

# EUTROPHICATION AND PREDATION RISK INTERACT TO AFFECT SEXUAL TRAIT EXPRESSION AND MATING SUCCESS

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Sexual traits are especially sensitive to low food resources. Other environmental parameters (e.g., predation) should also affect sexual trait expression by favoring investment in viability traits rather than sexual traits. We know surprisingly little about how predators alter investment in sexual traits, or how predator and resource environments interact to affect sexual trait investment. We explored how increasing phosphorous (P) availability, at a level mimicking cultural eutrophication, affects the development of sexual, nonsexual, and viability traits of amphipods in the presence and absence of predators. Sexual traits and growth were hypersensitive to low P compared to nonsexual traits. However, a key sexual trait responded to low P only when predator cues were absent. Furthermore, investment trade-offs between sexual traits and growth only occurred when P was low. The phenotypic changes caused by predator cues and increased P availability resulted in higher male mating success. Thus, eutrophication not only affects sexual trait expression but also masks the trade-off between traits with similar P demand. Sensitivity of sexually selected traits to changes in P, combined with the important roles these traits play in determining fitness and driving speciation, suggests that human-induced environmental change can greatly alter the evolutionary trajectories of populations.

**KEY WORDS:** Amphipoda, *Hyalella*, life-history evolution, phenotypic plasticity, sexual selection.

Since the time of Darwin, sexual traits have intrigued biologists because they are strikingly diverse and play an important role in speciation (Darwin 1871; Ritchie 2007). Although much research has examined sexually selected traits and their functions, we have a poor understanding of how environmental heterogeneity affects the expression of these traits by interacting with genes to shape development (Cornwallis and Uller 2009). Environmental heterogeneity favors the evolution of plasticity in a variety of traits (West-Eberhard 2003). Although several studies have addressed plasticity of sexually selected behaviors, sexually selected morphological traits have received far less attention (Andersson 1994). Plasticity research on sexually selected morphological traits has focused on the hypersensitivity

of these traits, relative to nonsexual traits, to low food quantity, and, in a few cases, food quality (a phenomenon termed “heightened condition dependence”; Rowe and Houle 1996; David et al. 2000; Cotton et al. 2004a,b; Bonduriansky and Rowe 2005; Kemp and Rutowski 2007; Parker and Ligon 2007; Contreras-Garduno et al. 2008; Judge et al. 2008; Kemp 2008; Punzalan et al. 2008; Cothran and Jeyasingh 2010). These studies clearly demonstrate that resource allocation to sexual traits is sensitive to the amount and quality of food acquired. However, we do not know if individuals plastically alter allocation patterns to sexually selected traits based on the relative importance of sexual selection versus natural selection (Cornwallis and Uller 2009).

Predation risk is a spatiotemporally heterogeneous environmental parameter that should alter allocations to sexual traits. Theory predicts that the expression of sexually selected traits will reflect a balance between the sexually selected benefits of trait expression and naturally selected costs (Fisher 1930; Endler 1980; Andersson 1994). The balance between these contrasting selection pressures has been elegantly demonstrated in guppies (*Poecilia reticulata*) where transplantation of individuals to sites with lower predation risk results in an evolved increase in courtship signals (Endler 1980; Kemp et al. 2009). Although genetically based adaptive evolution is one solution to environmental variation, selection may also favor phenotypic plasticity if the environment that induces phenotypes is predictable. For example, when variation in predation risk is the result of temporal changes in the density of predators, phenotypic plasticity in the amount of resources allocated to sexual traits may be favored. Such predator-induced plasticity is common in male mating behaviors. For example, male courtship signals are often tempered under high, perceived predation risk (e.g., Magurran and Seghers 1990; Candolin 1997). However, the role of perceived predation risk in the development of sexually selected morphological traits is unknown.

The sexes will likely differ in how they allocate resources under perceived predation risk, because they have very different strategies for achieving reproductive success (Andersson 1994; Hunt et al. 2004; Maklakov et al. 2008). Although males should invest more heavily in sexual traits across all environments, their ability to do so may be constrained by the availability of resources necessary to build sexual traits (Kay et al. 2005; Morehouse et al. 2010). Therefore, the environment may play a critical role in shaping variation in male sexual traits and consequently affect social interactions these traits are used to resolve.

Understanding environmentally induced changes in allocation patterns between sexual and nonsexual traits is not only important for advancing our knowledge about the evolution of sexually selected traits, but is also relevant given the dramatic rate of human-induced global change (Palumbi 2001). Anthropogenic releases of phosphorous (P) have caused dramatic changes in several ecosystem parameters, including the quantity and quality of primary producer biomass (Carpenter et al. 1992; Schlesinger 1997; Wetzel 2001), generally referred to as cultural eutrophication. Because P is critical for many biological structures and functions (Westheimer 1987), and the supply of P in the environment is often lower (micromolar) than somatic demand (millimolar), input from artificial sources drastically change biological processes at multiple levels of organization (Smil 2000).

The lack of sufficient P to autotrophs at the base of food webs results in the storage of photosynthesized carbon (C) as simple C-rich molecules (e.g., sugars) because P is required to fuel a variety of anabolic processes (Jeyasingh et al. 2011). As a

consequence, autotroph biomass in P-limited ecosystems is characterized by high C:P ratios (Sterner and Hessen 1994). When inorganic P supply is high (note that P supply to several ecosystems have increased by as much as 100-fold in the last century), autotroph biomass is characterized by low C:P ratios. Such situations are becoming increasingly common (Sterner et al. 2008). Specifically, in P-limiting oligotrophic conditions, autotroph biomass is characterized by high C: low P content, whereas low C: high P characterizes autotroph biomass under eutrophic conditions. Note that P supply to autotrophs also changes the abundance of other nutrients (e.g., nitrogen, N) relative to C in autotroph biomass, presumably because P limits the synthesis of proteins (a major percentage of somatic N; Elser et al. 1996). Although such subsequent effects of P supply on the expression of sexually selected traits is plausible, our focus here is on the primary factor driving the eutrophication of freshwaters, P loading (Schindler 2006).

Because the stoichiometry of autotrophs is flexible, and tracks the amount of inorganic P supply, herbivores are susceptible to stoichiometric imbalances between somatic demand and environmental supply (Sterner and Elser 2002). Heterotrophic organisms acquire P along with other elements in varied proportions as complex food resources (i.e., autotroph tissue). Changes in autotroph stoichiometry invoke a variety of responses at the individual level in herbivores including behavior (Plath and Boersma 2001), physiology (Jeyasingh 2007), and life history (Jeyasingh and Weider 2005), ultimately affecting growth (Elser et al. 2003). It follows that the consequences of P limitation should also manifest at the intraindividual level, as trade-offs in P allocation to traits differing in P demand. To date however, we know little about such mechanisms, not only in terms of sexual traits (Morehouse et al. 2010), but also traits in general (Kay et al. 2005).

We used an amphipod species in the *Hyaella azteca* species complex (referred to as OK-L species in Wellborn and Broughton 2008) to explore how perceived predation risk and P-availability interact to shape sexual trait development. OK-L is common in permanent aquatic habitats that lack predatory fish. In these habitats, larval dragonflies are important predators. Larval dragonflies are negative size selective predators that have affected the evolution of amphipod life-history traits, including selecting for higher growth rates (Wellborn 1994; Wellborn and Bartholf 2005). Larval dragonfly densities are variable; therefore, the strength of selection imposed by these predators varies over space and time (Jefferies 1994; van Buskirk and Relyea 1998). The rapid growth required to reach a size refuge from invertebrate predators is a P-expensive trait (Jeyasingh and Weider 2005). Moreover, P is important for developing large morphological traits including chelae in decapods, which are similar in form and location to the sexually selected posterior gnathopods (PGs) of amphipods (Færevig and Hessen 2003). Therefore, in aquatic habitats where P is often limiting, amphipods may alter resource

allocation to growth versus sexual traits depending on perceived predation risk.

We tested this possibility using a completely randomized, factorial design with two P-availability treatments (high-P algal discs or low-P algal discs) and two predator treatments (predator cue present or absent). Amphipods were reared to sexual maturity and sexual traits and nonsexual traits (female sexual traits and size-matched trait not under sexual selection) were measured. In addition, a subset of males and females was subjected to a mate competition and fecundity assay, respectively. This allowed us to assess whether P-availability and perceived predation risk affected sex-specific fitness components in addition to morphology.

Given that predation favors amphipod growth whereas female mate preference favors large male sexual traits, we predicted that amphipods would exhibit a trade-off between growth and sexual traits when resources are scarce. We also predicted that sexually selected traits would be more condition dependent than nonsexual traits in the absence of predators (i.e., when individuals prioritize these traits). However, we predicted that differences in sexual trait size between low- and high-P treatments would be tempered when perceived predation risk was high due to diversion of resources to growth (a viability trait). Finally, we tested whether changes in resource allocation to different traits resulted in fitness consequences. Specifically, we predicted that males raised in environments that select for decreased investment in sexual traits (i.e., low P, predator risk) would be inferior competitors for mates compared to counterparts raised in environments that favor increased investment in sexual traits (i.e., high P, no predation risk).

## Methods

### HYALELLA MATING BIOLOGY

The mating biology of *Hyalella* amphipods is tightly linked to the female molt cycle. A female's eggs can only be fertilized for a short period after she molts. This time limited receptivity to fertilization results in a strongly male-biased operational sex ratio and consequently intense competition among males for access to females. This is thought to have led to the evolution of precopulatory mate guarding as a male time investment strategy (Ridley 1983). Females become more receptive to pairing as their molt approaches (Wellborn and Cothran 2007; Cothran 2008a). Field collections and experiments have identified body size and PG size (after adjusting for scaling with body size) as sexually selected traits in this species complex (Wellborn 1995; Wellborn and Bartholf 2005). Moreover, experiments where male–male interactions are eliminated have demonstrated that mating biases for male traits are primarily driven by male–female interactions (Cothran 2008b; Cothran et al. 2010). Specifically, female resistance behavior selects for large male body size and large PG

size (Wellborn 1995; Wellborn and Bartholf 2005). In the focal species of this study, females that choose large males with large PGs receive both direct and indirect benefits (Cothran 2008c).

### EXPERIMENTAL ANIMALS

Amphipods (species OK-L in Wellborn and Broughton 2008) were collected from Lake Thunderbird (Cleveland County, OK) and kept in a 14-L plastic tub containing charcoal filtered, ultraviolet irradiated, well water. Shredded cardboard and waterweed (*Elodea* sp.) were added to each tub to provide substrate and refugia. Animals were housed in a temperature-controlled room (mean  $\pm$  1 SD:  $22.2 \pm 0.2^\circ\text{C}$ ) with a 12:12 day:night cycle. Twice each week, stock animals were fed a 4:1 mix of alfalfa and Tetramin<sup>®</sup> fish flakes (Melle, Germany) as well as high-P algal discs (see below for preparation of algal discs). From this stock population, 50 females with late-stage embryos in their marsupium were placed individually into 30-mL cups containing filtered well water and one high-P algal disc. The cups were checked daily for neonates. If neonates were found, the female was removed and returned to the stock population and the number of neonates was counted. Only families that produced a minimum of 12 neonates were used in the study (48 of the 50 females produced enough offspring). Each neonate was transferred to a separate 30-mL cup filled with filtered water. Individuals were then randomly allocated to treatments. All individuals used in the experiment hatched within one week. After the amphipod cups were assigned to treatments, they were placed into one of 28 empty plastic tubs and kept in a temperature-controlled room (mean  $\pm$  1 SD:  $22.2 \pm 0.2^\circ\text{C}$ ) with a 12:12 day:night cycle. Throughout the experiment, we randomly reassigned the position of the tubs to minimize any position effects.

### MANIPULATION OF P AVAILABILITY

P availability was manipulated by feeding amphipods either one low-P algal disc or one high-P algal disc every week. To prepare the discs, we collected attached algae, using clay pots as substrate for the algae to colonize, from three lakes (Lake LeBoeuf, Sandy Lake, and Crystal Lake all located in NW Pennsylvania, United States). After a 14-day colonization period, the algae were scraped from the pots to make an algal slurry. We then filled three 90-L wading pools with well water and seeded each pool with 450 mL of the algal slurry. We then spiked each pool with  $\text{NaNO}_3$  and  $\text{KH}_2\text{PO}_4$  to achieve approximate molar concentrations of 1000  $\mu\text{M}$  N and 2  $\mu\text{M}$  P, respectively. Our goal was to culture the algae onto discs that could be readily supplied to amphipods. Algal discs were prepared by cutting gardening paper into 6-mm discs and culturing the algal discs in the pools for 46 days. After 46 days, the discs were harvested from all three pools and thoroughly mixed together. We then manipulated the phosphorus content of the algal discs by spiking the discs for 24 h with

200 or 800  $\mu\text{L}$  of a 50 mM  $\text{KH}_2\text{PO}_4$  stock solution to achieve nominal molar concentrations of either 10 (low-P availability) or 40  $\mu\text{M}$  P (high-P availability), respectively. These discs were then stored in small batches at  $-80^\circ\text{C}$ . During the experiment, amphipods were given a new algal disc every week. We used a modified sulfuric acid digestion method that was verified using a spinach standard (NIST 1570a) to measure the P content of the discs. Phosphorus content of algal discs was  $30.67 \pm 4.25 \mu\text{gL}^{-1}$  (mean  $\pm$  SE) and  $116.78 \pm 7.9 \mu\text{gL}^{-1}$  for the low- and high-P discs, respectively. C content of discs was quantified using an automated elemental analyzer (Elementar Americas, NJ). Molar C:P of algal discs were  $383 \pm 18$  for low-P and  $103 \pm 13$  for high-P treatments. These values are representative of mesotrophic and eutrophic lakes, respectively (Wetzel 2001).

### PREPARATION OF PREDATOR CUES

We used predator-conditioned water to manipulate predation risk. To produce the predator cue, a single mid-instar larval dragonfly (*Anax junius*) was housed in a 1-L container and fed amphipods ad libitum. Predator cue containers were checked every three days and amphipods that had been consumed were replaced with individuals from control containers to return the total number of amphipods to 20. To produce a no-predator cue, we used two 1-L containers that contained amphipods from the stock population but no predators. Amphipods from the stock population were used to return the total number of amphipods in control containers to 20.

Roughly four weeks into the experiment, there was an outbreak of a lethal parasitic horsehair worm (Nematomorpha: Gorodioidea) that killed the *A. junius* larvae. New *A. junius* were not available; therefore, larvae of another dragonfly (*Libellula* sp.) were used to generate predator cues. Two *Libellula* were used per predator container. Although this switch in predator species was not ideal, both *A. junius* and *Libellula* sp. dragonflies are common at the locality where the amphipods were collected. Furthermore, all of the amphipods in the experiment were exposed to both predator species. *Libellula* dragonflies were used to generate cue for 16 days ( $\sim 28\%$  of the experiment) until new *A. junius* individuals were collected. *Anax junius* were used to generate cues for the remainder of the experiment.

Predator and no-predator cues were added to the experiment each day. Each day, we selected two *A. junius* containers that showed evidence of foraging. The water from these two containers was poured through a fine mesh net into a 14-L plastic box to remove the animals and was thoroughly mixed. For cups in the no-predator cue treatments, we followed the same procedure but collected water from the no-predator containers. Although collecting the predator and no-predator cues, we used separate mesh nets, pipettes, and plastic boxes to avoid cross-contamination. After a cue container was used in the experiment, fresh, filtered



**Figure 1.** Male and female *Hyalella* amphipods and traits measured. Traits are ordered from left to right according to the degree of sexual dimorphism: pereopod (P), anterior gnathopod (AG), second antenna (A), and posterior gnathopod (PG).

water was added to the container and the container was not used for approximately one week to ensure the presence of predator cues. Three milliliters of water were removed from each 30-mL cup ( $\sim 10\%$  of total volume) and replaced with either 3 mL of predator cue water or 3 mL of no-predator water. All organisms excrete P (Vanni 2002), giving rise to the possibility that experimental animals in the predator cue treatment may have access to higher concentrations of P. However, predator-treated water contained minimal concentrations of dissolved P (Jeyasingh and Weider 2005). Furthermore, in this experiment, we fed amphipods freeze-killed algae. Thus, even if there were significant P release from the predators, much of this excreted P would not be readily available to amphipods because dead algal cells cannot assimilate inorganic P.

### RESPONSE TRAITS

#### *Growth rate and morphological traits*

We measured five morphological traits in males and females to assess how investment in traits responded to P availability and predator cues. All traits were digitized using an Olympus SZX16 (Olympus America, Center Valley, PA) microscope fitted with a DP25 digital camera and measured using DP25 measurement software. We measured two traits (width of PGs in Fig. 1) and length of second antenna (A in Fig. 1) that are known targets of sexual selection in amphipods or in isopods (a taxon that has a similar mating biology; Wellborn 1995; Bertin and Cézilly 2003; Wellborn and Bartholf 2005). Empirical evidence indicates that female behavior produces mating biases in this species (Cothran 2008a; Cothran et al. 2010). We also measured the width of the anterior gnathopod (AG in Fig. 1), a trait that males use to hold females in the precopulatory position (a prerequisite for mating) but is not a known target of sexual selection. We also measured the basis segment (including the coxal plate) of the fifth pereopod (P in Fig. 1), a trait that has no known mating

function. Finally, we measured head length, a trait that is highly correlated with both total length and mass in amphipods (Edwards and Cowell 1992). Because of this relationship, we define amphipod growth rate as the change in head length over time. Linear measurements of each trait were taken on the right side of each animal (Fig. 1). All traits were log-transformed before data analysis. We tested the repeatability of our measurements by measuring each trait twice for 20 individuals of each sex and repeatability was found to be high for all traits (intraclass correlation coefficients [ICC]  $\geq 0.969$ , mean ICC for traits  $\pm$  SD:  $0.987 \pm 0.011$ ).

#### *Male pairing success assay*

We used a mate competition assay to assess whether responses to P-availability and perceived predation risk affected pairing success. A subset of mature males (assessed by the presence of enlarged PG) was used in the experiment and competed against males from the stock population. To differentiate between the focal male and the competitor male, both males were anesthetized with a clove oil solution (clove oil concentration of  $2.97 \times 10^{-4}$  mL mL<sup>-1</sup>; Venarsky and Wilhelm 2006) and the left or right fifth walking leg (pereopod) was clipped to remove two to three of the most distal segments. The side that was clipped on the focal and competitor male was alternated across trials. Stock males were 19%–24% larger than focal males (depending on the focal male treatment), and the size of the stock males used in the assay did not differ across treatments (all  $P \geq 0.143$ ; mean head length  $\pm$  SD:  $0.74 \pm 0.06$  mm). Recently, molted females from the stock population were selected to ensure that females experienced males for several days before pairing, because females are only receptive to pairing from approximately day 5–10 of their 10-day molt period (Strong 1973; Wellborn and Cothran 2007). Females were acclimated for 24 h in a 30-mL cup containing appropriate nutrient and predator treatments that were similar to the conditions in which the focal male developed. Half of the water from the original cup in which the focal male developed was used to acclimatize the competitor male in another 30-mL cup for 24 h. After acclimation, the competitor male and the female were added to the original cup with the focal male. Replenishment of predator cues continued daily. Each of these experimental cups was supplied with three algal discs every day. The cups were checked twice daily (0800–0900 and 1600–1700 EST) for pairs. If a pair was observed, the single male was removed and his leg clip was recorded. We defined the loser of the competitive interaction as the male that was last recorded as unpaired before oviposition occurred. This measure of male fitness is appropriate because females do not store sperm and the last male that pairs is in position to fertilize the female's eggs as they enter her brood pouch. We also recorded if an individual died or was cannibalized during the assay. After oviposition, all three indi-

viduals were preserved in 95% ethanol. Mature males that did not enter the mating trial were preserved in bulk on three consecutive days to minimize age differences. These animals were included in the analysis of treatment effects on morphology (see above).

#### *Female fecundity assay*

Once focal females had developed their first clutch of eggs (visible as a green pigmented spot on the dorsal side of the female), they were presented with a single male from the stock population to obtain fecundity data. A second algal disc was added to the cups to increase food availability for the pair. Two mating events were allowed to occur. The cups were dosed daily with predator cue or control water. Female maturity was recorded as the day of the female's first oviposition. After a female's second oviposition, neonates were counted as a measure of fecundity for the first reproductive event. During this portion of the experiment, there were numerous cases where the larger, stock male cannibalized the smaller, focal female. Not all females from the experiment were put through the fecundity assay due to a limited number of mature males in the stock population and the high levels of cannibalism. As a result, the females that did not go through a mating trial were preserved after they developed a visible clutch of eggs in the ovaries (visible on their dorsal side). These animals were included in the analysis of treatment effects on morphology (see above).

### STATISTICAL ANALYSES

#### *Growth rate and morphological traits*

Although individuals were reared separately, we expected responses by full-siblings to be correlated. Moreover, the number of individuals in each treatment per family ranged from one to three for females and one to six for males resulting in an unbalanced design. For these reasons, we used linear mixed models (LMMs, which use restricted maximum likelihood to estimate parameters; MIXED procedure in SPSS). These models can handle unequal samples sizes (i.e., unbalanced designs) and can correct for correlated data (SPSS Technical Report 2005). We included family as a random effect in these models to account for within family correlations. Separate analyses were performed for males and females because broods are typically male or female biased that led to incomplete overlap in families used for the male and female results. Only families that were represented in all four treatments were included in the analysis. For females, the dataset includes 110 individuals across 17 families. The male dataset includes 417 individuals across 38 families.

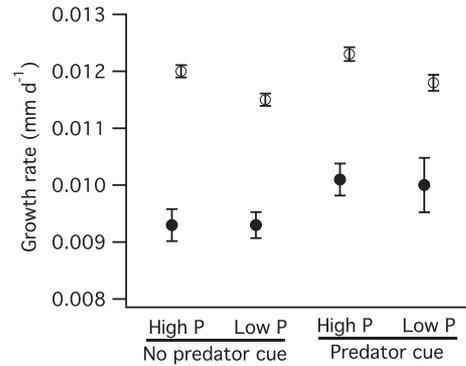
We used an LMM to assess the effects of P availability and predator cues on growth rates. A multivariate LMM was used to assess the effects of P availability and predator cues on morphological traits using head length (our measure of body size) as a

covariate.

For males, we also examined phenotypic correlations between size-adjusted sexually selected traits and growth rates to examine the sensitivity of trade-offs to predator and nutrient stress. To adjust traits for body size, we first used a multivariate analysis of covariance (MANCOVA) with P-availability and predator cue treatments as fixed factors and head length as a covariate and saved the residuals from the analysis. These residuals represented our size-adjusted measure of trait size. An assumption of this size correction method is that the allometric slopes for the relationship between each trait and body size are equal across treatments (McCoy et al. 2006). This assumption held true for antenna size but not PG size (P availability-by-body size interaction:  $F_{1,442} = 6.199$ ,  $P = 0.013$ ). Inspection of the relationship between body size, PG size, and P availability revealed that the interaction was driven by convergence of the allometric relationships at larger body sizes. Because we did not find an interaction over 86% of the range of body sizes (there was no interaction below  $\ln(\text{body size}) = -0.4307$ :  $F_{1,382} = 1.216$ ,  $P = 0.271$ ), we decided to go forward with this method. We calculated the mean size-adjusted trait size for male full-siblings and used these values in correlations. In 22% of cases, these values were point estimates rather than means because a treatment-by-family combination was only represented by a single individual. The number of families used varied across treatments because in a few cases a particular family had no male offspring in a particular treatment. The number of families used ranged from 42 (high P with predator cues) to 46 (high food without predator cues).

*Male pairing success assay*

We used chi-squared tests to assess whether there was an association between treatments (P availability and predator cues) and focal male pairing success (high P with cue  $n = 42$ ; high P without cue  $n = 42$ ; low P with cue  $n = 36$ ; low P without cue  $n = 45$ ). We first ran a test that included all four treatments to assess whether differences in pairing success exist among treatments. If this overall test was significant, we proceeded to subdivide the contingency table to identify that treatment(s) were responsible for effects (Glantz 1997, pp. 137–140). The sequential Bonferroni method was used adjust alpha to account for multiple testing. To assess whether known sexually selected traits (body size represented by head length and PG size) contributed to focal male pairing success, we used logistic regression analysis. We used size-corrected gnathopod size (see above) in this analysis. First, we standardized traits within P-availability and predator cue treatments in an attempt to avoid a redundant test of the effects of our treatments on male pairing success. Then, we used these standardized traits in a logistic regres-



**Figure 2.** Growth rate was sensitive to both P availability and perceived predation risk in males but only predation risk in females. Results are means for the same set of families across environments ( $\pm 1$  standard error). Open and closed circles represent male and female growth rates, respectively.

sion model with focal male pairing success as a binary response variable.

*Female fecundity assay*

We used a multivariate LMM to test if predator and P-availability treatments affected measures of female fecundity. Head length was included as a covariate in the model. This analysis included 80 females distributed across 17 families (one to three females per family per treatment).

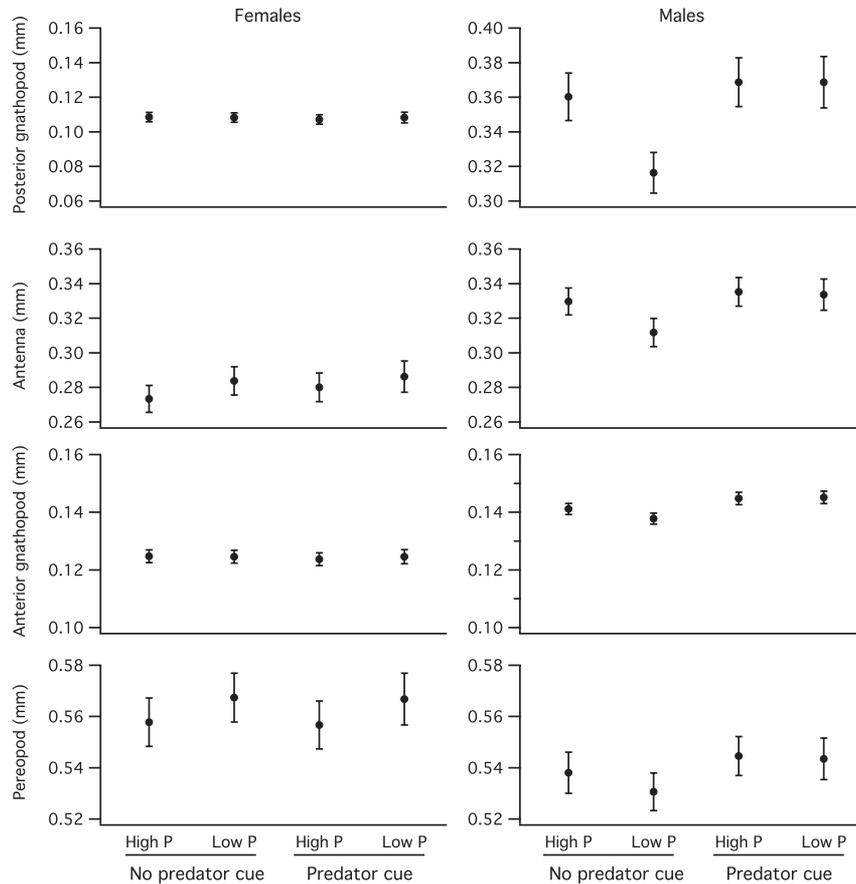
*Results*

**EFFECT OF P AVAILABILITY AND PREDATOR CUES ON GROWTH AND SEXUAL AND NONSEXUAL MORPHOLOGICAL TRAITS**

For males, increased P availability ( $F_{1,374.856} = 26.714$ ,  $P < 0.001$ ) and predator cues ( $F_{1,374.252} = 9.949$ ,  $P = 0.002$ ) both resulted in higher amphipod growth rates, but there was no effect of their interaction (Fig. 2). Family explained a significant amount of variation in growth rates (random effect, Wald  $Z = 3.356$ ,  $P = 0.001$ ).

Female growth rate was higher under perceived predation risk ( $F_{1,97.502} = 5.091$ ,  $P = 0.026$ ). However, we found no effect of P availability ( $F_{1,98.535} = 0.239$ ,  $P = 0.626$ ) or the interaction ( $F_{1,96.429} = 0.114$ ,  $P = 0.737$ ). Family did not explain a significant amount of variation in female growth rates (random effect, Wald  $Z = 0.746$ ,  $P = 0.455$ ; Fig. 2).

For male morphology (pereopod, AG, antenna, and PG), there was a multivariate effect of P availability ( $F_{4,369.359} = 6.633$ ,  $P < 0.001$ ), predator cue ( $F_{4,366.358} = 23.873$ ,  $P < 0.001$ ), and their interaction ( $F_{4,364.887} = 6.397$ ,  $P < 0.001$ ). Head length (i.e., body size;  $F_{4,402.258} = 507.814$ ,  $P < 0.001$ ) and family



**Figure 3.** Sexually selected traits responded more strongly to P availability and predator cues than nonsexual traits. Traits are arranged, top to bottom, from the most sexually dimorphic (posterior gnathopod) to least sexually dimorphic (pereopod). Results are estimated marginal means (assessed at a head length of 0.591 mm for males and 0.616 mm for females) for the same set of families across environments. Error bars are the 95% CI.

(random effect, Wald  $Z = 4.149$ ,  $P < 0.001$ ) also explained significant amounts of variation in the model. The estimates of fixed univariate effects revealed that the response of traits to P availability decreased with decreasing trait sexual dimorphism, but that this sensitivity was masked under perceived predation risk (P availability-by-predator cue interaction PG:  $t_{367.924} = 3.373$ ,  $P = 0.001$ ; A:  $t_{351.24} = 2.67$ ,  $P = 0.008$ ; AG:  $t_{369.298} = 2.45$ ,  $P = 0.015$ ; P:  $t_{371.829} = 1.037$ ,  $P = 0.3$ ; Fig. 3).

Female morphology was unresponsive to our treatments (Fig. 3). There was no multivariate effect of P availability ( $F_{4,104.493} = 2.042$ ,  $P = 0.094$ ), predator cue ( $F_{4,104.933} = 0.403$ ,  $P = 0.806$ ) or the P availability-by-predator cue interaction ( $F_{4,104.933} = 0.152$ ,  $P = 0.962$ ). Head length ( $F_{4,104.933} = 145.268$ ,  $P < 0.001$ ), but not family (random effect was too close to zero to estimate) explained a significant amount of variation in the model.

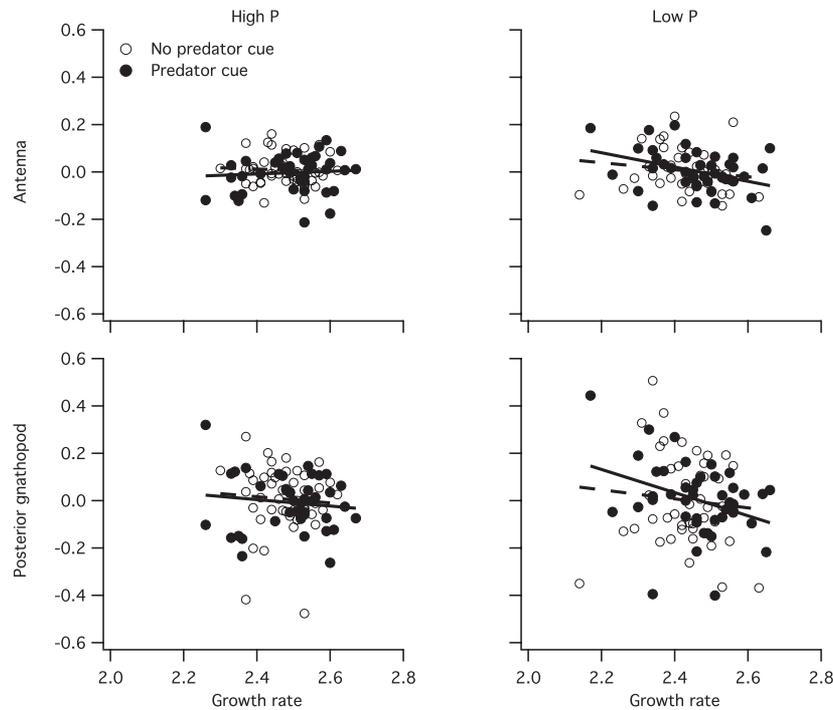
Overall, male sexual traits were more responsive to P availability than nonsexual traits. All of the traits that males use in sexual interactions were larger when P availability was higher and this effect was greatest for the most sexually dimorphic traits

(PG: 14% increase, A: 6%, AG: 2%). The nonsexual trait (P) and female morphological traits were not responsive to phosphorus availability.

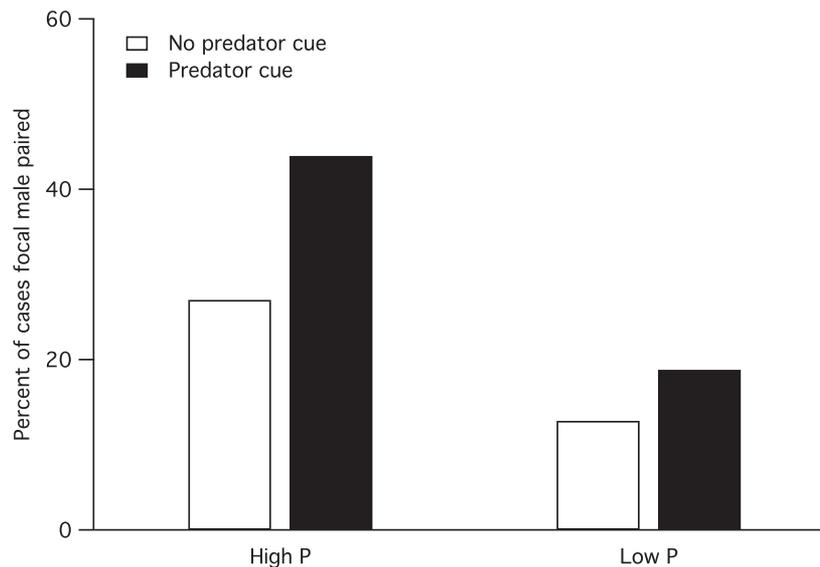
Next, we examined phenotypic correlations to look for potential trade-offs between size-adjusted sexual traits and growth rates. Under high-P availability, we found no relationship between growth rate and either of the sexual traits, regardless of predator treatment (all  $P \geq 0.518$ ; Fig. 4). Under low-P availability, however, we found a trade-off between each sexual trait and growth rate, but only when predator cues were present (with cue: antenna  $r = -0.376$ ,  $P = 0.017$ , PG  $r = -0.417$ ,  $P = 0.007$ ; without cue both  $P \geq 0.247$ ; Fig. 4).

**MALE PAIRING SUCCESS ASSAY**

Treatments differed in how successful focal males were at achieving pairing success against larger stock males ( $\chi^2 = 13.8$ ,  $df = 3$ ,  $P = 0.003$ ,  $\alpha = 0.016$ ; Fig. 5). Focal males raised on high-P food were twice as successful at pairing with females than males raised on low-P food ( $\chi^2 = 7.417$ ,  $df = 1$ ,  $P = 0.006$ ,  $\alpha = 0.016$ ). Animals raised with predator cues also had higher



**Figure 4.** Trade-offs between investment in a viability trait (growth rate) and residual sexual trait size (derived from regressing trait size against head length—our measure of body size) are only revealed when predator cues are present and P is in low supply. Data represent family means. Solid lines represent the fit when families were exposed to predator cues, whereas broken lines represent the fit when families were not exposed to predator cues. The results are for log-transformed traits.

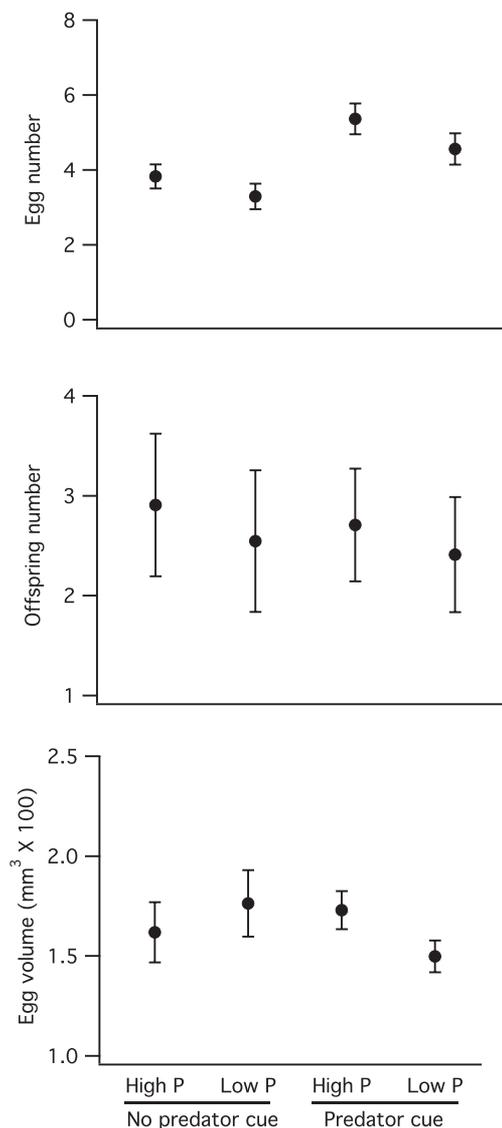


**Figure 5.** Focal males raised on high-P algae and in the presence of predator cues were more successful in competing for females against larger stock males. Mating success was measured as pairing success. Values represent the percent of cases the focal male paired for each treatment group.

pairing success than animals raised without cues ( $\chi^2 = 6.542$ ,  $df = 1$ ,  $P = 0.011$ ,  $\alpha = 0.016$ ). However, the logistic regression on traits standardized by P availability and predator cue treatments revealed that this success was not related to body size ( $P = 0.712$ ) or PG size ( $P = 0.229$ ).

**FEMALE FECUNDITY ASSAY**

Examining the three measures of female fecundity (number of neonates produced from the first reproductive event, and egg number and volume from the second reproductive event), we found a multivariate effect predator cues ( $F_{3,56,316} = 3.145$ ,  $P = 0.032$ )



**Figure 6.** Females produced more eggs under perceived predation risk, but other measures of female fecundity were not responsive to P availability or predator cues. For egg number and egg volume, there was no relationship between trait and body size; therefore, results are means for the same set of families ( $\pm 1$  standard error). For offspring number, results are estimated marginal means (assessed at a head length of 0.625) for the same set of families across environments. Error bars are the 95% CI.

and a marginally significant effect of body size (measured as head length;  $F_{3,56.316} = 1.785$ ,  $P = 0.16$ ). P availability ( $F_{3,56.316} = 1.209$ ,  $P = 0.315$ ) and the P availability-by-predator cue interaction ( $F_{3,56.316} = 0.8$ ,  $P = 0.499$ ) had no effect on female fecundity. Examination of the estimated of fixed univariate effects revealed that females produced larger clutches under perceived predation risk ( $t_{66} = 2.126$ ,  $P = 0.037$ ; Fig. 6). Body size was correlated with offspring number ( $P = 0.041$ ) but not the other traits (all  $P \geq 0.345$ ).

## Discussion

Our study shows that resource quality and the threat of predation interact to shape sexually selected traits and viability traits that share a common resource pool. Male sexual traits were highly sensitive to P availability whereas nonsexual traits showed little response. In addition, the presence of predator cues induced males to increase their growth rate and the relative size of sexual and nonsexual traits. For the PG (one of the most important sexual traits; Wellborn 1995; Wellborn and Bartholf 2005), increased P caused the male trait to become relatively large, but only if predator cues were absent. Phenotypic correlations indicated that when P was in low supply, males that invested more in growth under the threat of predation produced smaller sexual traits. However, this trade-off was only revealed when P was in low supply. Males raised on high-P algae were better competitors for mates than males raised on low-P algae. Also, males raised with predator cues were more likely to achieve pairing success than males raised without cues. However, we found no evidence that the success of these males was necessarily the result of changes in sexually selected traits. Although perceived predation risk affected female life-history traits, eliciting faster growth and greater egg production, P availability had no effect on female morphological traits.

The sexes demonstrated strikingly different responses to the availability of P and perceived predation risk. For example, male but not female growth rate was extremely sensitive to P. Female growth rates were not sensitive to increased P availability, suggesting that the sexes have different nutritional requirements (Maklakov et al. 2008; Morehouse et al. 2010). In *Hyalella*, sex-specific responses to P availability may be due to males exhibiting higher growth rates, and thus greater P demand, than females. Sex differences in P sensitivity have important implications for interactions between the sexes. Large body size is important for male mating success in *Hyalella* (Wellborn 1995; Wellborn and Bartholf 2005) and, in at least one species, females gain both direct and indirect benefits for mating with large males (Cothran 2008a). Under low-P conditions, high growth rates may also signal a male's ability to acquire or efficiently use this limiting nutrient. Moreover, sexual conflict over guarding duration exists in *Hyalella*, with males preferring longer guarding durations than females (Cothran 2008b). To the extent that body size determines fighting ability (Parker 1974; Thornhill and Alcock 1983), low P may limit the ability of males to exploit females. Finally, data indicate that the nutritional environment interacts with the predation environment to produce sex-specific responses. For example, we found that while perceived predation risk induced faster growth in both sexes, the P environment did not affect this response in females, indicating differential P demands between the sexes. Similarly, females invested more into eggs under perceived predation risk, and this response was independent of the amount of P avail-

able. As such, these sex-specific adaptive responses to alterations along two important ecological axes point to a large potential for environmentally mediated impacts on sexual interactions.

From a receiver's perspective, sexual traits are only reliable when they signal information about an individual's quality (e.g., ability to fight or benefits an individual can offer a mate; Zahavi 1975; Grafen 1990; Johnstone 1995; Maynard Smith and Harper 2003). In *Hyalella*, females often resist male pairing attempts and this resistance selects for males with larger PGs (Cothran et al. 2010). Moreover, in the species studied here, females gain both direct and indirect benefits from choosing large males with large PGs (Cothran 2008a). These results clearly indicate that gnathopods are important in signaling male quality to females. Although the importance of antennae in mating interactions has not been documented in *Hyalella*, it is likely that this trait does play a role in sexual interactions. First, in aquatic isopods, which share a similar mating biology with amphipods, larger antennae increase mating success (Bertin and Cézilly 2003). Second, this trait is sexually dimorphic in *Hyalella* (17% larger in males than females). Sexually selected traits, including the PG of male amphipods, often grow at a faster rate than other traits (Kodric-Brown et al. 2006; *but see* Bonduriansky 2007); as a result, their development will impose strong demands on an individual's nutrient budget (Morehouse et al. 2010). By extending the growth rate hypothesis (Elser et al. 1996) to traits within organisms, we would predict that fast growing sexual traits would have a high-P demand to support protein synthesis (Kay et al. 2005). We discovered that sexual traits were indeed more sensitive to P availability than nonsexual traits and this sensitivity increased with trait sexual dimorphism (response by  $PG > A > AG > P$  in Fig. 3). These results suggest that the availability of nutrients can have profound effects on the development of sexual traits. Under low nutrient availability, sexual traits that are sensitive to nutrient stress may inform females about a male's resource acquisition and assimilation efficiency (Harris et al. 2008; Wolf et al. 2008). However, under high nutrient availability, typical of eutrophic lakes, the utility of male sexual traits as signals may be compromised because all males can sequester enough nutrients for sexual trait development.

Predator environments caused trade-offs between sexual traits and a viability trait (i.e., growth rate) under low-P conditions. For many species, predation risk decreases with increased prey size favoring rapid prey growth early in life (Werner and Gilliam 1984). Given that rapid growth requires more P (Elser et al. 1996), predator induction of rapid growth would cause strong P demand (Jeyasingh and Weider 2005). It follows that growth and sexual traits should compete for similar resources. Thus, we should expect negative correlations between these traits if individuals vary in how they allocate P, but only when P is in low supply (van Noordwijk and de Jong 1986). Indeed, under the threat of predation and low-P availability, we discovered that growth rate

was negatively correlated with the size of sexually selected traits. In the absence of predator cues, however, this relationship was weak and nonsignificant. Our study clearly demonstrates that under low-P availability males are forced to alter their investment in sexual traits and viability traits depending on the presence of predator cues.

Our results also suggest that P availability and predator cues affect male mating success. Males raised on high-P algae were more successful at competing for mates than males raised on low-P algae. However, we found no evidence that this success was the result of changes in sexually selected traits. Males fed high-quality diets may have been more vigorous in their attempts to mate with females. The lack of a relationship between male sexual traits and pairing success in our experiment does not necessarily mean that changes in allocations to sexual traits observed in our study will not have evolutionary consequences. First, our experiment only assessed competitive interactions between males that differed greatly in body size (focal males were always much smaller than competitor males). As a result, focal males were rarely successful (i.e., they paired in 26% of cases across all treatments). Second, it is well established that the traits that were sensitive to P availability and predator cues (PGs and antennae) are important determinants of mating success in nature and in the laboratory (Wellborn 1995; Bertin and Cézilly 2003; Wellborn and Bartholf 2005). Finally, although we tried to statistically tease apart the relative importance of behavioral (due to differences in the quality of food) and morphological traits in explaining predator and nutrient-induced effects on mating success, we suggest that a robust test requires further experimentation. A growing number of studies have found a link between limiting nutrients and sexual trait expression (Bertram et al. 2006, 2009; McGraw et al. 2006), and future studies should focus on how these environmentally driven developmental changes affect mating success.

Collectively, our study shows that the environment causes plastic responses in how individuals invest in sexually selected traits. Although recent studies have discovered that sexual traits are hypersensitive to resource stress and that sexual trait variation available for selection can be affected by resource supply (Cotton et al. 2004b; Bonduriansky and Rowe 2005; Kemp and Rutowski 2007; Parker and Ligon 2007; Contreras-Garduno et al. 2008; Kemp 2008; Punzalan et al. 2008; Cothran and Jeyasingh 2010), our results demonstrate for the first time that predator cues and resource availability interact to shape phenotypic variation available for selection and phenotypic trade-offs. Increased nutrient availability at levels commensurate with rates of cultural eutrophication appears to alleviate nutrient stress imposed by investing in nutrient-rich sexually selected traits. Thus, a nutrient-rich environment can mask genetic variation in sexual traits among males, and compromise the effectiveness of sexual traits as

indicators of male quality. Understanding such environmentally driven alterations to the expression and maintenance of fitness-relevant phenotypes, while particularly relevant to contemporary evolutionary theory, should also illuminate the microevolutionary impacts of anthropogenic global change on biota.

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