth (Wellborn, 1994a, b; Wellborn et al., 2005; Wellborn & Broughton, 2008). Because P is important for growth, we further tested the prediction that P content of males from the large ecomorph, which exhibits prolonged, rapid growth should respond more strongly to dietary P supply.

Materials and methods

Survey of P composition in the bodies of field-collected and lab-raised amphipods

Our first goal was to quantify the body P composition of the sexes for the two ecomorphs collected directly from field environments or collected in field and then raised in laboratory environments. Both of the field environments (Lake Thunderbird, Cleveland County, OK; Lake LeBoeuf, Erie County, PA, USA) are classified as eutrophic lakes with total phosphorus measurements exceeding 40 $\mu\text{g/l}$ (OWRB, 2005; Butkas & Ostrofsky, 2006). Eutrophic lakes have carbon:phosphorus ratios typically lower than 100 (Sterner et al., 2008), indicating that these lakes have high P availability, although we did not directly measure P availability in the lakes. It is well known that the P content of autotrophs tracks inorganic P supply especially in aquatic ecosystems (Elser et al., 2000). Thus, it is likely that field-collected amphipods were experiencing a high P diet.

Large ecomorph amphipods (species OK-L in Wellborn & Broughton, 2008) were collected from Lake Thunderbird in October 2011 and raised in the laboratory using 10-l plastic tubs containing water treated with Tetra Aquasafe[®] (Tetra Werke, Melle, Germany) and spiked with 1,000 μM NaNO_3 and 5 μM KH_2PO_4 to promote periphyton growth.

Matala[®] aquatic filter media were added to each tank to provide cover. Animals were housed in a temperature-controlled room (mean \pm SD = 20 \pm 1°C) with a 16:8 day:night cycle. The animals were fed twice weekly with a 2:1:1 mix of ground rabbit pellets, Tetramin[®] fish flakes (Tetra Werke, Melle, Germany), and Spirulina (Nutrex Hawaii Inc., Kailua-Kona HI, USA) that was suspended in Bacto agar. The carbon:phosphorus ratio of periphyton in laboratory tanks (mean \pm SD = 611 \pm 229), and agar-based pellets (mean \pm SD = 351.6 \pm 30.0) was high, indicating a P-limited diet for amphipods with body stoichiometry (mean \pm SD) of 140.6 \pm 31.3 (Frost et al., 2006). Our eutrophic field environments, however, likely exhibit C:P ratios of <100 (Sterner et al., 2008). Stock animals were raised in the laboratory for 7 months to ensure that only individuals born and raised in the laboratory were used for elemental analysis. To assess the elemental composition of field animals, additional large ecomorphs were collected from Lake Thunderbird in April 2012.

Small ecomorph amphipods (species C in Wellborn & Cothran, 2004) were collected from Lake LeBoeuf (Erie County, PA, USA) in June 2011 and raised in a 90-l outdoor wading pool. These animals were subsequently overwintered in a single 1,000-l cattle watering tank filled with 800 l of well water and then placed back into a 90-l wading pool the following spring. Thus, these animals had been exposed to wading pool conditions for several generations and elemental composition was assayed on animals that were born and raised in the wading pool. The pool was filled with approximately 80 l of well water, inoculated with algae from Lake LeBoeuf, and provided with approximately 13 kg of washed sand. We added 0.968 μM P (as KH_2PO_4), and 53.55 μM N (as NaNO_3) to promote algal growth. To assess the elemental composition of field animals, additional small ecomorphs were collected from Lake LeBoeuf in June 2012.

We acknowledge that the two ecomorphs were raised in very different laboratory conditions prior to estimation of body elemental content. Large ecomorph amphipods were housed indoors in multiple containers, while small ecomorphs were reared in a single, outdoor wading pool. Thus, direct comparisons between the ecomorphs in P content should be interpreted with caution because it is likely the P environments vary between the two species. However, our design allows for robust comparisons of P content

within ecomorphs (i.e., sex-specific, and environment-specific effects).

To assess body P content, all amphipods were sexed at 4× magnification using a Swift SM90 stereo microscope and then dried individually in a 60°C incubator for 48 h. To control for the effect of female reproductive stage on body elemental composition, only females with clearly visible eggs in their ovaries were selected. This ensured that all females used were undergoing the same stage of oogenesis, a particularly P-intensive process for females (Markow et al., 1999, 2001). Twenty-three large ecomorph males, 24 large ecomorph females, and 8 of each sex of small ecomorph amphipods from each environmental group (i.e., lab and field) were analyzed for phosphorus content. A modified sulfuric acid digestion method (APHA, 1992) was used to estimate %P, and verified with a spinach standard 1570a certified by the National Institute of Standards and Technology.

Because direct species comparisons cannot be made, we ran separate two-way ANOVAs of sex and environment for each species. Statistical analyses were conducted using R 2.14.1 (R Foundation for Statistical Computing).

Food-choice experiment

To determine the extent to which species- and sex-specific variation in P acquisition drives differences in body P composition, we gave amphipods a choice between high-P (HP) and low-P (LP) food. All of the food-choice experiment trials were performed at the University of Pittsburgh. Small ecomorph and large ecomorph animals were collected from Lake LeBoeuf and Lake Thunderbird, respectively. Both ecomorphs used in the following experiment were raised in the laboratory under identical conditions, in 14-l tubs filled with carbon-filtered and UV irradiated water. Sand was provided as a substrate while waterweed (*Elodea* sp.) and fake macrophytes made of polypropylene rope served as refugia for amphipods. The tubs were kept in a temperature-controlled (mean ± 1 SD: 22.2 ± 0.2) room with a 16:8 day:night cycle. Amphipods were fed a 3:1 mixture of ground Tetramin® fish flakes and alfalfa (Spring Valley, Bohemia, NY, USA), supplemented with 0.5 g of *Spirulina* and 20 ml of high-phosphorus *Scenedesmus* algae suspended in 20 ml Bacto®-agar solution three times a week. Large and small ecomorphs were kept

under laboratory conditions for at least 3 and 7 months, respectively, prior to behavioral testing. Given a time to maturity of ~21 days, this assured that all adults tested were descendants of wild-collected adults and raised under identical laboratory conditions (Wellborn & Bartholf, 2005).

For each sex-by-ecomorph combination we gave groups of five amphipods a choice between HP and LP foraging stations and quantified the proportion of each foraging station consumed by the amphipods. To account for the large variation in size in the large ecomorph and avoid biases due to exploitative intra-specific competition (Wellborn, 1994b), we also tested for size effects on foraging preferences using two size classes for each sex, using head length an indicator of body size (mean ± 1 SD mm): small males: 0.58 ± 0.07, small females: 0.59 ± 0.06, large males: 0.83 ± 0.09, large females: 0.83 ± 0.063. For the large ecomorph, we performed 17 foraging trials for each sex. For the small ecomorph, we performed 7 and 12 foraging trials for females and males, respectively.

Experimental units were 48-ml plastic cups containing carbon-filtered, UV-irradiated water, and washed play sand. Each cup housed two 1-cm² Nitex mesh foraging stations (mesh size = 1 mm) containing green algae (*Scenedesmus* sp.) grown under conditions with either low (5.94 μM P) or high added inorganic phosphorus (59.37 μM P) concentrations (Kilham et al., 1998) and then mixed with 20 ml of Bacto®-agar. These manipulations assured that P availability between our two treatments was much different. To confirm that our algae manipulations were effective, we analyzed carbon using an elemental analyzer (Elementar, Hanau, Germany) and phosphorus via sulfuric acid digestion. Algae reared under different P conditions differed in %P content (mean ± SD; LP 0.044% ± 0.012%, HP 0.061% ± 0.008%, $t_5 = 1.989$, $P = 0.05$), but not in %C content (LP 39.6 ± 0.9%, HP 39.1% ± 0.5%, $t_5 = 0.746$, $P = 0.489$).

Amphipods were starved for 4.5–6 h in 48-ml plastic cups filled with filtered water to clear their guts (Hargrave, 1970). Each trial was terminated after approximately 40–60% of the total food available was foraged. Foraging stations were removed and digitized using an Olympus SZX16 microscope fitted with a DP25 digital camera (Olympus America, Center Valley, PA, USA). We quantified the proportion of each food type consumed by counting the number of

Table 1 Two-way ANOVA results showing the effects of environment, sex and their interaction on %P

Source	Large ecomorph			Small ecomorph		
	<i>F</i>	df	<i>P</i>	<i>F</i>	df	<i>P</i>
Environment	7.113	1,90	0.009	12.788	1,28	0.001
Sex	41.503	1,90	<0.001	0.994	1,28	0.327
Environment × sex	30.552	1,90	<0.001	1.068	1,28	0.310

F statistics, degrees of freedom and *P* values are reported for each ecomorph

Bold values indicate significance at $P < 0.05$

empty squares on the mesh and dividing by the total number of squares. Although there was some variation among foraging stations in the number of 1-mm mesh squares, this did not differ statistically between food types (large ecomorph: paired $t_{33} = 1.878$, $P = 0.07$; small ecomorph: paired $t_{15} = 1.3$, $P = 0.215$). Eight control foraging arenas, without amphipods, were set up to confirm that absence of food from the squares was due to consumption by amphipods.

Separate experiments were performed for the large ecomorph and small ecomorph; therefore, we analyzed the data for each ecomorph separately. For each ecomorph-by-sex combination (and for size in large ecomorph), we used a Wilcoxon signed-rank for paired samples to test whether amphipods preferred HP algae over LP algae. We then calculated the difference between the proportion of LP and HP food consumed in each replicate and applied a Mann–Whitney *U* test to test for sex differences, and for the large ecomorph size differences, in the magnitude of preference for HP food. Finally, for each experimental unit we divided the number of HP squares consumed by the number of days amphipods foraged to assess acquisition rates (number of squares consumed per day) of HP algae. We used a Mann–Whitney *U* test to test for sex differences, and for the large ecomorph size differences, in acquisition rates. All statistical analyses for the foraging experiment were conducted using IBM SPSS Statistics 20.

Results

P content of field-collected and lab-raised amphipods

For the large ecomorph, we found that body P was affected by sex, environment, and their interaction (Table 1). When we compared the two environments,

we found that male body P decreased by 26% when raised in the laboratory compared to animals collected from the field, whereas females did not differ (Fig. 1a). When we compared the two sexes, we found that females had higher body P when raised in the lab but there was no difference between the sexes in %P when collected from the field.

For the small ecomorph, we found that body P was affected by the environment, but not by sex or the sex-by-environment interaction (Table 1). Across both sexes, %P decreased by 7% when amphipods were reared in the laboratory compared to amphipods collected from the field (Fig. 1b).

We acknowledge that our sample size for the small ecomorph is small ($N = 32$). Therefore, we conducted a post-hoc power analysis using a small, medium, and large effect size (0.2, 0.5, and 0.8, respectively). This power analysis yielded powers of 0.194, 0.779, and 0.992 for the small, medium, and large effect sizes, respectively. The effect size for the large ecomorph was 0.569, falling between the medium and large effect sizes in the power analysis. Given this information, we had a sufficient small ecomorph sample size (giving us a power > 0.8) to uncover an effect of similar magnitude to that observed in the large ecomorph.

Food-choice experiment

For both ecomorphs, males and females strongly preferred HP algae to LP algae, as did both size classes of the large ecomorph (Table 2; Fig. 2). However, the strength of this preference did not differ between the sexes in either ecomorph (small ecomorph: $Z = -0.844$, $P = 0.398$; large ecomorph: $Z = -0.753$, $P = 0.452$) or size class in the large ecomorph (females: $Z = -1.092$, $P = 0.275$; males: $Z = -0.2$, $P = 0.842$).

We also found no evidence for sex differences in acquisition rates of HP food (small ecomorph:

Fig. 1 Large (a) and small (b) ecomorph responses in male (filled circles) and females (open circles) in %P. Markers represent means and error bars ± 1 SE

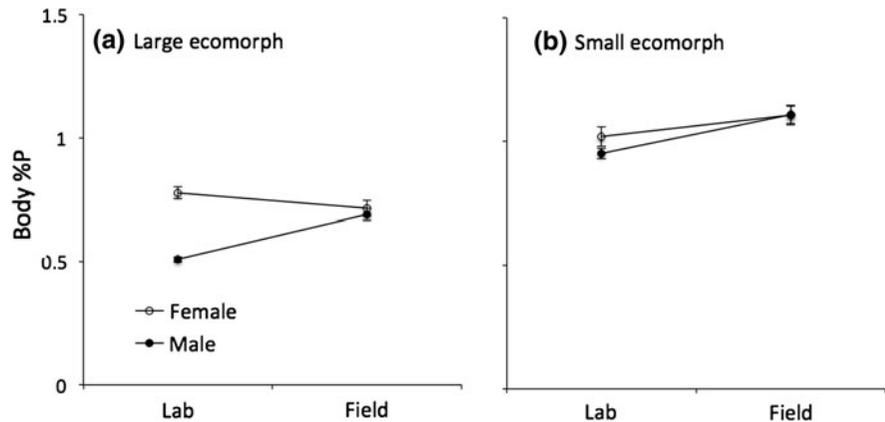


Table 2 Foraging preference results comparing the proportion of high P and low P algae squares consumed for each sex and also size class for the large ecomorph

Ecomorph	Sex	Size class	Z	P
Large	Female	Small	2.94	0.003
		Large	2.032	0.042
	Male	Small	2.923	0.005
		Large	2.371	0.018
Small	Female		2.521	0.012
	Male		2.366	0.018

In all cases, more high P squares were consumed than low P squares. Wilcoxon sign-rank Z and P values are presented

Bold values indicate significance at $P < 0.05$

$Z = -1.504$, $P = 0.152$; large ecomorph: $Z = -0.919$, $P = 0.663$). However, large females of the large ecomorph consumed HP food at a higher rate than small females (females: $Z = -2.209$, $P = 0.027$); a similar pattern was found in males, although it was not significant ($Z = -1.66$, $P = 0.109$). For both large ecomorph sexes, large amphipods consumed on average four more HP food squares per day than small amphipods (mean ± 1 SD squares consumed per day: large females = 12 ± 3 , small females = 8 ± 3 , large males = 11 ± 6 , and small males = 7 ± 3). Male and female small ecomorph amphipods consumed on average 5 ± 2 and 7 ± 3 HP food squares per day, respectively.

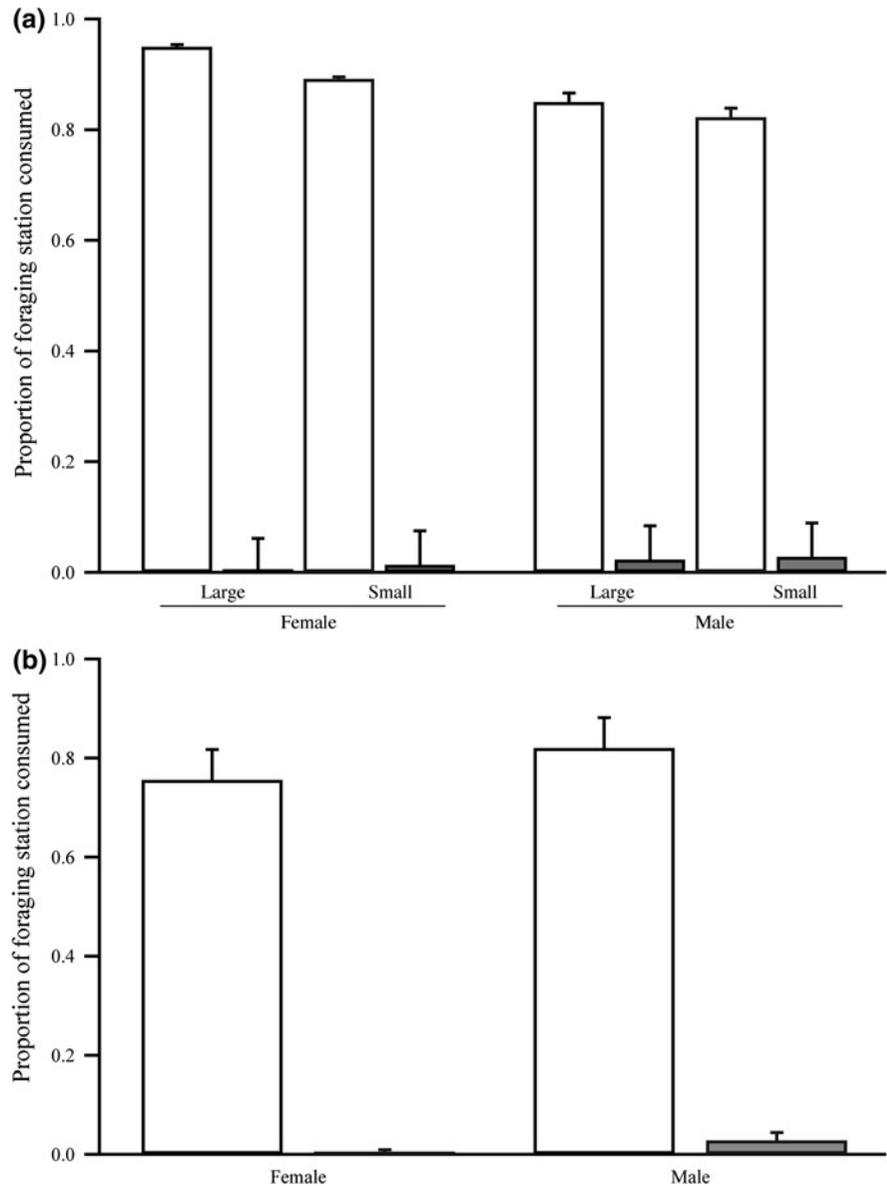
Discussion

We found that two *Hyalella* ecomorphs with different life histories exhibited divergent sex-specific plasticity in P content in response to rearing environment.

Specifically, large ecomorph males exhibited plasticity in P content in response to rearing conditions (i.e., lab-reared or field-caught), while no such plasticity was observed in large ecomorph females or either sex of the small ecomorph. In addition, a food choice experiment revealed that such sex-specific environmental responses do not cause different foraging behaviors. Although interspecific variation in body stoichiometry is often explained by broad phylogenetic differences (Fagan et al., 2002; Jaenike & Markow, 2003; Woods et al., 2004), the two ecomorphs used in this study were once considered the same species, *Hyalella azteca*, until recent molecular and life history studies uncovered substantial species diversity within the group (Witt et al., 2006; Wellborn & Broughton, 2008). This suggests that even species that are morphologically quite similar can have divergent and environmentally responsive P content.

Phosphorus content of both species of amphipods was dependent on the environment. Amphipods raised in the laboratory fed primarily on periphyton growing in the tanks, supplemented with agar-based food pellets that were low in P, indicating a P-limited environment. On the other hand, both lakes from which animals were sampled are currently recognized as eutrophic (OWRB, 2005; Butkas & Ostrofsky, 2006), indicating high availability of P, and C:P ratios of autotrophs typically <100 (Sterner et al., 2008). Although we did not directly quantify P content of periphyton from these lakes, it is a safe assumption that our lab environment, with C:P ratios between 350 and 600, was considerably P poor than these eutrophic lakes. Thus, it is likely that variation in P supply, and associated shifts in composition of macromolecules (e.g., classes of carbohydrates, lipids) in the diet of

Fig. 2 Proportion of high phosphorus (*white bars*) and low phosphorus (*gray bars*) foraging stations consumed by amphipods. Results for both sexes of **a** large ecomorph and **b** small ecomorph are presented. The large ecomorph species was further split into small and large size classes. Bars represent means \pm 1 SE



aquatic consumers (Jeyasingh et al., 2011) contributed to differences in elemental composition between laboratory-reared and field-caught amphipods. Furthermore, we acknowledge that other sources of variation (e.g., temperature, sunlight, community structure) in sex specific responses in P content to altered environments should be important, because our laboratory environment for the small species involved outdoor mesocosms. However, the design of this study to explore sex specific variation precludes us from isolating such sources of variation.

Species-level differences in how P content of the sexes responds to changes in dietary supply of P may be due to disparate patterns of sexual selection, yet further study is warranted that directly compares species collected from lakes with known resource C:P and reared under the identical laboratory conditions. In the large ecomorph, larger males have higher mating success, whereas this pattern is weaker in the small ecomorph and countered by positive size-selective predation by fish (Wellborn, 1994a; Wellborn, 1995; Wellborn & Bartholf, 2005). Sexual selection on male

body size in amphipods, which have indeterminate growth, is a combination of selection on growth rate and age. Growth rate is strongly associated with P content within organisms as P is a major component of the rRNA necessary to fuel protein synthesis (Elser et al., 1996). Therefore, we might expect higher P demand in large ecomorph males than small ecomorph males, which may explain the sensitivity of large ecomorph males to changes in P availability. Note that counter to predictions (Elser et al., 1996), we found that fast growing large ecomorph amphipods had lower P content. It is likely that the size-dependency of organismal P content drives this effect, because P content decreases with an increase in size as the fraction of rRNA in the body decreases relative to other P-rich molecules (Gillooly et al., 2005).

In addition to differences in growth rates, the importance of exaggerated sexual traits and their demand for P may explain ecomorph differences in sensitivity to the nutrient environment. Large PGs increase mating success in both ecomorphs, however, only large ecomorph males must also continue to invest P to fuel growth after maturation because of selection of smaller individuals by their predators and the resource competition advantages of large body size (Wellborn, 1994a, 2002). Large ecomorph PGs also have steeper allometric slope than small ecomorph PGs probably because of the greater returns in mates for investment in PGs at larger size classes in the large ecomorph (Wellborn, 1995; Wellborn & Bartholf, 2005; Bonduriansky, 2007; Cothran & Jeyasingh, 2010). Further, a previous study by Cothran et al. (2012) has shown that PG expression is sensitive to low P availability. The greater demand for P to fuel high relative growth rates of PGs combined with competing demands for increasing body size may explain why large ecomorph males are sensitive to changes in the nutrient environment.

While body P analysis revealed sex-specific responses to rearing environment in the large ecomorph amphipods, we found no sex differences in preference or acquisition rate when amphipods were offered foods of different P content in either ecomorph (Fig. 2). Both sexes in each ecomorph preferred HP to LP food and these results were consistent across size classes in the large ecomorph. We did find significant differences in acquisition rates between the two ecomorphs, with large ecomorph amphipods acquiring

food at a higher rate. These differences are to be expected, however, given the significant size differences between the ecomorphs, and previous work showing that the large ecomorph consumes algal resources at a higher rate (Wellborn, 1994b). These results show that amphipods are able to select food based on relative P content. The ability to choose food based on P content has been observed in *Daphnia* and is believed to be an important in mitigating stoichiometric imbalances between food and consumer (Schatz & McCauley, 2007). However, this ability is not sex-dependent, suggesting that sexual dimorphism is not driven by differential ability to discern food based on P content. Phosphorus availability has been shown to have many effects on the structure and biochemistry of algae (Tillberg & Rowley, 1989; Theodorou et al., 1991; Theodorou & Plaxton, 1993), and it is possible that the amphipods are showing preference for algal characteristics resulting from increased phosphorus. Further study is needed to examine the specific algal characteristics preferred by amphipods.

In summary, we found that closely related and morphologically similar ecomorphs have divergent body P content. Further, P content was plastic but only in males of the large ecomorph while the P content of small ecomorph males, and females of both ecomorphs were not plastic. However, further studies are warranted in which lineages from multiple species are subjected to identical environmental treatments to examine plasticity in P content across species. Variation in P content is most likely driven by differences in the life history of the ecomorphs and sexes studied. Further, differences in body P content were not explained by differences in food choice or acquisition of P, and are most likely a function of differential processing of P. Elucidating the mechanisms underlying sex-specific responses to changes in the supply of key elements, such as phosphorus, should reveal much about how the environment can affect the evolution of freshwater organisms that inhabit environments experiencing major shifts in key abiotic parameters such as phosphorus loading.

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