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ECOLOGICAL CONTEXT AND THE EVOLUTION OF MATING BIASES IN A FRESHWATER AMPHIPOD SPECIES COMPLEX

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

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ABSTRACT

Mating traits are among the most extravagant traits found in nature. As with any trait, understanding the diversification of mating traits requires information on both direct and indirect selection acting on the traits and quantification of the genetic variation available for selection. This dissertation is an attempt to elucidate the possible direct and indirect selection pressures acting on mating traits in the *Hyalella azteca* species complex. These amphipods are found in a variety of freshwater habitats and thus different species and populations are exposed to different ecological pressures. Here, I focus on two reproductively isolated, undescribed species that are found in disparate habitats and differ with respect to body size (hereafter referred to as large species and small species) and several life history traits. This condition sets the stage for divergence in traits that impact fitness because ecology determines the form and magnitude of natural selection acting on populations. Thus, the genus *Hyalella* is a useful group for studying the evolutionary ecology of mating biases.

In the first chapter, I explore the potential for sexual conflict over guarding duration in two species that occur under disparate ecologies. I find evidence for conflict over precopulatory mate guarding duration in both species. When female resistance behavior is experimentally removed, guarding duration increases for both species. Furthermore, experimental reduction of female resistance results in an increase in the number of male grasping attempts that are successful and these interactions last longer than when female resistance is unencumbered. Therefore, sexual conflict over precopulatory guarding duration may play a role in

the diversification of mating traits in *Hyalella.* Future studies of the traits mediating this conflict and of the female fitness consequences of mating with successful males will be needed to determine the evolutionary importance of this conflict.

In chapter 2, I examine the mechanistic basis of the large male mating advantage in *Hyalella*. The well-documented large male mating bias persists even when male-male interactions are limited experimentally, suggesting that male-female interactions are the primary driver of this effect in *Hyalella*. Forcible takeovers, however, do occur and seem likely to accentuate the advantage of large male size. I argue that future work should focus on sexual conflict and female choice as the primary mechanisms of sexual selection operating in *Hyalella*.

In chapter 3, I take a comprehensive look at the female fitness benefits gained from mating with successful males in the species that shows the strongest large male mating bias. I find evidence for both direct and indirect fitness benefits and no cost associated with mate choice. Thus, the strong mating biases observed in this species are likely the result of female choice. The lack of costs explains the highly skewed size-based pairing distributions and male-biased sexual size dimorphism observed in this species.

This dissertation raises many questions. 1) What role has sexual conflict played in the evolution of mating traits? In the small species, single females are more susceptible to fish predation while paired than when single. This is not the case for the large species resulting in asymmetries between the two species in the costliness of pairing (Cothran 2004). In chapter 1, I found that the percent increase in pairing duration when females were unable to resist male guarding attempts was greater in the small species than the large species. This result suggests that the degree to which male and female interests differ over guarding duration is greater in the small species. Previous work has shown that paired females are at greater risk of predation in small species populations where they co-occur with positive, size-selective predatory fish. Thus, I argue that sexual conflict may be more important in the small species where the costs to females of early pairing are greatest, thus resulting in greater intersexual asymmetries in optimal pairing durations. 2) What does the genetic architecture of body size and posterior gnathopod size look like, and does heritability of these traits vary across environments? The posterior gnathopod is an anterior appendage that is greatly enlarged in males. In nature, males with larger posterior gnathopods have higher pairing success, which, along with the strong sexual dimorphism, suggests this trait has been shaped by sexual selection. Theory predicts that sexually selected traits will often show heightened condition dependence. An individual’s condition has been defined as the total pool of resources acquired for allocation to various traits that impact fitness. This recent emphasis on condition dependency of sexually selected traits highlights the importance of understanding the resources necessary to build sexual traits and how such resources vary over space and time. Specifically, under benign conditions genetic variation associated with mating traits may be masked. Understanding how traits, and their additive genetic variation, vary across environments may shed light on the large disparity among species in within-population trait variation. 3) What are the female fitness consequences of mating with large males in the small species? In chapter 3, I found that in the large species, mating with large males increases female fitness both directly, through decreased predation risk while paired, and indirectly, through the superior mating success of sons. At least the direct benefit is unlikely to manifest in small species populations where fish predators select for small size prey. Therefore, it is possible that the mechanistic basis of size-biased pairing patterns differs between species, potentially explaining species differences in the strength of sexual selection on male traits and sexual size dimorphism.

CHAPTER 1

REMOVAL OF FEMALE RESISTANCE BEHAVIOR REVEALS POTENTIAL FOR SEXUAL CONFLICT OVER PAIRING DURATION IN TWO FRESHWATER AMPHIPOD SPECIES (*HYALELLA* SPP.)

Formatted for Behavioral Ecology and Sociobiology

ABSTRACT

Sexual conflict is common in nature, but detailed behavioral studies on the role of female resistance behavior in shaping mating patterns are rare. I manipulated female resistance to examine its effects on pairing dynamics in two ecologically different freshwater amphipods. I found evidence for female behavior playing an important role in both the outcome of pre-pairing interactions and the initiation of pairing in both species. In these species, the male optimum pairing duration is greater than the value preferred by females or compromised pairing durations observed under natural conditions, thus indicating sexual conflict. Furthermore, the proportion of male-female encounters producing male grasping was greater and the duration of such interactions was longer when female resistance was reduced. Thus, sexual conflict over pairing duration may select simultaneously for female resistance and for male persistence both of which mediate the outcome of pre-pairing interactions in *Hyalella*. Contact precopulatory mate guarding and the interactions that precede it are common components of crustacean and insect mating systems, suggesting that such conflicts may play an important role in the evolution of mating traits in many taxa.

INTRODUCTION

Sexual conflict over mating is expected to be common in nature (Chapman et al. 2003, Arnqvist and Rowe 2005), although its detection and analysis can be difficult in practice. Rowe and Day (2006) recently proposed three elements for demonstrating that sexually antagonistic selection is acting within a population: 1) identification of the trait over which conflict occurs (shared trait), 2) identification of the traits in each sex that mediate the outcome of conflict over the shared trait (antagonistic traits), and 3) an understanding of the fitness consequences of the antagonistic traits for each sex (Thornhill 1980). Although point 3 makes uncovering sexually antagonistic selection a daunting task, significant progress has been made for some groups, particularly species in which females store sperm and sexes experience conflict over mating rates (*reviewed in* Arnqvist and Rowe 2005). In many of these taxa, researchers have identified traits that mediate the outcome of conflict over mating rate, and the effect of these traits on fitness components has been demonstrated in many cases (*reviewed in* Arnqvist and Rowe 2005). However, evidence for pre-mating sexual conflicts is far less common and often incomplete.

Local ecological conditions across a species' range can result in variation in sexual conflict dynamics and lead to divergence in mating patterns across populations. The opportunity for, or intensity of, sexual conflict varies across populations. Population structure, i.e. density and sex ratios, will determine male-female encounter rates, and thus play an important role in determining the degree of sexual conflict within populations. In fact, population structure is often manipulated in sexual conflict studies to vary the opportunity for conflict. These studies have shown increases in resistance and persistence behaviors in treatments with more male-female interactions (Arnqvist 1992, Martin and Hosken 2003), and decreases in female fitness components under high density situations (Martin and Hosken 2004). Furthermore, predation risk and environmental resource levels can affect a female's willingness to mate, thereby affecting the value of male persistence traits (Rowe et al. 1994, Magurran and Seghers 1994a).

In many crustaceans, a female's receptivity to fertilization is limited to a brief period after she molts, a temporal restriction that automatically produces a male-bias in the operational sex ratio. This skew is thought to have created fitness incentives for male precopulatory mate-guarding, which is widespread in the group (Ridley 1983). In species that form precopulatory pairs, sexual conflict occurs because males and females are likely to disagree about the optimal duration of pairing (Parker 1979). Sex-specific costs while paired may be associated with predation risk (Cothran 2004), foraging efficiency (Robinson and Doyle 1985), depletion of stored energy (Jormalainen et al. 2001) and missed mating opportunities (*reviewed in* Jormalainen 1998), any or all of which may affect the relative value being paired. Similarly, the value of entering precopula at a particular time may differ greatly between the sexes because of sex differences in the ability to find a mate (Jormalainen et al. 1994). This topic has received considerable theoretical treatment and conflict is generally predicted to be most intense near the middle of a female's molt cycle (Jormalainen et al. 1994, Yamamura and Jormalainen 1996). The consensus seems to be that males have more to gain from pairing earlier in the female’s molt cycle than do females.

Freshwater amphipods in the genus *Hyalella* are common inhabitants of permanent freshwater habitats in North America (Bousfield 1958). Two ecomorphs are commonly found regionally: a small-bodied ecomorph (hereafter 'small species') found in habitats with visual, size-selective predators (e.g. *Lepomis* spp.), and a large-bodied ecomorph (hereafter 'large species') found in habitats where they experience little or no fish predation (Wellborn 1994). These ecomorphs represent reproductively isolated, undescribed species and each ecomorph is represented by multiple species within the *Hyalella azteca* species complex (Wellborn et al. 2005). Each ecomorph has morphological, behavioral and life history phenotypes that are adaptive in their respective environment (Wellborn 1994). Furthermore, the costs of being paired (vs. being single) differ between the two ecomorphs. For the large species, pairing decreases predation risk by larval dragonflies, a common predator in large-species habitats, whereas, it increases predation risk by bluegill sunfish, *Lepomis machrochirus*, a common predator in small-species habitats (Cothran 2004). Asymmetries in the costs females pay while paired may translate into different levels of sexual conflict over the onset of precopula between the two species.

In this study, I manipulated female behavior to determine the potential for sexual conflict over the onset of precopula (a shared mating trait) in large and small species *Hyalella*. Females were lightly sedated and the onset of precopula and several behavioral interactions were recorded. An earlier onset of precopula when females were sedated would indicate that females normally have some degree of control over pairing. Also, I tested whether female behavior is important in determining the outcome of pre-pairing interactions, including the frequency and duration of male-initiated grasping behavior, in both *Hyalella* species.

METHODS

Amphipods used in this study were collected in late May. Large species *Hyalella* were collected from a spring seep adjacent to the flowing portion of Cowen Creek, Marshal County, Oklahoma (33° 55’ N, 96° 51’ W). Small species *Hyalella* were collected from the vegetated littoral region, mostly composed of *Potamogeton* and *Chara*, in a farm pond at the University of Oklahoma Kessler Farm Field Laboratory, McClain County, Oklahoma (35°03' N, 97°32' W). Amphipods were kept in 80 L aquaria at the University of Oklahoma Biological Station greenhouse, using water and vegetation from their source habitats.

Female behavior and pairing dynamics

Males and females were randomly assigned as pairs to 150 ml beakers. Each of these females had recently deposited eggs into their marsupium (recently fertilized eggs are dark green and easily distinguished from older embryos) a requirement ensuring that females experienced males for nearly an entire molt interval and that standardized the reproductive condition of females used in the experiment. Each beaker contained beach sand plus water from the animals' source habitats. At 12 hr intervals (between 0800 and 1200 and 2000 and 0000 hrs) beakers were checked for precopulatory pairs. At each check, unpaired females were removed from beakers and placed in small dishes (5 cm diameter) filled with either lake water (control females) or lake water containing a sedative (experimental females; see below). Females remained in these dishes for 5 minutes, after which they were returned to their respective 150 ml beaker. Sedated females were immobile for several minutes after treatment and a reduction in activity was noticeable throughout the experiment. Beakers were checked for pairs 30 min after females were treated. I recorded two response variables to compare pairing dynamics between treatments. First, I recorded the time remaining to the female molt when the onset of pairing was observed, even if the pairing was transient, for each pair of amphipods. This was necessary because the first pairing was often unstable (when a pair separated before the female molt, the pair was scored as having had a switch in pairing state) in the sedated female treatment. I also recorded pairing duration, defined as the interval between stable (no observed switch in pairing state) pair formation and separation of the pair coinciding with the female molt and oviposition. For each individual, I measured head length, a measure of body size (Edwards and Cowell 1992, Pickard and Benke 1996), and male posterior gnathopod width, a sexually dimorphic appendage in *Hyalella*, using a dissecting microscope fitted with an ocular micrometer.

For the large species, water treated for 10 min with a constant supply of CO_2 was used to sedate females. For the small species, mortality was high for females exposed to CO_2 treated water, therefore a clove oil solution (0.002 ml clove oil ml^{-1}, 0.001 ml ethanol ml^{-1}, water solution) was used in its place. The clove oil solution and CO_2 had similar effects on female behavior. Female mortality was higher in the sedated treatment for both the large (control 4.7% vs. sedated 44.2%; $\chi^2_1 = 18.21$, $P < 0.001$) and small (control 7.1% vs. sedated 26.2%; $\chi^2_1 = 5.49$, $P = 0.019$) species. Only trials where females survived were used in analyses.

Within treatments, I first tested for correlations between response variables and female body size, male body size and male gnathopod size. For male gnathopod size, partial correlations were used to control statistically for the covariance between male body size and gnathopod size. I then tested for an effect of reduced female activity level on pairing dynamics using independent samples *t* -tests or Welch's *t* when homogeneity of variances could not be achieved via transformation of data (Quinn and Keough 2002). First, I tested whether the reduction in female activity level affected the timing of the onset of pairing (defined as the male grasping the female with his anterior gnathopods in the precopula position). For this analysis, I corrected for the time-to-female molt (response variable = time of the onset pairing / time-to-female molt) because this time determines the maximum possible pairing duration and was correlated with the onset of pairing in the sedated female treatment. The time-to-female molt did not differ between treatments for either the large or small species, and ranged from 120 – 240 hrs in the large species and 120 – 264 hrs in the small species. Pearson Chi-squared tests were used to compare the proportion of trials where a switch in pairing state was observed for control and sedated female treatments. Finally, I tested whether female activity level affected stable pairing duration. This response variable was not corrected for time-to-female molt because there was no correlation between these two variables.

Pre-pairing behavioral observations

Behavioral observations were performed on a random subset (half of the pairs set up for each treatment) of beakers each day during either the morning or evening observation (alternated for each beaker each day). During each 5 min observation I recorded each case of physical contact between the sexes, whether this led to an interaction (defined as the male grasping the female in an attempt to pair), the duration of each interaction, and pairings. Data used in analyses represent mean values for all observations recorded for each pair of amphipods.

From behavioral observations, I compared the proportion physical contacts that led to a male grasping the female as well as the duration of these interactions for females with normal (control females) and reduced (sedated females) activity levels using independent samples *t* -tests or Welch's *t* when homogeneity of variances could not be achieved via transformation of data.

RESULTS

Pairing dynamics differed between treatments for both the large and small species. When female activity levels were reduced, the onset of precopula increased by 152% in the large species (Welch's $t_{30.42} = 6.41$, $P < 0.001$) and 125% in the small species ($t_{68} = 3.74$, $P < 0.001$; Figure 1). The initial precopula was often transient in the sedated female treatment, as indicated by the fact that switches in pairing state were much more common in this treatment than the control treatment for both the large (control: 1/41, sedated: 15/24; $\chi^2_1 = 29.43$, $P < 0.001$) and small (control: 3/39, sedated: 13/31; $\chi^2_1 = 11.49$, $P = 0.001$) species. Stable pairing duration was longer for sedated females than control females for both the large (49% longer: $t_{63} = 2.57$, $P = 0.012$) and small (43% longer: $t_{68} = 2.9$, $P = 0.005$; Figure 2) species. Neither male body size, size-corrected gnathopod size, nor female body size were correlated with onset of pairing or duration of stable pairs in either species (Table 1).

Sedated females experienced a higher proportion of encounters that led to their being grasped in the large species (Welch's $t_{13.64} = 7.62$, $P < 0.001$), a pattern that could not be demonstrated statistically in the small species (Welch's $t_{19.08} = 1.96$, $P = 0.07$; Figure 3). Interactions between the female and male lasted longer when females were sedated for both the large ($t_{29} = 5.67$, $P < 0.001$) and small ($t_{34} = 2.26$, $P = 0.03$; Figure 4) species. There were no significant correlations between response variables and male body and size-corrected gnathopod size and female body size for pre-pairing behavioral data (Table 2).

DISCUSSION

In this study, phenotypic manipulation of female activity level, including the capacity to resist male pairing attempts, revealed disagreement between the sexes over paring duration in *Hyalella* amphipods. In the sedated female treatment, pairings occurring early in the female molt cycle were often transient in this study, probably because females eventually recover from sedation and invest in resistance behavior when paired too early. Thus, I interpret the occurrence of these first pairings as an indicator of the maximum guarding duration males are willing to accept, which provides information on the degree of disparity between male optimal guarding durations and female-driven or compromised guarding durations. The onset of pairing occurred earlier in the female molt cycle when females were unable to resist male pairing attempts (Figure 1), indicating that male optimal pairing durations are longer than those preferred by females. This result has now been documented in several peracarid crustaceans (Jormalainen and Merilaita 1995, Sparkes et al. 2000), and highlights the importance of female behavior in what was traditionally considered to be a male decision making process (Jormalainen 1998).

Theoretical and empirical studies suggest that local ecological conditions will affect the opportunity for sexual conflict within populations (Gavrilets 2000, Martin and Hosken 2004, Härdling and Kaitala 2005). High population density and male-biased operational sex ratios result in high intersexual encounter rates and thus increased male harassment of females (Krupa and Sih 1993, Magurran and Seghers 1994b). The structure of *Hyalella* populations is conducive for intersexual conflict over pairing duration in these key respects. Both the small and large species occur at high densities (small species from 8,300 to 18,100; large species from 700 to 8,400 individuals m^{-2}; Wellborn 1994), which combined with male-biased operational sex ratios (Wellborn and Cothran 2007), sets the stage for intense male-male competition for mating opportunities. The optimal duration for mate-guarding is expected to be longer for males than for females because males are likely to benefit from even slight increases in pairing duration, but such increases are detrimental to females (Parker 1974, Jormalianen 1998). The extent to which male and female interests differ, however, will depend on the costs associated with pairing.

Intersexual asymmetries in the costs of pairing in the small species may result in intense sexual conflict, whereas, the opportunity for conflict appears to be weaker in the large species. In *Hyalella*, predation risk associated with pairing differs between species. In the small species, pairing increases male and female susceptibility to predation by *Lepomis* sunfish, which are size-selective predators preferring larger prey items (Strong 1972, Wellborn 1994). The magnitude of this cost is greater for females than males, because females are not as susceptible to fish predation while single compared to males (Cothran 2004). On the other hand, large species females are less likely to fall prey to larval dragonflies while paired than when single. This is probably due to lower activity levels while paired, and thus decreased encounter rates with these sit-and-wait predators (Cothran 2004). These results suggest that asymmetries between the sexes in predation costs are less likely to play a significant role in sexual conflict over pairing duration in the large species (Cothran 2004). Therefore, small species females have more to lose and are expected to invest more in resistance behaviors than large species females. This argument is in agreement with observations of field guarding durations where large species females pair earlier in their molt cycle than small species females (Wellborn 1995, Wellborn and Bartholf 2005).

Female resistance behavior may be important in moderating the negative effects of male guarding attempts. Pre-pairing interactions with males may be costly for females resulting in decreased energy reserves and fecundity, as has been observed in the isopod *Idotea baltica* (Jormalainen et al. 2001). Furthermore, pre-pairing interactions involve considerable movement, which may increase the conspicuousness of the interacting pair to predators. A higher proportion of encounters led to the male grasping the female in the large species (Figure 3), and these grasps lasted longer when females were unable to resist in both the large and small species (Figure 4). Thus, control females were more efficient at avoiding male grasps and quickly dislodging males compared to sedated females, suggesting that female behavior is important in mediating pre-pairing interactions in *Hyalella*.

To understand the evolutionary implications of conflict over precopula requires a functional understanding of the traits that mediate its outcome and knowledge about how these traits impact the fitness of each sex (Pizzari and Snook 2003, Rowe and Day 2006). Mating biases are common in *Hyalella*, with both male body size and posterior gnathopod size increasing male pairing success in some populations (*reviewed in* Wellborn and Cothran 2007). It is likely that sexual conflict has played at least an indirect role in the evolution of such biases. Clearly, females resist male pairing attempts early in their molt cycle to avoid the costs of early pairing (Jormalainen 1998). Thus, natural selection has favored the phenotype in females, resistance to pairing, which is responsible for filtering male phenotypes later in the female molt cycle. In addition to resistance early in the molt interval, however, females may also practice selective resistance (i.e. favoring some male phenotypes over others) during the period they are receptive to male guarding. If this is the case, then the mating biases that emerge from such a process are best explained as a form of traditional sexual selection via female choice.

In the large species, females receive fitness benefits from mating with large males with large gnathopods, (Chapter 3), suggesting that traditional sexual selection through female choice is important in maintaining mating biases in large species populations. In the small species, mating biases with respect to male body and posterior gnathopod size are weaker. Intermediate and larger males have equal pairing success and large gnathopods increase pairing success only for smaller males (Wellborn 1995, Wellborn and Bartholf 2005). Currently, we do not know how male traits influence female fitness in the small species, but because sexual conflict is expected to be most intense in this species, this issue certainly deserves attention. Sexual conflict over pairing duration has potential to shape mating traits in *Hyalella* species; therefore studies on the fitness consequences of intersexual interactions in this group are necessary to shed light on the evolutionary potential of this conflict.

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TABLES

Table 1. Correlation coefficients for time to female molt of the onset of pairing and stable pairing duration with respect to male and female traits. For each pair of variables the *P* value of the correlation is given in parentheses below the coefficient. For male gnathopod size partial correlations are reported accounting for male body size.

	Onset of pairing		Stable pairing duration	
	Control	Sedated	Control	Sedated
Large species	N = 41	N = 24	N = 41	N = 24
Male head length	0.16 (0.32)	0.06 (0.80)	0.21 (0.18)	-0.02 (0.91)
Gnathopod width	-0.10 (0.54)	0.30 (0.17)	-0.01 (0.96)	-0.32 (0.13)
Female head length	-0.09 (0.58)	0.2 (0.35)	0.05 (0.76)	0.09 (0.69)
Small species	N = 39	N = 31	N = 39	N = 31
Male head length	0.34 (0.04)	-0.08 (0.69)	0.20 (0.23)	0.08 (0.69)
Gnathopod width	0.00 (0.98)	0.03 (0.86)	-0.10 (0.54)	-0.05 (0.81)
Female head length	0.08 (0.62)	-0.13 (0.49)	-0.07 (0.65)	0.14 (0.46)

Table 2. Correlation coefficients for proportion of encounters that led to the male grasping the female and average grasp duration with respect to male and female traits. For each pair of variables the *P* value of the correlation is given in parentheses below the coefficient. For male gnathopod size partial correlations are reported accounting for male body size.

	Proportion Grasp		Average Grasp Duration	
	Control	Sedated	Control	Sedated
Large species	N = 21	N = 13	N = 21	N = 13
Male head length	0.42 (0.06)	0.19 (0.55)	-0.01 (0.97)	-0.22 (0.51)
Gnathopod width	0.13 (0.60)	-0.24 (0.50)	0.15 (0.56)	0.31 (0.38)
Female head length	-0.32 (0.16)	-0.29 (0.33)	0.09 (0.72)	0.00 (1.0)
Small species	N = 25	N = 15	N = 25	N = 15
Male head length	-0.32 (0.12)	-0.19 (0.53)	-0.07 (0.76)	-0.29 (0.36)
Gnathopod width	-0.03 (0.91)	-0.41 (0.21)	-0.19 (0.40)	-0.51 (0.11)
Female head length	-0.23 (0.28)	0.28 (0.34)	-0.09 (0.67)	0.31 (0.32)

FIGURE LEGENDS

Figure 1. Proportion of time remaining to female molt when the onset of pairing was observed for control vs. sedated females. Each box represents the 25th and 75th percentiles. Whiskers represent the 10th and 90th percentiles. The dashed line represents the mean and the solid line the median. Closed circles represent outliers. Sample sizes are given above each box.

Figure 2. Stable pairing durations for control vs. sedated females. Symbols as in Figure 1.

Figure 3. Proportion of encounters that resulted in the male grasping control vs. sedated females. Symbols as in Figure 1.

Figure 4. Average male grasp durations for control vs. sedated females. Symbols as in Figure 1.

Figure 1

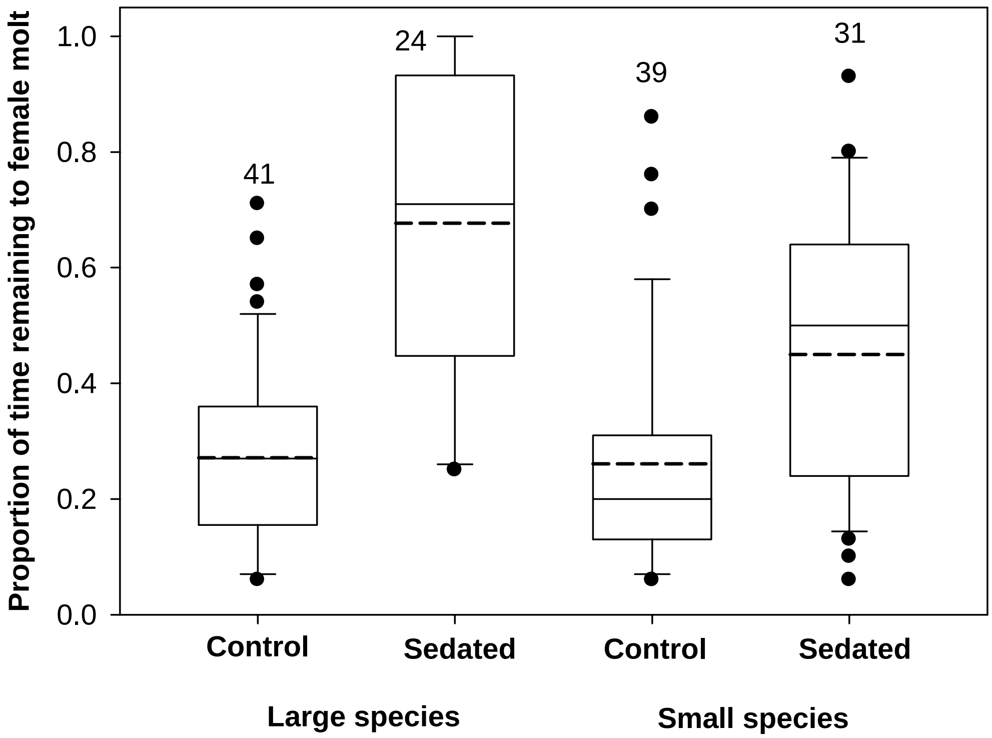
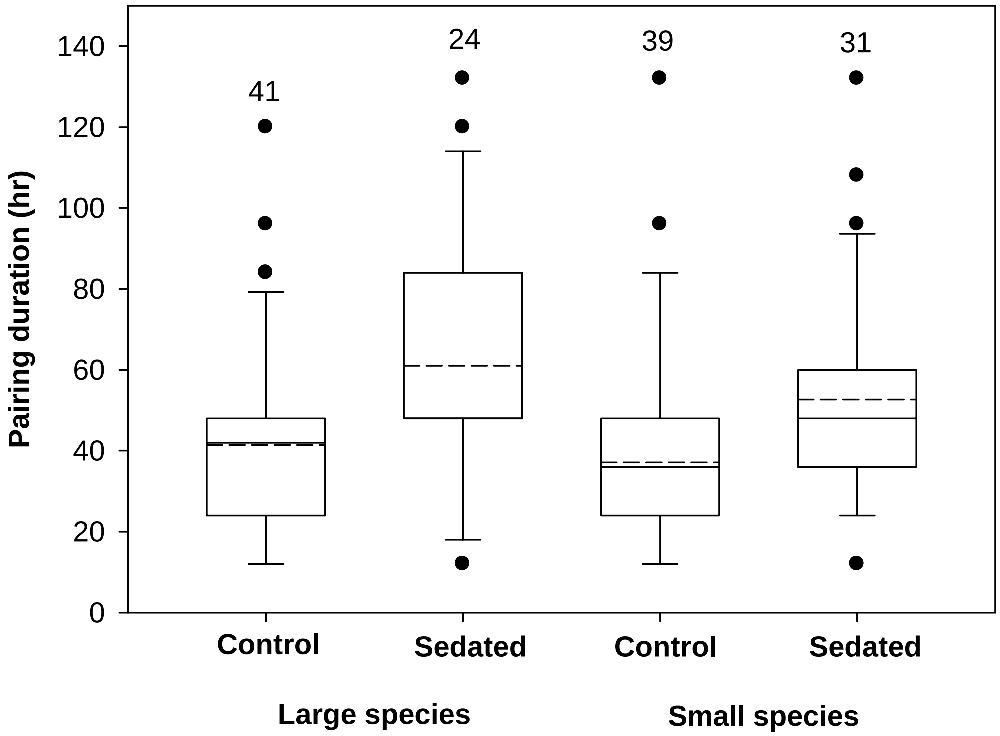

Proportion of time remaining to female molt
1.0
0.8
0.6
0.4
0.2
0.0
41
24
39
31
Control
Sedated
Control
Sedated
Large species
Small species

Figure 2



Pairing duration (hr)
0
20
40
60
80
100
120
140
41
24
39
31
Control
Sedated
Control
Sedated
Large species
Small species

Figure 3

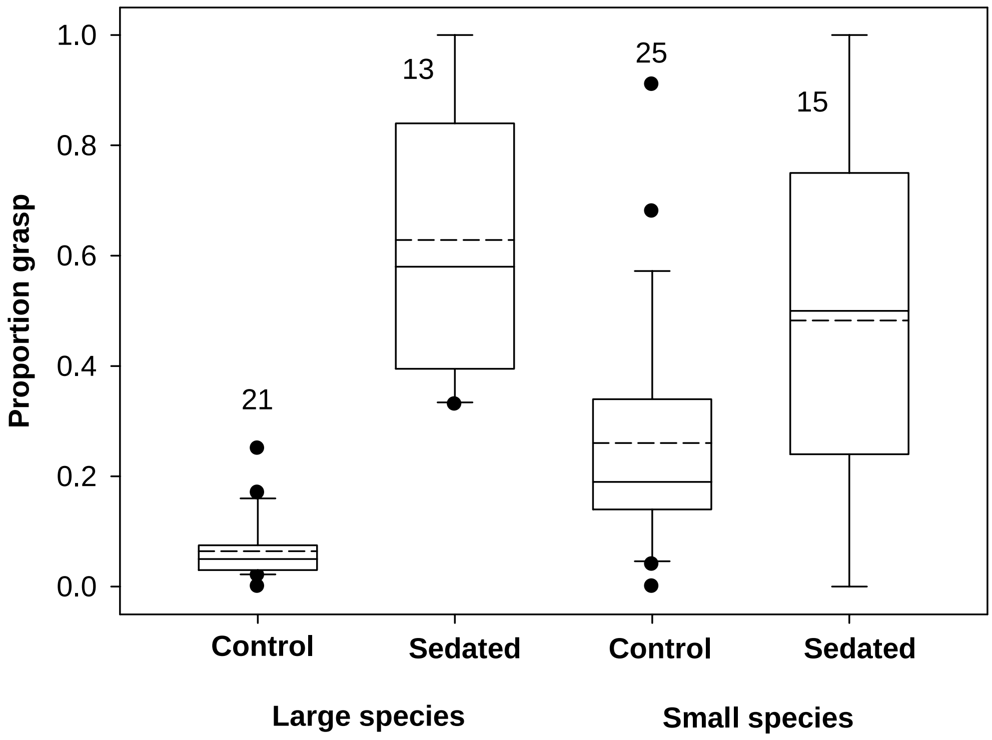
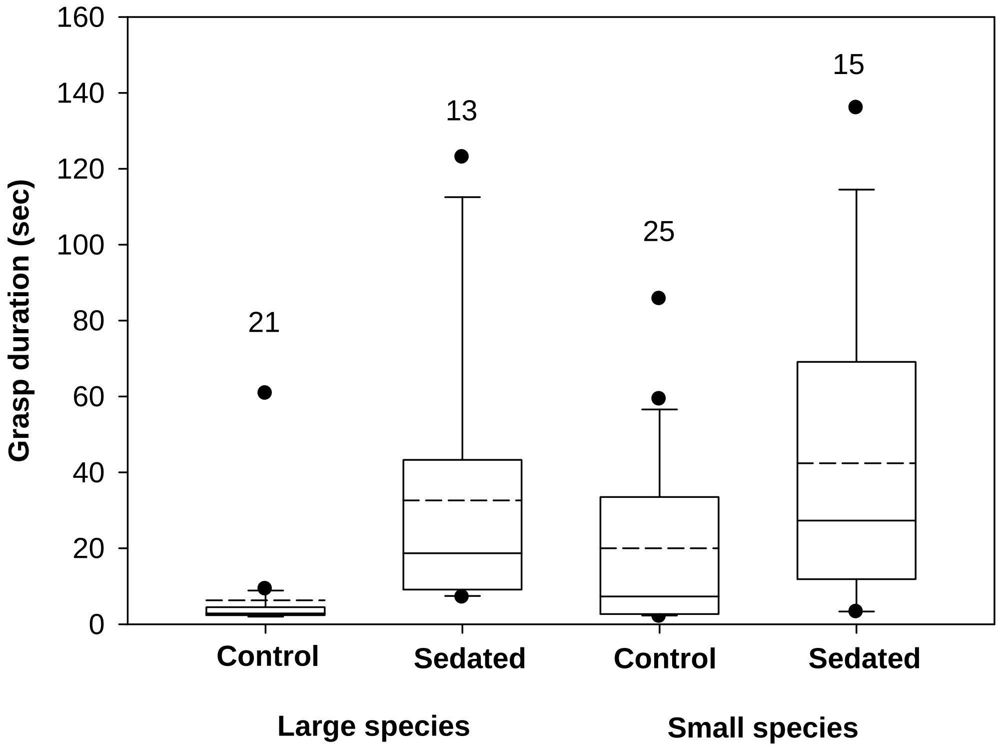

Proportion grasp
1.0
0.8
0.6
0.4
0.2
0.0
21
13
25
15
Control
Sedated
Control
Sedated
Large species
Small species

Figure 4


Grasp duration (sec)
0
20
40
60
80
100
120
140
160
21
13
25
15
Control
Sedated
Control
Sedated
Large species
Small species

CHAPTER 2

THE MECHANISTIC BASIS OF A LARGE MALE MATING ADVANTAGE IN TWO FRESHWATER AMPHIPOD SPECIES

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ABSTRACT

In many animals, body size plays an important role in determining both ecological success and mating success. Thus, the expression of body size within a population is often the result of the interaction between natural selection and sexual selection. Here, I examine the mechanistic basis for a large male mating advantage in two freshwater amphipod species that differ ecologically. Traditionally, size-biased mating patterns in amphipods have been attributed to the advantage of large size in male-male competition for females. Here, I report the results of a male tethering experiment. When male-male interactions were eliminated experimentally, the mating patterns observed were similar to those observed under control conditions (males interacting freely), while also matching the patterns reported in previous field surveys and laboratory trials. There was, however, some evidence for male takeovers in the species that shows the stronger size-based mating bias. Takeovers occurred in 33% of trials when smaller males were in the position of defender, i.e. paired with the female. Therefore, takeovers by larger males may also contribute to the strong size-based mating biases observed in this species.

INTRODUCTION

Body size often plays an important role in mediating interactions within animal mating systems (Andersson 1994). Like other sexually selected traits, its expression is ultimately determined by the combined effects of natural selection and sexual selection. Thus, studies that focus on ecologically disparate species that differ with respect to sexual size dimorphism can provide insights into how sexual selection and natural selection interact to shape trait diversity.

Body size may affect mating success through several mechanisms. In many animals, contests between competing males are decided by power asymmetries that determine resource holding potential (RHP, Parker 1974a), which generally increases with body size (Parker 1974a, Thornhill & Alcock 1983). Large size also may be favored in male-male scramble competition for females. Both search activity (Carroll & Salamon 1995) and size of sensory structures (e.g. antennae, McLain 1982, Hanks et al. 1996, Bertin & Cézilly 2003) that affect search efficiency have been shown to increase with body size, resulting in a large male mating bias. Moreover, intersexual interactions, either through female choice or female resistance to male mating attempts, may select for large male body size (Andersson 1994, Arnqvist & Rowe 2005).

Natural selection may often select against large body size, thus counterbalancing the effects of sexual selection. The development of large size requires investment in growth as a juvenile through either extending the pre-reproductive period of development or increasing the rate at which resources are acquired to convert to growth. To the degree that growing large involves substantial net costs, viability selection may oppose large body size (*reviewed in* Blanckenhorn 2000). Moreover, survival selection against large adult size (e.g. positive size-selective predation) may counterbalance sexually selected advantages (Lande 1981, Wellborn & Cothran 2007). Thus, variation in body size is expected to be sensitive to resource levels and predator assemblages, both of which vary across space and time. Such disparate selection regimes likely play an important role in stabilizing the variation in the relationship between body size and mating success observed both among populations and closely related species (e.g. Olson et al. 1986, Ward 1986, Rowe et al. 1994, Carroll & Salamon 1995, Wellborn 1995, Bertin & Cézilly 2003).

The relationship between body size and pairing success has received considerable attention in amphipods and isopods (Ridley & Thompson 1979, Birkhead & Clarkson 1980, Ward 1984, Adams & Greenwood 1987, Wellborn 1995). In these crustaceans, female receptivity to fertilization is restricted to a brief period after molt. This pattern of female receptivity results in male-biased operational sex ratios (OSR; Emlen & Oring 1977), a condition that favors the evolution of precopulatory mate guarding (Ridley 1983) and imposes selection on other male traits (Ward 1988, Bertin & Cézilly 2003, Bollache & Cézilly 2004). Precopulatory mate guarding is a ubiquitous mating strategy within this group and consists of the male carrying the female for a period (ranging among species from hours to days) before she molts and fertilization takes place (Jormalainen 1998). The precopulatory phase is preceded by an interaction between the male and female that does not always end in pairing, with females often rejecting male pairing attempts (Jormalainen 1998, Wellborn & Cothran 2007). In some species, single males attempt to displace paired males and the frequency of takeovers is variable among species (Jormalainen 1998). These details of amphipod and isopod mating biology along with behavioral observations led Shuster & Wade (2003) to describe the mating system as coercive polygynandry. Despite extensive work on this group we lack a mechanistic understanding of size-related mating biases. The mating biology of amphipods and isopods points to selection favoring the evolution of large male body size through three mechanisms: 1) large males may be more efficient at locating receptive females, 2) large males may hold an advantage in taking over and defending against takeovers of receptive females, and 3) female resistance or assessment may select for large male size (Jormalainen 1998, Wellborn & Cothran 2007).

Hyalella Study System

Amphipods in the genus *Hyalella* are common inhabitants of permanent freshwater habitats throughout North America (Bousfield 1958). This group represents a complex of genetically diverse, undescribed species (Witt & Hébert 2000, Wellborn & Cothran 2004, Wellborn et al. 2005, Witt et al. 2006). Regionally, at least two species' ranges commonly overlap, segregating among habitats based on the intensity of size-selective predation imposed by fish (primarily *Lepomis* spp.; Wellborn et al. 1994). In habitats with intense fish predation, a small-bodied ecomoprh (hereafter 'small species') is found, whereas a large-bodied ecomoprh (hereafter 'large species') occurs in habitats with little or no fish predation. In addition to body size and life history differences (Strong 1972, Wellborn 1994, Wellborn et al. 2005), these species are characterized by dissimilarities in the relationship between male body size and pairing success. In the small species smaller males have low pairing success, but pairing success is similar for medium and larger male size classes, whereas in the large species pairing success increases throughout the range of male body sizes (Wellborn 1995, Wellborn & Bartholf 2005).

Explanations for large male mating advantages in amphipods have typically focused on the contexts of scramble competition and takeovers of paired females (Ward 1988, Bollache and Cézilly 2004). Behavioral observations (Strong 1973, Wellborn 1995) and results from experiments (Chapter 1), however, suggest that female behavior also helps mediate pair formation in *Hyalella*. Females become more receptive to male guarding attempts as their molt approaches (Strong 1973, Wellborn 1995). Furthermore, experimental manipulation of female resistance behavior results in an increase in guarding duration (Chapter 1). Given that females have some control over pair formation, it is likely that if males vary in quality (in terms of expected direct or indirect benefits) females may use selective resistance to bias pairing patterns. Moreover, takeovers of paired females are rarely observed under lab conditions (Strong 1973), and if similarly uncommon in nature, pairing success may be a good indicator of mating success.

The goal of this study was to assess the mechanistic basis for pairing patterns in *Hyalella* amphipods. First, I experimentally manipulated male behavior by restricting male search area and excluding the possibility of takeovers. If pairing patterns result from size-related male variation in search or takeover ability, then the large male mating advantage should vanish when male mobility is limited. For the large species, which shows a stronger relationship between male size and pairing success, I also examined the frequency of takeovers with respect to absolute and relative male and female body size. Previous studies have shown that takeovers are most likely to occur when the attacking male is substantially larger than the defender (large asymmetry in RHP Ridley & Thompson 1979, Sigurjónsdóttir & Parker 1981, Dick & Elwood 1990). Also, although small males are significantly less likely to pair than large males in laboratory trials, they do sometimes succeed (Wellborn & Bartholf 2005). Therefore, I concentrated on situations where smaller males had a positional advantage over larger competitors to determine if these pairings were stable or likely to end with usurpation by a larger attacker.

METHODS

Collection and Housing of Animals

Large and small species amphipods were collected in late May and early June. The large species was collected from a spring seep adjacent to the flowing portion of Cowen Creek, Marshal County, Oklahoma (33° 55’ N, 96° 51’ W). The small species was collected from the vegetated littoral region, mostly composed of *Potamogeton* and *Chara*, of a small farm pond at Kessler Farm Field Laboratory, McClain County, Oklahoma (35°03' N, 97°32' W). Amphipods were maintained in water and vegetation from their source habitat in 80 L aquaria in a greenhouse facility at the University of Oklahoma Biological Station.

Male Tether Experiment

This experiment was performed for both the large species and small species. For both the large species and small species, a sample of individuals was collected from stocks using a large dip net. Individuals were sexed, by inspection for enlarged posterior gnathopods (a sexually dimorphic trait in *Hyalella* that is enlarged in males), using a dissecting microscope. This assured that smaller male size classes were included in the experiment. These males were then haphazardly assigned to experimental arenas with the only condition that the two males assigned to any given arena (defined below) had to differ in size. To standardize the time-to-female-molt across replicates, I used single females with clearly visible eggs in the ovaries, and thus were close to becoming receptive to male guarding attempts.

I experimentally manipulated male search activity by tethering both males to small rocks using superglue and a single cotton thread (approx. 2 cm in length) from a cotton ball. This treatment allowed males to move locally and to form precopula pairs with females while limiting their ability to search for females and preventing male-male interactions. The two males were placed at opposite ends of a plastic container filled with lake water (13 cm X 13 cm). Each tethered male had access to roughly 7% of the total area of the container. After the tethering procedure, males were given 24 hours to recuperate before addition of a receptive female. Pairing patterns observed in this treatment were compared to those from a control where the two males were sham-tethered. Sham tethered males experienced the same pre-experiment conditions as males in the tethered treatment except that they were not affixed to a small rock (i.e. they received both the spot of superglue and a cotton thread), but rather were allowed to interact freely with each other and the focal female within the arena. The experiment was checked every 6 hours for precopula pairs. To be included in the analysis, males had to be paired on two consecutive checks. A total of 38 and 40 replicates were set up for each treatment for the large and small species, respectively. A replicate was discarded if 1) either male escaped tethering, 2) an individual died, or 3) a female oviposited eggs before a pairing was observed on two consecutive checks. At the conclusion of the experiment, I measured head length, an indicator of body size (Edwards & Cowell 1992), for all animals to the nearest 0.02 mm using a dissecting microscope fitted with an ocular micrometer.

I analyzed whether experimental manipulation of male activity altered pairing patterns in *Hyalella*. I used backward elimination stepwise logistic regression to test whether the occurrence of pairings achieved by the smaller of the two males in a replicate depended on tethering treatment, larger male body size, smaller male body size, and female body size (Hardy & Field 1998). I used a cubic spline estimation procedure (*see* Schluter 1988) to visualize the relationship between explanatory variables retained in the logistic regression analyses and the occurrence of smaller male pairings. Finally, I tested for a large male mating advantage using a binomial sign test. For this analysis, data from control and tether treatments were combined if there was no effect of tethering ($p > 0.05$) on male pairing patterns.

Takeover Study

For the large species, I also examined whether smaller males were able to defend successfully against takeover attempts. I created situations where a smaller, paired male (defender) was placed with a larger single male (potential attacker) and recorded the frequency of takeovers. A large sample of males was collected from a stock tank and measured to the nearest 0.02 mm. Males were then haphazardly assigned as pairs (1 male larger than the other) to replicates. The smaller of the two males was housed with a randomly selected mid-molt female in a 150 ml beaker, and the larger of the two males was housed under similar conditions but in the absence of a female. Once the smaller male paired with the female, both the pair and larger male were transferred to a common beaker. I then checked the beakers daily at 0800, 1200, 1600, and 2000 and recorded which male, if any, was paired with the female. I changed water and fed amphipods (commercial shrimp pellets) every two days. Once the female molted and the pair separated, individuals were preserved in 95% ethanol. Head length, a measure of body size, was measured as in the tethering experiment.

I used logistic regression to test whether takeover frequency depended on size asymmetries between competing males. I also used female size as a predictor in this analysis, because female fecundity increases with size (Strong 1972), thus larger females are more valuable in terms of expected eggs fertilized than smaller females. Two models were analyzed. In the first model, I determined whether takeovers depended on absolute size measures of the larger and smaller male. If size is correlated with RHP and if males use self-assessment to make decisions concerning persistence in fights, the absolute size of males should be associated with takeover probability (Taylor & Elwood 2003). In the second model, I analyzed whether takeover probability depended on the relative size of the two males. If males use mutual-assessment to determine fight persistence then this relative measure of size should be associated with takeover probability.

RESULTS

Male Tether Experiment

For the small species, the occurrence of pairings by the smaller male did not depend on tethering treatment, female size or larger male's size (Table 1). There was, however, a positive trend between the size of the smaller male and the probability that he achieved pairing success (Table 1, Fig. 1). In the large species, the occurrence of pairings by smaller males did not depend on tethering treatment or female size, but increased with his size and decreased with the size of the larger male (Table 1, Fig. 2).

There was a large-male mating advantage in both the large and small species (Fig. 3). In the large species, the larger of the two males paired in 67% of trials (sign test, p = 0.01, n = 61); in the small species, 65% of trials (sign test, p = 0.05, n = 51).

Takeover Study

Takeovers occurred in 33% (15/46) of trials, with 80% of these occurring within 24 hours of the female’s molt [pairing duration (mean ± 1 SD) = 40 ± 18 hrs]. The occurrence of takeovers appeared not to depend on measures of absolute size or relative size of competing males, nor female size (Table 2).

DISCUSSION

Large-male mating advantages are common in crustaceans, and are typically thought to result from the dynamics of male-male competition for mates. While studies have demonstrated sexual selection on male body size in isopods and other amphipod species (Ward 1988, Bertin & Cézilly, Bollache & Cézilly 2004), we lack an understanding of the mechanistic basis of large male advantages in most species (Wellborn & Cothran 2007). Studies that manipulate the opportunity for selection by increasing encounter rates, either through manipulations of the OSR or population density, are powerful demonstrations of the plastic nature of pairing patterns (Jormalainen 1998), but these studies seldom differentiate between male-male and male-female mechanisms. Results from the male tethering experiment in this study suggest that male-male interactions contribute little to size-based variance in male mating success in *Hyalella* populations. This experiment eliminated direct male-male interactions and greatly restricted male search area, yet patterns of male mating success relative to size did not differ from controls and were similar to those observed previously in field and laboratory studies (Wellborn 1995, Wellborn & Bartholf 2005). Thus, male-female interactions appear to be important in driving size-based mating biases within *Hyalella.*

Although the male tethering experiment demonstrates that male-female interactions are important in producing mating patterns in *Hyalella*, an understanding of the specific mechanism at work requires further work. Large males may hold an advantage over their smaller counterparts because they are favored by females or are more successful at overcoming female resistance to mating. The idea that female behavior is an important determinant of size-biased pairing has been discussed in the literature, but has generally been considered ancillary to male-male competition (Ridley & Thompson 1979, Jormalainen 1998). Studies on several crustaceans (Jormalainen and Merilä ita 1995, Diaz & Thiel 2003), including *Hyalella* (Chapter 1), have demonstrated that female behavior is important in determining the onset of guarding. Given that females can thwart male guarding attempts (Strong 1973, Jormalainen 1998), it seems likely that selective resistance based on male phenotype will evolve if significant variation in male quality exists within populations and the costs of choice are not too high (Kokko et al. 2003, 2006).

Species differences in mating patterns observed in this study were largely in agreement with previous studies on *Hyalella* mating behavior, and may have resulted from differences between these two species in the value to females of choosing males on the basis of size (Wellborn & Cothran 2007). In small species populations, sexually selected advantages to large size will be counterbalanced by survival selection imposed by differential predation by fish on larger males. In the large species, however, multiple fitness components (fecundity, viability, and survival) scale positively with body size, suggesting that there may be no immediate countervailing selection pressure on large size in this species (Strong 1972, Wellborn 1994). Assuming that size is heritable, the stronger relationship between size and pairing success observed in the large species may reflect the greater benefits these females gain from exerting choice (Wellborn & Cothran 2007).

The intensity of female choice depends on both the costs of exercising choice and variation in male quality within a population (Parker 1983, Kokko et al. 2003), both of which may differ between large and small species. In *Hyalella*, females likely use the time they are receptive to pairing as a window to select mates. In parallel, in brine shrimp, *Artemia salina*, which also exhibits pairing prior to fertilization, limiting female sampling intervals results in a breakdown of non-random pairing by size (Forbes et al. 1992). Pairing duration appears akin to search duration, which is expected to show a positive relationship with choosiness (Real 1990). In *Hyalella*, pairing durations are longer in the large species than the small species, probably because pairing increases predation risk in the small but not the large species (Cothran 2004). In the small species, predation risk imposed by fish may constrain female sampling periods dampening their choosiness for large male size (Real 1990). Moreover, because males of the small species are more similar in size to one another than males of the large species (Cothran unpub. data), small species females have less variation to choose from, perhaps lowering their acceptance thresholds (Parker 1983).

Mating biases may also result from larger males having an advantage over smaller males in overcoming female resistance to pairing (Jormalainen 1998). Sexual conflict over pairing duration should be common in these crustaceans because males should typically benefit from extending pairing duration (Parker 1974b), whereas females only incur costs from longer pairing durations (Jormalainen et al. 2001, Cothran 2004). When female ability to resist male pairing attempts was experimentally impaired (through sedation), guarding duration increased for both the large and small species, suggesting that males and females generally differ with respect to optimal onset of precopula (Chapter 1). That study stopped short of analyzing how body size mediates such conflicts. Theoretical models, however, predict that physical power, which often depends on body size (Parker 1974a, Thornhill & Alcock 1983), is important in resolving conflicts over precopula duration (Yamamura & Jormalainen 1996, Jormalainen 1998). Ultimately, differentiating between female choice and sexual conflict requires information on the female fitness consequences of mating with large males (Pizzari & Snook 2003, Rowe & Day 2006).

In the large species, takeovers by bigger males may amplify the already pronounced size-related male mating biases initiated by male-female dynamics. Previous work on *Hyalella* (Strong 1973, Wen 1993) reported no takeovers, however, each of these studies only allowed single males to contact pairs for a short period and did not follow interactions until the female molt. Here, I found frequent takeovers (33% of trials when smaller males were defending against larger rivals), which were most likely to occur toward the end of the female molt cycle.

Higher takeover frequency near the end of the female molt is expected, both from the temporal details of mating in *Hyalella* and from theoretical work on male decision-making. Strong (1973) suggested that paired males may be in danger of losing females during the later stages of the female molt interval because paired males may have physical difficulty holding on to females during the female molt, which immediately precedes fertilization. Interestingly, a recent study in the amphipod *Gammarus pulex*, found that multiple bouts of copulation during the female's receptive period (after the molt and before the exoskeleton hardens) are necessary for successful insemination (Hume et al. 2005), which may provide ample time for takeovers to occur (suggested in Strong 1973). Moreover, male time investment decreases by pairing close to the time of female molt, resulting in lower opportunity costs (Jormalainen 1998). Thus if males can assess a female's time to molt, perhaps via leaky molting hormones (Borowsky 1985, Borowsky & Borowsky 1987), they could adjust their takeover effort accordingly and escalate their effort when the female is most valuable (Grafen and Ridley 1983, Härdling et al. 2004). Chemical communication in *Hyalella* is contact based (Strong 1973, Wellborn & Cothran *unpublished data*), which may explain why interactions between single males and pairs are common, but rarely result in takeovers (Strong 1973, Ward 1983, Elwood et al. 1987, Wen 1993). Future work on crustacean takeovers should focus toward the end of the female molt interval, when male payoffs of winning contest are expected to be highest. Finally, it should be noted that the estimate of takeover prevalence found here might be conservative. A more revealing approach might employ molecular markers to assess paternity of broods, specifically to detect the frequency that a paired male near the end of the precopulatory phase is unrelated to part or all of the brood.

Ultimately, the response of a complex trait like body size to selection will depend on the form and intensity of selection acting on the trait and the amount of additive genetic variation available for selection (Lande 1976, Falconer & Mackay 1996). In *Hyalella*, it is clear that large male body size is advantageous for mating in both the large and small species. In the small species, however, this advantage in mating competition is apparently counterbalanced by strong size-selective predation imposed by predatory fish, which is absent in the large species populations. These selection patterns may explain why the large species has a larger size at maturity and shows strong male-biased sexual size dimorphism, whereas females are slightly larger than males in small species populations (Wellborn & Cothran 2007). Measuring the intensity of selection in nature (Arnold & Wade 1984) for these amphipods, however, is confounded by the covariance between size and age due to indeterminate growth (Wellborn & Cothran 2007). Thus, selection experiments are necessary to understand the how this important fitness-related trait responds to selection (Fuller et al. 2005).

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TABLES

Table 1. Logistic regression results for the male tethering experiment. Coefficients, standard errors, and p values are presented for each species (separate models). Large species- n_{tether} = 29, $n_{control}$ = 32; small species- n_{tether} = 15, $n_{control}$ = 36.

Large Species (overall model: χ_2^2=9.98, p=0.007)				Small Species (overall model: χ_1^2=3.69, p=0.06)			
Variable	β	SE	p	Variable	β	SE	p
Larger male size	-22.66	8.94	0.01	Smaller male size	17.72	9.82	0.07
Smaller male size	23.60	9.90	0.02	Tethering*	-0.84	0.73	0.19
Female size*	-5,19	4.67	0.27	Larger male size*	-11.08	9.76	0.26
Tethering*	0.56	0.56	0.31	Female size*	-3.51	5.15	0.50

*Tethering and female size were dropped from the large species model, and tether, female size, and larger male size were dropped from the small species model.

Table 2. Logistic regression results for the takeover experiment. Models for the probability that a takeover occurred with respect to absolute sizes and relative size are presented. Coefficients, standard errors, and p values are given for each model. $n_{\text{no takeover}} = 31$, $n_{\text{takeover}} = 15$.

Absolute size (overall model: χ_3^2=3.62, p=0.31)				Relative size (overall model: χ_1^2=2.00, p=0.16)			
Variable	β	SE	p	Variable	β	SE	p
Larger male (attacker) size	2.94	5.46	0.59	Relative size (smaller/larger)	-5.63	4.04	0.16
Smaller male (defender) size	-8.19	9.54	0.39				
Female size	7.88	6.65	0.24				

FIGURE LEGENDS

Figure 1. Smaller male’s pairing success as a function of his size for the small-species tethering experiment. The solid line represents the nonparametric estimated curve and dashed curves indicate ± SE of predicted values from 50 bootstrap replicates (Schluter 1988). Open circles represent raw data (0 = not paired, 1 = paired). $n_{tether} = 15$, $n_{control} = 36$; $p = 0.07$. To reveal overlapping data points 0.0005 units were added to values occupying the same position on the graph.

Figure 2. Smaller male’s pairing success as a function of a) the size of the larger male ($p = 0.01$) and b) and his size ($p = 0.02$) for the large-species tethering experiment. The solid line represents the nonparametric estimated curve and dashed curves indicate ± SE of predicted values from 50 bootstrap replicates (Schluter 1988). $n_{tether} = 29$, $n_{control} = 32$.To reveal overlapping data points 0.0005 units were added to values occupying the same position of the graph.

Figure 3. Percent of cases where the larger (black bar) and smaller (white bar) male paired when males were allowed to interact freely vs. when they were tethered (thus restricted from interacting). Results are presented for the large-species (top) and small-species (bottom).

Figure 1

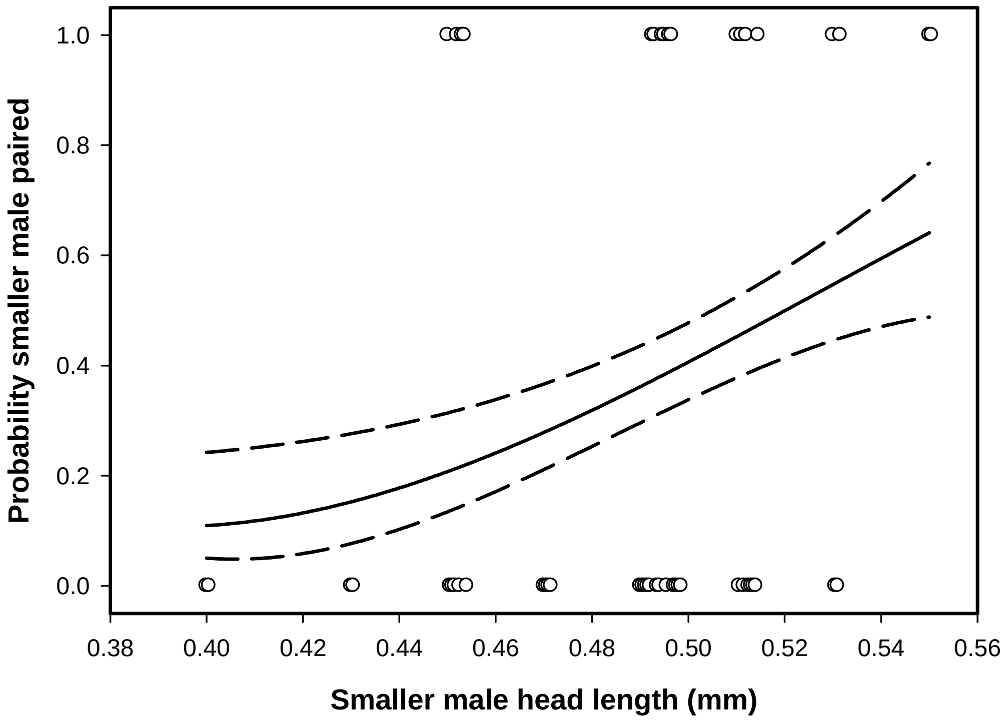

Probability smaller male paired
1.0
0.8
0.6
0.4
0.2
0.0
0.38
0.40
0.42
0.44
0.46
0.48
0.50
0.52
0.54
0.56
Smaller male head length (mm)

Figure 2

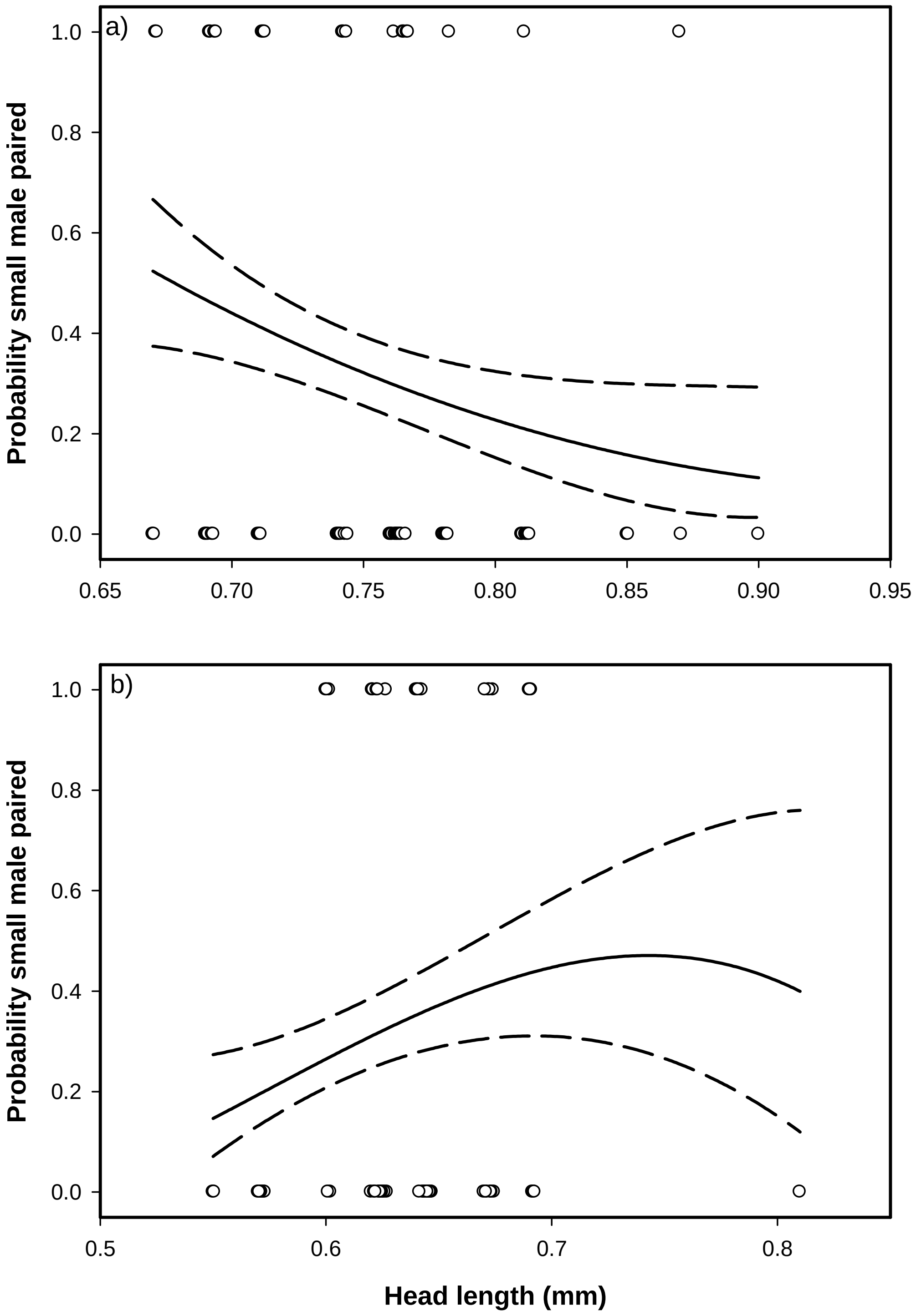
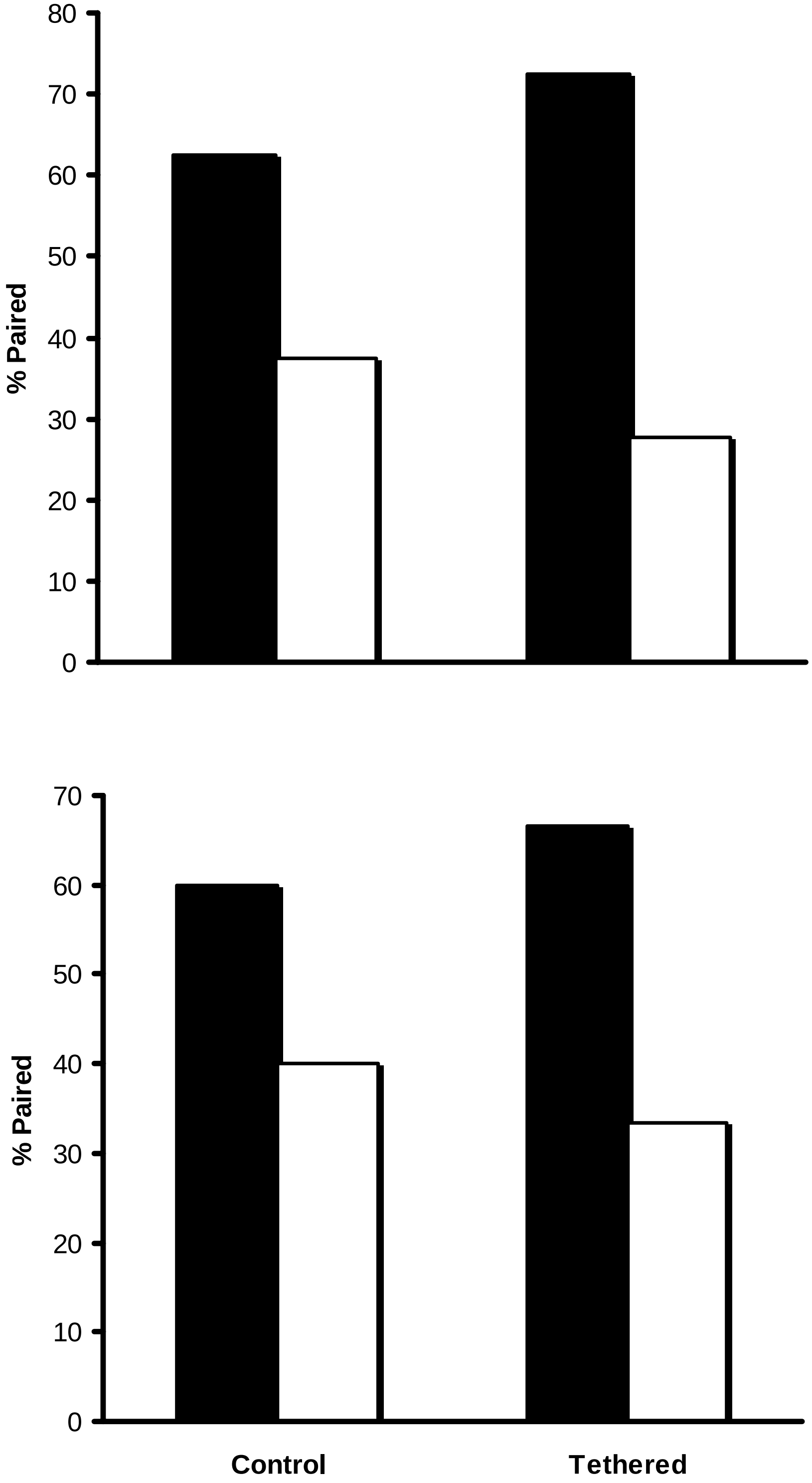

a)
Probability small male paired
1.0
0.8
0.6
0.4
0.2
0.0
0.65
0.70
0.75
0.80
0.85
0.90
0.95
b)
Probability small male paired
1.0
0.8
0.6
0.4
0.2
0.0
0.5
0.6
0.7
0.8
Head length (mm)

Figure 3


80
70
60
50
40
30
20
10
0
% Paired
70
60
50
40
30
20
10
0
% Paired
Control
Tethered

CHAPTER 3

DIRECT AND INDIRECT FITNESS CONSEQUENCES OF MATE CHOICE IN A CRUSTACEAN

Formatted for Evolution

ABSTRACT

The evolution of female mate choice has received considerable attention in behavioral ecology, yet studies that consider both direct and indirect costs/benefits of female mate choice are rare. Ultimately, an understanding of the relative magnitude of direct and indirect benefits is necessary to shed light on the evolution of female mating preferences and their contribution to male trait diversification. In this study, I assessed the fitness consequences of female mate choice in a freshwater crustacean. In *Hyalella* amphipods, males attempt to form precopulatory pairs with females. Large males, bearing large posterior gnathopods, tend to be over-represented in precopulatory pairs. I show that females receive both direct and indirect benefits from mating with these males. Furthermore, the behavioral mechanisms used to filter male phenotypes carry no detectable energetic cost for females. Thus, females that choose males with successful phenotypes are expected to have higher Darwinian fitness than females that mate at random. This study shows that direct and indirect selection act together to favor large male size, which explains the sexual size dimorphism and size-based mating biases observed in this species.

INTRODUCTION

Although the study of mate choice has a rich theoretical and empirical history (Andersson 1994; Jennions and Petrie 1997; Widemo and Saether 1999; Kokko et al. 2006), the role of sexual selection in explaining the origin and maintenance of mating preferences is unclear (e.g. Cameron et al. 2003; Cordero and Eberhard 2003; Kokko et al. 2003, Arnqvist and Rowe 2005; Kokko et al. 2006). The debate largely concerns how to explain costly female choice in systems where material (‘direct’) benefits are absent. Research in this area has historically concentrated on genetic (‘indirect’) benefits females gain from being choosy (Andersson 1994). Indirect benefits models predict that costly female preferences can be compensated for by the production of offspring with high fitness, which they inherit, in part, from their attractive father (Fisher 1930; Zahavi 1975; Eshel et al. 2000; Kokko 2001; Kokko et al. 2002). The effect of indirect selection, however, depends on genetic correlations between the male trait and fitness, as well as between the female preference and male trait, which may often be weak. Furthermore, strong directional selection imposed by females should deplete variation in male traits making choice obsolete (the lek paradox, Borgia 1979). Therefore, indirect benefits should be of minor importance when compared to direct selection on female mating preferences (Kirkpatrick 1996; Kirkpatrick and Barton 1997; Cameron et al. 2003).

Strong mating biases in species that apparently lack direct benefits may also be explained by cryptic direct selection on female mating preferences (Reynolds and Gross 1990). For example, in the absence of male parental care or nuptial gifts, females may acquire direct benefits if mating with attractive males increases fertility (*reviewed in* Møller and Jennions 2001). Furthermore, in species that have prolonged mating interactions, either pre- or post-copulatory, pairing with attractive males may increase survival or foraging efficiency (Rowe et al. 1994, Cothran 2004, Wellborn and Cothran 2007).

Mating biases may also result if males vary in their ability to overcome female resistance to mating (*reviewed in* Arnqvist and Rowe 2005). In this case, exaggeration of male and female phenotypes stems from the coevolutionary arms race between resistant females and persistent males. This differs from the aforementioned selection for direct benefits because males succeeding through this process are affecting female fitness in a negative way (i.e. by adding costs). Empirical support for the importance of sexual conflict in shaping reproductive traits comes from instances where male traits are negatively correlated with female fitness components (reviewed in Chapman et al. 2003). These studies, however, often fail to consider the indirect fitness benefits females may gain from producing effectively manipulative sons (Cordero and Eberhard 2003).

Recent theory has stressed the interconnectedness of mate choice models, highlighting the fact that both direct costs and indirect benefits are likely to play a complex and integral role in the net fitness consequences of mate choice (Kokko et al. 2002; Cordero and Eberhard 2005; Kokko et al. 2006). The ecological context in which females choose mates will largely shape the form, balance and intensity of selection on female mating preferences. The direct costs of choice including— time, energy costs, and vulnerability of females to predators when choosing mates (Reynolds and Gross 1990; Magnhagen 1991; Rowe 1994; Watson et al. 1998)— vary across different ecological contexts. These costs are expected to be important in determining the choosiness of females in a population (Andersson 1994; Kokko et al. 2002). When choice is cheap and male mating success is highly uneven, females can gain sufficient indirect benefits through the production of sons that have high mating success, even at the expense of their sons' viability. Conversely, as the costs of mate choice increase, choosy females would have to receive benefits via several offspring fitness components (e.g. high survival, daughter fecundity, and son mating success) as compensation. Finally, as the costs associated with mate choice become very high, females are expected to mate randomly because benefits of choice are outweighed by its costs (Kokko et al. 2002).

Several empirical studies have demonstrated that female preferences for attractive males increase some fitness components of their offspring (Reynolds and Gross 1992; Sheldon et al. 1997; Welch et al. 1998; Brooks 2000; Kotiaho et al. 2001; Hine et al. 2002; Evans et al. 2004; Byers and Waits 2006; Rundle et al. 2007), while other studies have demonstrated that male traits can decrease components of female fitness (*reviewed in* Holland and Rice 1998; Arnqvist and Rowe 2005). Studies that consider both direct selection (costs or benefits) and indirect benefits of mate choice are rare (*e.g.* Jones et al. 1998; Iyengar and Eisner, 1999; Head et al., 2005).

Studies attempting to measure the effect of mate choice on offspring fitness components often have two shortcomings, which the current study attempts to avoid. First, variation in males is typically considered with respect only to a single trait, despite consensus that females use multiple traits when choosing mates (Heisler 1985; Candolin 2003; Head et al. 2005). Second, females are often paired experimentally with the male extremes in attractiveness. Although this protocol increases the chance of obtaining effect sizes sufficient to reject the null, it provides little information on whether choosing mates is favored over the alternative strategy of mating at random (Andersson 1994).

The System

North American *Hyalella* amphipods are a genetically diverse group that includes several undescribed species (Witt and Hebert 2000; Wellborn and Cothran 2004; Wellborn et al. 2005; Witt et al. 2006). The current study focuses on a large-bodied, late-reproducing species found in Oklahoma habitats where individuals experience little or no fish predation (Wellborn et al. 2005). This species shows strong mating biases with respect to body size and posterior gnathopod size, a sexually dimorphic appendage, with pairing success increasing monotonically with both traits (Wellborn and Bartholf 2005).

Hyalella mating behavior includes a period of precopulatory mate guarding (hereafter ‘precopula’) where males use their anterior gnathopods to carry females in a ventral position (Strong 1973; Borowsky 1984). Behavioral observations and results from experiments suggest that female behavior plays an important role in pair formation. Specifically, females become more receptive to males as their molt approaches (Strong 1973; Wellborn 1995), and removal of female resistance behavior through anesthesia increases precopula duration (Chapter 1). Also, in an experiment where male-male interactions were limited, larger males still enjoyed a pairing advantage over smaller males (Chapter 2). These results suggest that the large male mating advantage is primarily driven by male-female interactions.

In this study, I assess the fitness consequences of selective mating by female *Hyalella*. Mating biases are likely due to female choice of male phenotypes during the interaction preceding pair formation. Males attempt to pair with most individuals they encounter by grasping individuals, and these interactions are a common and conspicuous component of *Hyalella* mating behavior (Strong 1973). I examined whether such male-female interactions impose an energetic cost to females, and thus constitute a potential fitness disincentive to selective mating. I also evaluated the benefits females gain from selective mating. The precopula phase in amphipods and isopods provides an opportunity for females to gain direct fitness benefits for choosing males (Wellborn and Cothran 2007). Therefore, I examined whether the size of a female's mate affects her predation risk while paired. Finally, females may benefit indirectly from choice if offspring sired by successful males have higher fitness than offspring sired by males chosen at random from the population. Thus, I examined whether females given a choice of mates produced offspring with higher viability, growth rate, fecundity and mating success relative to females mated at random.

METHODS

Collection and Housing of Animals

Amphipods were collected from the shallow littoral region of Lake Thunderbird, Cleveland County, Oklahoma (35° 16' N, 97° 18’ W). The amphipods were transported to a greenhouse at the University of Oklahoma, Aquatic Research Facility or a wet lab on the main campus. Animals were held in stock tanks (54 X 44 X 13 cm) with water and vegetation from the lake. Lighting conditions in the wet lab were 14L : 10D. Diets were supplemented weekly with flaked fish food (Tetra Fin®).

Experiment 1: Energetic Costs of Male-Female Interactions

The goal of this experiment was to determine the energetic expenditures of mating for female *Hyalella*. Females may bias pairing in the direction of large males by resisting male guarding attempts with sufficient vigor that only large males can succeed. Such resistance may require increased levels of energy expenditure constituting a cost of mate choice. This study examines whether mating interactions are more energetically costly than non-sexual social interactions.

Thirty-six recently molted females were randomly assigned to be housed with either a size-matched male or a female companion. These dyads were kept in 200 ml jars filled with lake water, and fed flaked fish food *ad lib* every two days. Male-female jars were checked twice a day (between 0800-1000 and 2000-2200) for pairing. If paired, the male and female were removed from the jar, along with a female-female replicate and stored at -80˚ C for subsequent glycogen and lipid extractions. Females housed with males experienced pre-guarding interactions but not guarding itself, therefore any observed reduction in energy reserves compared to the female-female treatment is due to these interactions and not a reduction in foraging while in precopula. Whole animal glycogen and lipid levels were quantified using the methods described in Van Handel (1965, 1985a, 1985b) and Warbug and Yuval (1997). In arthropods, glycogen provides a highly accessible form of energy, whereas lipids are used for longer-term storage (Beenakkers 1969; Downer and Matthews 1976), therefore assessment of both forms of energy gives a complete measure of the available energy budgets available to an animal. Animals were dried (20 hrs at 60˚ C) and then weighed to the nearest 0.001 μg using a Cahn microbalance before quantification of energy reserves. A multivariate GLM was used to test for treatment differences in glycogen and lipid reserves using individual dry weight as a covariate.

Experiment 2: Direct Benefits of Choice: Predation

This experiment tested whether a female's predation risk while paired was dependent on the size of her mate. A large sample of males was collected from a stock tank. Each male was assigned a female and the pair was kept in a 100 ml jar with lake water. Jars were checked daily for precopula pairs and amphipods were fed flaked fish food every two days.

Larval dragonflies (*Erythemis simplicicollis*) used in this experiment were collected in late June from marshy habitats in the Sutton Wilderness Area of

Norman, Oklahoma, and kept in individual (12 X 12 X 4 cm) plastic containers. Larval dragonflies were fed *Hyalella* daily, except the day before they were used in the experiment.

As soon as a precopula pair was observed, it was transferred to a Petri dish (8.5 cm diameter) containing one larval dragonfly in a mesh cage (7 cm diameter). After 2 min the cage was removed and the foraging response of the dragonfly was recorded. I recorded whether the predator attacked the pair, whether the attack was successful, and if so which individual(s) (male, female or both) was captured. If an attack did not occur within 7 min, the observation was recorded as no attack. After each trial, I measured the size of the dragonfly (mm total length, range: 7.5 mm to 19 mm) and the head lengths (an indicator of body size: Edwards and Cowell 1992) of both amphipods. I used backward elimination logistic regression to examine the relative contributions of these predictive variables (Hardy and Field 1998). I first tested whether dragonfly attacks depended on the size of the dragonfly predator and the size of the individual amphipods involved. For cases where attacks were observed, I tested whether a female’s probability of being captured depended on her size, the size of her mate or the size of the dragonfly involved in the attack. Finally, I used a cubic spline estimation procedure to determine the form of the relationship between the size of a female’s mate and her predation risk while paired (Schluter 1988).

To understand how predation risk may differ for females that mate randomly as opposed to selectively, I weighted the estimated predation risk given the size of a female’s mate by the frequency that females are found mated with males of that size class for both females that were allowed to choose a mate and females that were assigned a mate a random. Mate size frequency distributions for randomly-mated and choice-mated females used in this analysis were obtained from experiment 3 (see below). These weighted probabilities were then summed across male size classes for each mating treatment. I then divided the summed total for females given a choice by the summed total for females mated at random to determine the relative direct benefit of mating selectively.

Experiment 3: Indirect Benefits of Choice

Choice- and random-mating treatments

Amphipods used in this experiment were collected from Lake Thunderbird in late June and were transported to the greenhouse facility on the main campus of the University of Oklahoma. Immediately upon arrival at the greenhouse, the collected sample was thoroughly mixed and half allocated to each of two 54 X 44 X 13 cm plastic holding containers. Starting the day after collection, precopulatory pairs were collected from one of these containers over a four-day period. Fifty of these females were randomly assigned to each of two treatments: given a choice or mated at random. Choice-mated females were teased apart (by gently applying pressure between the paired individuals) and allowed to re-pair with their originally paired male. Random-mated females were similarly teased apart from their originally paired male and assigned a new male at random from the holding container that was not used to collect pairs. The second holding container was used for collection of these males to assure that the pool of males in the random-mated treatment included a full array of male attractiveness. Pairs were housed in 100 ml jars containing 50 g of sand and lake water that had been aged for 2 weeks to allow development of a periphyton community as a resource base (hereafter referred to as a 'conditioned jars'). Diets were supplemented with a weekly addition of flaked fish food (approx. 10 mg). Once eggs had been fertilized, indicated by separation of the pair and eggs visible in the female’s marsupium, males were removed and preserved in 95% ethanol. Upon release of broods, females were removed and preserved in 95% ethanol. I measured head length, a measure of body size, for all adult participants plus gnathopod width for males to the nearest 0.02 mm. I also recorded the number of neonates produced (fecundity) by each female.

For parents, I tested for treatment differences for traits that are important determinants of fitness. For sires and dams, I tested for differences in body size using GLM. For dams, I also compared size-specific fecundity using an *ANCOVA* with dam body size as a covariate. For sires, I also compared size-specific gnathopod size. I originally planned an *ANCOVA* for this comparison, but a body size X male group interaction ($F_{3,138} = 149.04$, $p < 0.001$) indicated heterogeneous slopes (Quinn and Keough 2002). Therefore, I used the Wilcox procedure (Wilcox 1987; Quinn and Keough 2002) to determine the range of body sizes for which differences in gnathopod size among male groups exists with 95% confidence. No difference in dam body size was expected because dams in each treatment’s sample was drawn from the same population of paired females. Dam fecundity may differ as a function of treatment (e.g. if females increase reproductive output when paired with attractive mates: Thornhill 1983; Burley 1986, 1988). Choice sires and originally paired males should be similar in body size given they are both representative of males paired under natural conditions. Random-treatment sires should be smaller (and have smaller size-specific gnathopods), on average, than choice-treatment and originally paired males because of the strong large male pairing advantage observed in this species (Wellborn and Bartholf 2005).

Juvenile growth rates and viability

First instar offspring were transferred as groups of ten to conditioned 100 ml jars. Jars were kept in a flow-through system supplied with water at a constant 23° C. Offspring diets were supplemented weekly with 10 mg of fish flakes. At age 21-26 d (ca 1 week before reaching sexual maturity) two haphazardly selected male and female offspring were measured to obtain juvenile growth rate data. I also recorded the number of surviving offspring as a measure of offspring viability, as well as the sex ratio of each brood. I used student or Welch’s (when variances were not equal) *t*-tests to test for differences in juvenile growth rate, viability and brood sex ratio (arcsine transformed) between random- and choice-mating treatments.

Daughter fecundity and son gnathopod size and mating success

Each daughter measured for juvenile growth rate was transferred to an individual conditioned 100 ml jar to complete development to adulthood. Starting at six weeks of age, daughters were checked daily for presence of eggs in the marsupium. As soon as eggs were observed females were preserved in 95% ethanol. The number of eggs in a female's marsupium (fertility) was used as a proxy for fecundity. I had planned an *ANCOVA* to test for daughter size-specific fecundity differences between choice-mated and random-mated females, but a treatment X daughter size interaction ($F_{2,61} = 27.70$, $p < 0.001$) revealed heterogeneous slopes (Quinn and Keough 2002). Therefore, I used the Wilcox procedure to determine the daughter body size interval for which treatments differed in size-specific fecundity with 95% confidence (Wilcox 1987; Quinn and Keough 2002).

I used GLM to compare size-specific gnathopod size for the sons of choice-mated and random-mated females females. Only families for which at least two sons reached adulthood were used in this analysis. I calculated mean son size and gnathopod size for each family and used these variables to test for treatment differences. First, I tested for a body size X choice treatment interaction. If no interaction was not detected, I used an *ANCOVA* to test whether sons from choice- and random-mated females differed in gnathopod size, using mean son body size as a covariate.

To compare pairing success of sons from choice- and random-mating treatments I placed one son from each of the two treatments in competition with each other for access to a female. Sons were assigned as age-matched (36 days old) pairs to experimental arenas. I clipped the 3 most distal segments from one of the 4th pair of walking legs to identify individual males in this experiment. Males are often found with missing appendages or segments of appendages in nature (personal observation). One male was clipped on the right side, his opponent on the left, with choice alternating between replicates. After clipping, males were given 24 hrs in isolation to recover. Next, the male dyad plus a single early-molt female were added to a 100 ml conditioned jar and provided with 10 mg of flaked fish food weekly. The jars were checked twice a day (between 0600-0800 and 1800-2000 hrs) for pairing. When a pair was observed, the leg clip of the remaining male was recorded using a dissecting microscope, after which he was returned to the test jar. Once the female oviposited, both males were removed from the jar and preserved in 95% ethanol. I used ordinal regression to compare pairing success of sons from random-mated and choice-mated females. For each family, the success of sons was recorded as ‘unsuccessful’ (both sons failed to pair), ‘somewhat successful’ (1 of 2 sons paired), or ‘very successful’ (both sons paired). Choice treatment, average son head length, and average son residual gnathopod size (correcting for body size) were then used as explanatory variables in the model. To determine the effect size for this test I weighted the proportion of matings achieved by sons (unsuccessful = 0, somewhat successful = 0.5, very successful = 1.0) by the frequency of occurrence for each treatment (choice and random). I then summed these weighted proportions and divided the summed total for sons from choice-matings by the summed total for sons from random-matings to determine the relative indirect benefit females gain from selective mating.

RESULTS

Experiment 1: Energetic Costs of Male-Female Interactions

Females interacting with males prior to pair formation did not have lower energy reserves than females interacting with other females (Table 1, Fig. 1). Energy reserves were positively correlated with female dry weight (Table 1a), primarily due to the positive covariance between lipids and female dry weight (Table 1b).

Experiment 2: Direct Benefits: Predation

Smaller larval dragonflies were less likely to attack pairs than larger larvae, but the size of the paired individuals had no effect on the likelihood of attack (Table 2a). When attacks did occur, the probability that a female was captured decreased with the size of her mate (Table 2b, Fig. 2), and females that mate at random are 33% more at risk than females given a choice of mates. The probability that a female was captured did not depend on her size or the size of the larval dragonfly (Table 2b).

Experiment 3: Indirect Benefits of Choice

Comparisons of sire and dam traits for choice and randomly mated treatments

As expected, choice sires and males originally paired with randomly mated females were larger than sires in the random-mating treatment ($F_{2, 135}$ = 9.66, $p < 0.001$; Table 3). Comparing gnathopod size among male groups (choice sires, random sires and originally paired males) produced a significant size X male group interaction ($F_{2, 132}$ = 19.41, $p < 0.001$), driven by the convergence of the gnathopod size-body size relationship at larger male size classes (Fig. 3). There was a difference among male groups in size-specific gnathopod size for small and intermediate male size classes (Fig. 3). The Wilcox procedure indicated that gnathopods were similar in size for random-sires and choice-sires between 0.67 and 0.78 mm head length, whereas smaller choice-sires (those with head lengths < 0.67 mm) had disproportionately larger gnathopods than random-sires of similar size. A similar pattern was found comparing random-sires to males originally paired with randomly-mated females. For this comparison, no difference in gnathopod size was found for males with larger heads (0.66 to 0.72 mm in this sample), while for smaller males (< 0.66 mm) originally paired males had larger gnathopods than random-sires. Patterns outside the upper limit are more difficult to interpret because fewer data points are available in that region. For the choice-sire vs. random-sire comparison, nothing can be said about observations above the 0.78 mm head length upper limit because the random-sire group is not represented in that region (Fig. 3). For the random-sire vs. originally paired male comparison, random-sires had larger gnathopods above the 0.72 mm head length upper limit, however, the random sire group is only represented by 5 observations and the originally paired group by 4 observations in this region (Fig. 3).

There was no interaction between dam size and choice treatment ($F_{1,\ 88}$ = 0.40, p = 0.53) justifying the use of *ANCOVA* to test for differences in fecundity between choice and randomly-mated females. Dam body size did not differ between the choice- and random-mating treatments (t_{90} = 0.11, p = 0.91; Table

3). I also did not detect a difference in dam size-specific fecundity between choice- and random-mating treatments (*ANCOVA*- $F_{1, 88} = 1.89$, $p = 0.17$; Table 3)

Comparison of offspring traits for choice and randomly mated treatments

Offspring viability ($t_{92} = 0.23$, $p = 0.82$) and juvenile growth rates (sons: Welch's $t_{71.34} = 0.54$, $p = 0.64$; daughters: Welch's $t_{79.37} = 0.73$, $p = 0.47$) in the random-mating treatment were similar to those observed in the choice-mating treatment (Table 4). Brood sex ratios were slightly more male-biased in the choice-mating treatment than the random-mating treatment, although the difference was not statistically significant ($t_{80} = 1.73$, $p = 0.09$; Table 4).

There was a significant daughter size X choice treatment interaction ($F_{1, 57} = 6.93$, $p = 0.01$) when comparing fecundity of daughters from choice- and randomly-mated dams. The Wilcox procedure revealed no treatment differences in daughter fecundity between 0.43 mm and 0.70 mm head length. I interpret this as indicating no treatment differences in daughter fecundity, because there are no observations below the lower limit (0.43 mm head length) and the region above the upper limit (0.70 mm head length) is represented by only 3 observations (Fig. 4).

There was no interaction between son body size and choice treatment when comparing gnathopod size of sons ($F_{1, 42} = 0.12$, $p = 0.74$), justifying the use of *ANCOVA* to test for treatment differences in size-specific gnathopod size of sons. No difference could be demonstrated in size-specific gnathopod size between sons from choice- and randomly-mated females (*ANCOVA*: $F_{2, 43} = 1.83$, $p = 0.18$; Table 4).

Sons from choice-females, however, were 59% more likely to achieve pairing success than sons from randomly mated females (Table 5; Fig. 5). Successful males in this experiment also had larger size-specific gnathopods (Table 5).

DISCUSSION

The evolution of any trait is dependent on the combined effects of direct and indirect selection acting on available genetic variation. Thus, to understand the evolution of selective mating one must assess 1) the costs associated with choice; 2) the direct fitness consequences of mating with successful males; and 3) the indirect fitness consequences of these matings. In this study, females given a choice of mates outperformed females mated at random. Females that were allowed to choose a mate produced sons that were 59% more likely to achieve pairing success than sons from randomly mated families (Fig. 5). In addition, choosy females experience roughly 33% less predation by larval dragonflies, their major predators, than females that mate at random. There was no antagonisitic selection against mating with successful males in terms of other offspring fitness components (e.g. viability, juvenile growth rates, and daughter fecundity; Rice and Chippindale 2001). These results suggest that the net effect of mating with successful males is positive, and so females should be selective even if choice is somewhat costly.

Do Females Pay a Cost for Choice?

The costs of selective mating are an important component of mate choice models (Pomiankowski 1987; Kokko et al. 2003; 2006), however they have rarely been empirically evaluated. When females are the mate-searching sex, it is clear that search effort requires time and energy and may increase the susceptibility of the searching female to predators and pathogens (Daly 1978; Reynolds and Gross 1990; Jennions and Petrie 1997; Wickman and Jansson 1997; Byers et al. 2005). In many species, however, males do the brunt of the searching (Hammerstein and Parker 1987), thus freeing females from these costs and promoting female choosiness (Kokko and Johnstone 2002). Under these circumstances females may still pay for selective mating if the behavioral mechanisms they use to filter male phenotypes require substantial energetic expenditure or time (Watson et al. 1998; Jormalainen et al. 2001). In *Hyalella*, the interaction between males and females that precedes pair formation produces a large male mating advantage (Chapter 2), thus playing a central role in selective mating. In this study, females that interacted with a male prior to the precopula phase had energy budgets comparable to females that did not experience such interactions (Fig. 1). Thus, females did not pay a measurable cost associated with selective mating. This result contrasts with a similar study on the aquatic isopod, *Idotea baltica*, which found a significant female energetic cost associated with male-female interactions (Jormalainen et al. 2001). In *I. baltica*, interactions with males can last several minutes with females flexing and extending their bodies several times (over 200 flexes have been observed) to thwart male guarding attempts (Jormalainen et al. 2000; 2001). In *Hyalella*, however, both vigorous, active resistance and more passive resistance occur, the latter being common in the species studied here (personal observation). Passive resistance consists of the female curling her abdomen anteriorly, a position that appears to block male pairing attempts (similar behavior has been reported in the isopod, *Lirceus fontinalis* [Sparkes et al. 2000]). It seems reasonable that such passive resistance is less energetically demanding than active resistance. Also, when struggles do ensue, they last only seconds, compared to the minutes observed in *I*. *baltica* (Chapter 1). Thus, females appear to have mechanisms to control the onset of pairing without investing in what may be more costly forms of active resistance.

Costs of mate choice, however, depend also on the environment in which females mate. For example, high encounter rates due to high population densities and male skewed sex ratios may result in high levels of male harassment (Watson et al. 1998). The costs of resisting male guarding attempts may be especially high under these contexts and may even result in a drastic reduction in female-driven mating biases (Rowe et al. 1994). In the current study, males and females were maintained in containers with surface areas of about 15 cm^2. Surface area is a better indicator of space available than volume because amphipods are benthic and spend most of their time on the bottom. Therefore, the density of amphipods in these containers was much lower than field estimates (ranging from 700-8,400 individuals m^{-2}; Wellborn 1994), which means the opportunity for costs to accrue via sexual harassment was lower in this study than under natural conditions. In previous experiments, encounters between a single male and female in these containers were common, and females clearly were able to spoil male guarding attempts by swimming away from males upon contact (Chapter 1). It is possible that avoidance of males costs females in terms of foraging efficiency, but it seems unlikely that an assessment of both readily accessible (glycogen) and long-term (lipids) energy sources would not detect a treatment difference in resource acquisition rates over a period of several days. These results, combined with the fact that males are the more active mate-searching sex (Wellborn and Cothran 2007), suggest that costs of female choice for the *Hyalella* species studied here are low.

Do Females Benefit Directly from Choice?

Given the potential for direct selection to shape mating biases, there is a clear need to assess it in non-resource based mating systems (Reynolds and Gross 1990; Kirkpatrick 1996; Cameron et al. 2003). Here, I discuss how mating with different male phenotypes directly impacts female fitness through current (fertility) or future (fecundity or survival) reproductive success (*reviewed in* Møller and Jennions 2001; *also see* Jones and Elgar 2004). In *Hyalella*, direct selection may act on female mating preferences if the costs or benefits a female accrues while paired depends on the phenotype of her mate. Pairing typically lasts 3-5 days, presenting a considerable window during which costs of making the wrong choice can acccumulate (Wellborn and Cothran 2007). Predation risk while paired may be particularly important given that amphipod predators, e.g. larval dragonflies, often occur at high densities and have a large impact on *Hyalella* populations (Wellborn 1994). Contact mate guarding, whether it be pre- or postcopulatory, has been shown to affect predation risk in several invertebrate species (Andersson 1994), including amphipods (Ward 1986; Cothran 2004). For the species studied here, pairing decreases predation vulnerability to larval dragonflies (Cothran 2004), a result corroborated in this study by the reduced attacks on pairs by smaller larval dragonflies. In this study, larger dragonfly larvae attacked pairs with varying success. Females paired with larger males were less at risk of predation by larval dragonflies than females paired with smaller males (Fig. 2). Thus, results from this study suggest that *Hyalella* females can further decrease their chances of being depredated if they pair with a larger male. The direct benefit gained by pairing with large males, however, will depend on the availability of these males in the population. Quantification of the direct benefit revealed that choosy females are 33% less likely to be captured than females that mate randomly. This effect is driven by the over-representation of large males (> 0.70 mm head length) and under-representation of small males (< 0.60 mm head length) in cases where females were given a choice relative to cases where females were assigned a male at random (Fig. 2). This benefit of mate choice holds regardless of the underlying genetics of body size, unlike indirect benefits that require a heritable component and genetic correlations between the male trait, female preference and fitness (Kirkpatrick 1996; Kokko et al. 2003).

Do Females Benefit Indirectly from Choice?

Empirical examples of indirect benefits are common in the recent mate choice literature (e.g. Head et al. 2005, Byers and Waits 2006, Rundle et al. 2007), and highlight the potential importance of indirect effects in the evolution of female mating preferences. Experimental demonstration of significant indirect effects does not necessarily mean that they are responsible for the origin of preferences. In fact, theoretical formulations suggest that indirect benefits will rarely outweigh direct effects, and thus are expected to play only a minor role in the evolution of female mating preferences (Kirkpatrick and Barton 1997; Cameron et al. 2003). Recent empirical work, however, has come to the opposite conclusion (Head et al. 2005). Thus, studies that assess the relative strength and direction of direct and indirect effects are important for advancing our knowledge of mate choice (Kokko et al. 2003). In this study, the directions of both direct and indirect effects were positive. In addition to having sexy sons, females paired with larger males were less often captured by larval dragonflies, an important predator in *Hyalella* habitats (Wellborn 1994). With no evidence for countervailing direct selection against female choice, indirect benefits female amphipods gain from the production of sexy sons may play an unusually important role in the maintenance of female mating preferences (Kirkpatrick and Barton 1997).

Indirect benefits models require that females can assess male quality via some heritable component of the male’s phenotype (Andersson 1994). In *Hyalella*, male mating success depends strongly on body and posterior gnathopod size (Wellborn 1995; 2000; Wellborn and Bartholf 2005), suggesting that these traits are good candidates as indicator traits. In the species studied here, foraging success increases and predation mortality drops with increasing body size (Wellborn 2002), thus to the extent that body size is heritable, male size may indicate genetic quality (Wellborn and Cothran 2007). In the current study, however, sons produced by females given a choice had juvenile growth rates similar to sons from females mated at random, therefore sons from choice- and random-matings did not differ in body size. Lack of treatment differences in body size may result from the favorable conditions under which offspring developed. Heritable variation is sensitive to environmental conditions, with some hypotheses predicting a decrease in phenotypic differences among genotypes under benign conditions (*reviewed in* Hoffmann and Merilä 1999). Juvenile growth rates observed here (0.017 mm d^{-1}) are higher than those observed under conditions where both resource quantity and quality were high (Othman and Pascoe 2001; 0.014 mm d^{-1}) suggesting that the developmental environment provided in the greenhouse represented quite favorable growing conditions. Thus, a benign environment may have masked the genetic potential for variation in juvenile growth rates between offspring from choice- and random-mated females.

Posterior gnathopods are large muscular structures that may be costly to produce and maintain, thus may provide females with information about a male’s genetic quality (Hunt et al. 2004). Again, however, no difference in size-specific gnathopod size was found between sons from choice- and random-mated females. Variation in condition dependent traits is expected to be sensitive to resource levels in the environment, with higher variation expected when resources are limited (Cotton et al. 2004a). Under high resource levels variation in condition dependent traits may be negligible, thus providing females no information about male genetic quality. In this study, sons grown under conditions in the greenhouse had significantly larger gnathopods than sires collected from the field (paired t on gnathopod size/body size by family; $t_{73} = 10.15$, $p < 0.001$). Furthermore, variation in gnathopod size, measured as the percent dispersion from the gnathopod size-body size allometry, was much higher in males collected from the field (sires: 8% allometric dispersion, a measure of trait variablity) than males raised in the greenhouse ([sons: 3% allometric dispersion], Cotton et al. 2004b). Future studies that focus on the heritability of body and posterior gnathopod size across a resource gradient will shed light on whether heritable variation in these traits can be “hidden” by the environment in which offspring develop.

Despite similarities in body and posterior gnathopod size, sons from females given a choice had higher pairing success than sons from randomly mated females. This result suggests that trait(s) other than body and posterior gnathopod size may be important in determining pairing success in *Hyalella*. Here, females in the choice treatment had access to several males that surely varied in traits other than body and posterior gnathopod size. Chemical communication during mating is common in crustaceans (Diaz and Thiel 2004; Kelly and Snell 2004; Moore and Bergman 2005) and, to the extent that chemical signatures vary across males (Sappington and Taylor 1990), may be important in female mate choice decisions. Also, genetically based behavioral differences (e.g. the level of persistence) among males may contribute to differences in pairing success. Studies that assess the chemical and tactical cues conveyed during male-female interactions prior to guarding are necessary to understand the role these traits play in determining male mating success in *Hyalella*.

Synthesis

Assessment of both direct and indirect selection acting on choosy mating is necessary to understand the evolution of mate choice (Kokko et al. 2003). Here, I used a comprehensive approach to study mating biases in a freshwater amphipod species. Females gain both direct and indirect fitness benefits from mating with males with successful phenotypes without incurring a detectable cost associated with choice. Under these circumstances, heavily skewed male mating distributions may contribute to the elaboration of sexually selected traits (Kokko et al. 2003). Consistent with theory and results found in this study, pairing biases with respect to male size and gnathopod size are strong in this species. Furthermore, sexual size dimorphism, males larger than females, is common in populations of this species (Wellborn and Cothran 2007). *Hyalella* amphipods, however, occur over a wide range of ecologies and the evolutionary outcome of the interplay between natural selection and sexual selection in terms of female choice and the male traits they select for is likely to vary across ecological contexts (Schluter 2001). Interestingly, mating biases and sexual size dimorphism are less extreme in some *Hyalella* (Wellborn and Cothran 2007). Studies that place both female preferences and male traits within a phylogenetic framework are now needed to understand the broader significance of female choice in driving the evolution of male traits within the *Hyalella* species complex.

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TABLES

Table 1. Multivariate GLM results comparing energy reserve levels for females housed with another female (N = 15) or a male (N = 15). a) provides multivariate statistics and b) univariate between-subjects effects.

a) Multivariate Tests

Source of variation	**Df**	**Wilks' lambda**	***F***	**p**
Sex of companion	2, 26	0.87	1.82	0.18
Size of focal female	2, 26	0.68	6.25	0.006

b) Tests between-subjects effects

Source of variation	**Type III SS**	**df**	***F***	**p**
Corrected model				
Glycogen	1318	2	2.92	0.07
Lipids	5421	2	6.57	0.005
Sex of companion				
Glycogen	590	1	2.62	0.12
Lipids	583	1	1.41	0.25
Size of focal female				
Glycogen	614	1	2.72	0.11
Lipids	4517	1	10.95	0.003

Table 2. Backward elimination logistic regression results for the direct benefits: predation experiment. a) model testing whether the probability of an attack occurring was dependent on the size of the dragonfly predator, size of the female, and size of the male. b) model testing if a female's probability of being captured depends on her size, the size of her mate, or the size of the dragonfly involved in the attack. Coefficients with standard errors (S.E.), Wald statistics, degrees of freedom (df), and p-values are provided.

a) Probability attack occurred (0 = no attack, 1 = attack)
(overall model: $\chi^2_1 = 32.92, p < 0.001, N = 123$)

Explanatory variable	***ß***	**S.E.**	**Wald**	**df**	**p**
Dragonfly size	0.61	0.13	22.71	1	< 0.001
Female size	-1.10	3.21	0.12	1	0.73
Male size	1.31	4.02	0.11	1	0.74

*Female size and male size were dropped from the model

b) Probability female captured (0 = not captured, 1 = captured)
(overall model: $\chi^2_1 = 4.88, p = 0.03, N = 69$)

Explanatory variable	***ß***	**S.E.**	**Wald**	**df**	**p**
Male size	-10.13	4.90	4.28	1	0.04
Dragonfly size	0.95	0.14	0.49	1	0.48
Female size	-2.21	3.67	0.36	1	0.55

*Female size and dragonfly size were dropped from the model

Table 3. Descriptive statistics and results from post-hoc tests comparing traits of parents used in the indirect benefits experiment. For sire and dam size means and standard deviations (SD) are provided. For sire gnathopod size and dam fecundity least square means (LSM) and 95% confidence intervals (CI) are provided after correcting for the effect of body size. When more than two groups are being compared, pairwise comparison results are indicated by superscripts (same letter = no statistical difference between means). Sample sizes were 49 and 45 for the choice and randomly mated females, respectively.

Trait	**Originally paired male**	**Choice**	**Random**
Sires			
Body size			
Mean	0.68^{a}	0.69^{a}	0.64^{b}
(SD)	(0.041)	(0.045)	(0.062)
Gnathopod size*			
LSM	0.567^{a}	0.573^{a}	0.557^{b}
(CI)	(0.559-0.575)	(0.565-0.581)	(0.549-0.566)
Dams			
Body size			
Mean		0.71	0.71
(SD)		(0.055)	(0.055)
Fecundity **			
LSM		18.01	16.32
(CI)		(16.30-19.83)	(14.51-18.13)

* evaluated at 0.67 mm head length
** evaluated at 0.71 mm head length

Table 4. Descriptive statistics comparing offspring fitness components and traits from choice and random matings. For viability, son JGR, daughter JGR, and brood sex ratio means and standard deviations (SD) are provided. For daughter fecundity and son gnathopod size least square means (LSM) and 95% confidence intervals (CI) are provided after correcting for the effect of body size.

Fitness component or trait	**Choice**	**Random**
Viability[*]		
Mean	0.95	0.95
(SD)	(0.07)	(0.09)
Son JGR[**]		
Mean	0.017	0.017
(SD)	(0.0014)	(0.0022)
Daughter JGR[**]		
Mean	0.016	0.016
(SD)	(0.0017)	(0.0022)
Brood sex ratio (male:female)		
Mean	0.54	0.46
(SD)	(0.20)	(0.22)
Daughter fecundity[***]		
LSM	18.02	18.66
(95% CI)	(16.13-19.92)	(16.97-20.35)
Son gnathopod size[****]		
LSM	0.59	0.59
(95% CI)	(0.581-0.598)	(0.587-0.602)

[*] viability = proportion of surviving offspring to age 21-26

[**] JGR = head length mm-day^{-1}

[***] assessed at 0.64 mm head length

[****] assessed at 0.66 mm head length

Table 5. Ordinal regression results comparing pairing success of sons from choice mated and randomly mated females. Coefficients with standard errors (SE), Wald statistics, degrees of freedom (df), and p-values are provided.

a) Probability of pairing success (0 = unsuccessful, 1 = somewhat successful, 2 = very successful)
(overall model significance: $\chi^2_3 = 9.39$, p = 0.025, N = 46)

Explanatory variable	***ß***	**SE**	**Wald**	**df**	**P**
Treatment					
Choice	1.55	0.62	6.26	1	0.01
Random[a]	0	.	.	.	.
Head length	-6.23	10.48	0.35	1	0.55
Residual gnathopod size	26.08	12.08	4.66	1	0.03

a. This parameter is set to 0 because it is redundant.

FIGURE LEGENDS

Figure 1. Glycogen and lipid reserves for females that were housed with males (gray bars) and females that were housed with females (white bars). Bars represent means and error bar 95% confidence intervals. Both glycogen and lipids were measured in µg.

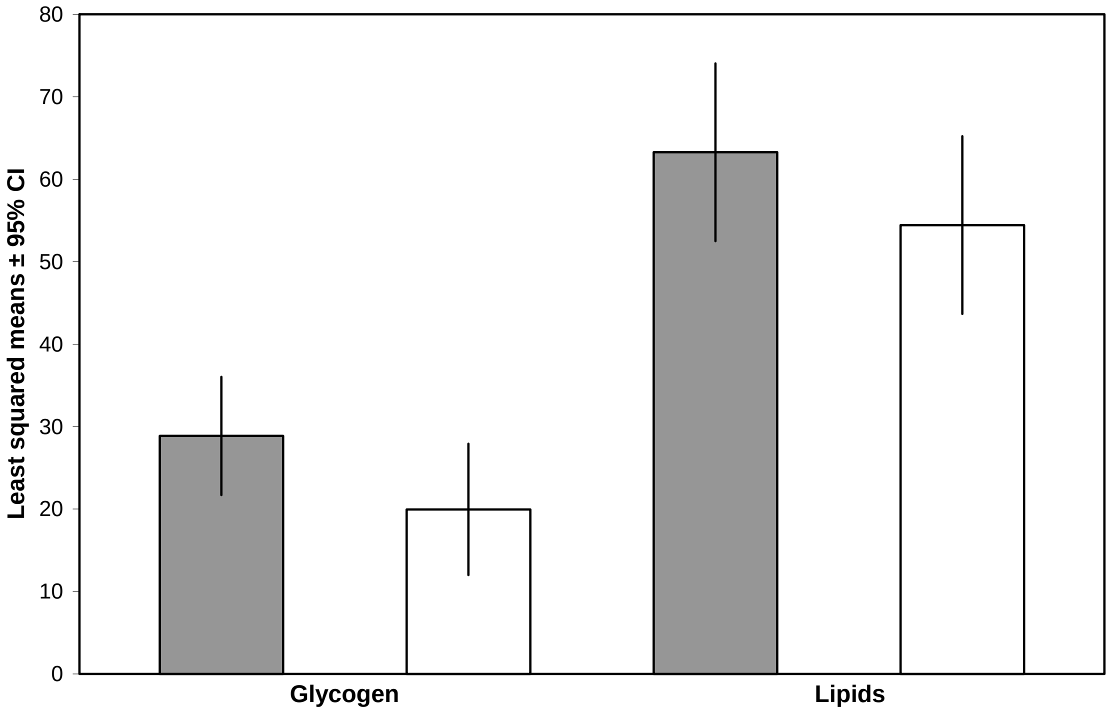
Figure 2. Female predation risk as a function of her mate's size. The solid line is the predicted function based on a cubic spline estimation procedure (Schluter 1988). The dashed lines represent ± 1 SE. The function was estimated using a fixed lambda of -10 and 200 bootstraps. Open circles represent raw data (1 = female captured, 0 = female not captured) that was used to estimate the curve. The open bars represent the frequency of occurrence for each male size class for cases where females were allowed to choose a mate (top) and assigned a mate at random (bottom).

Figure 3. The relationship between body size and gnathopod size for randomly- and choice-mated sires, as well as males that were originally paired with females in the random-mated treatment. Overlapping data points were 5% jittered to reveal their position on the graph.

Figure 4. The relationship between body size and fecundity for daughters from the choice- and randomly-mated treatments. Fecundity was measured as the number of eggs in a female's marsupium.

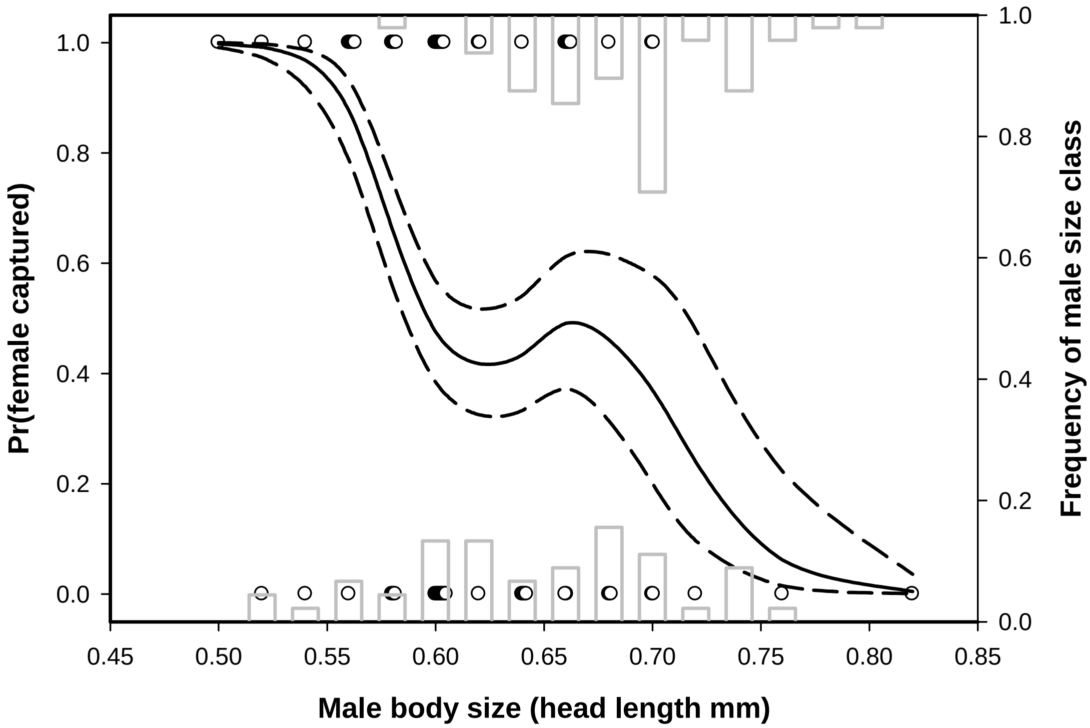
Figure 5. Results comparing pairing success of sons from choice- and randomly-mated families. *Unsuccessful* represents cases where both sons from a given family did not pair. *Somewhat successful* represents cases where 1 out of 2 sons achieved pairing success. *Very successful* represents cases where both sons achieved paring success.

Figure 1



80
70
60
50
40
30
20
10
0
Least squared means ± 95% CI
Glycogen
Lipids

Figure 2



Pr(female captured)
1.0
0.8
0.6
0.4
0.2
0.0
Frequency of male size class
1.0
0.8
0.6
0.4
0.2
0.0
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
Male body size (head length mm)

Figure 3

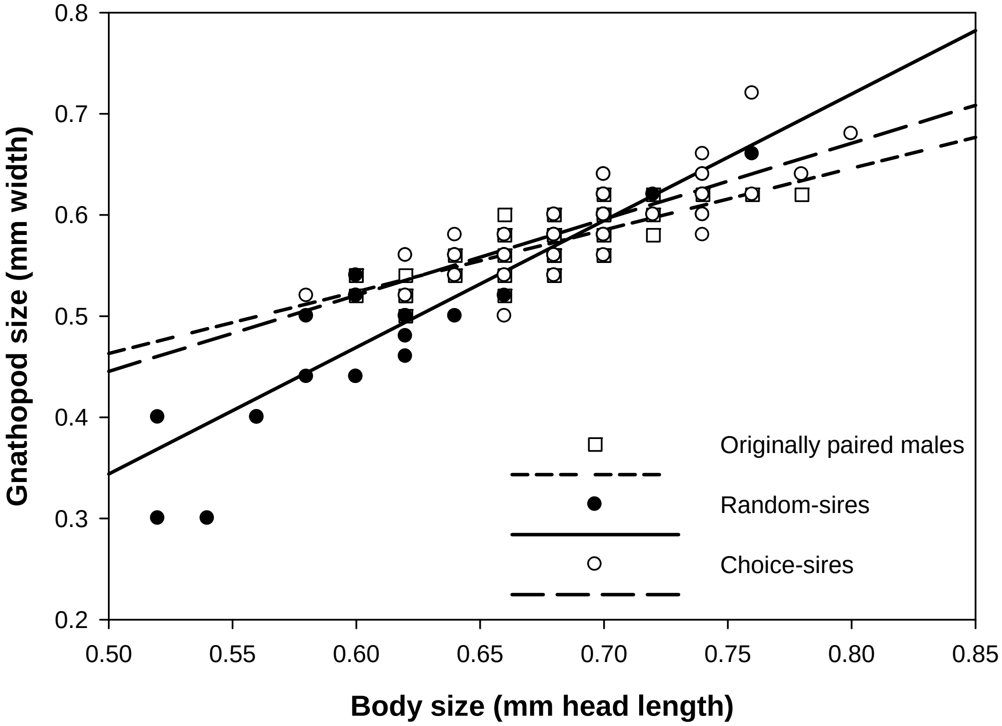
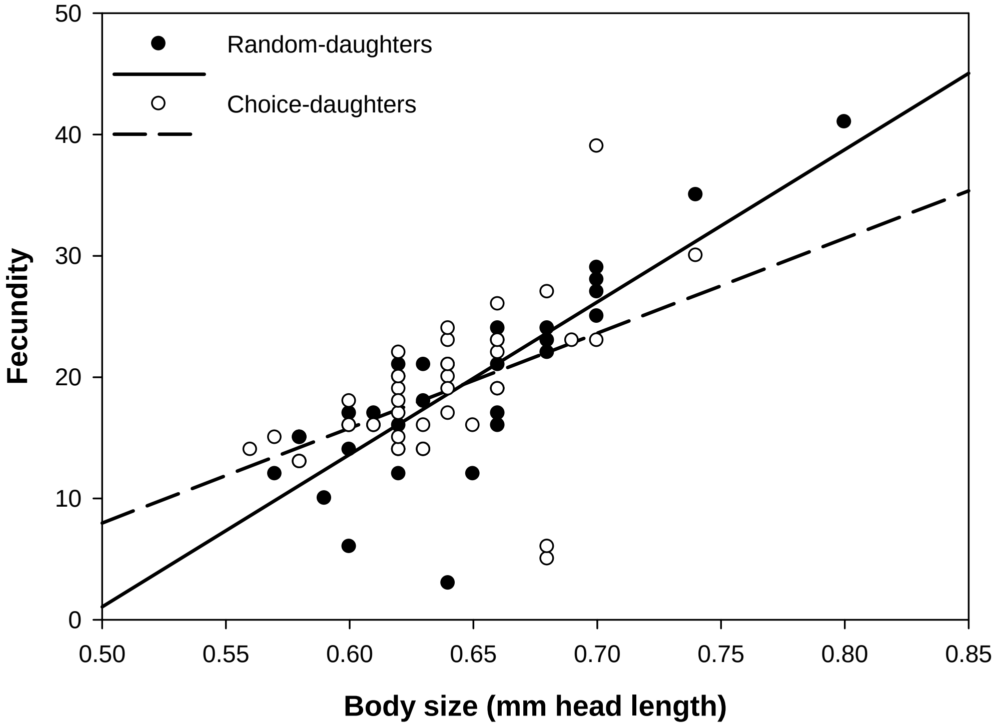
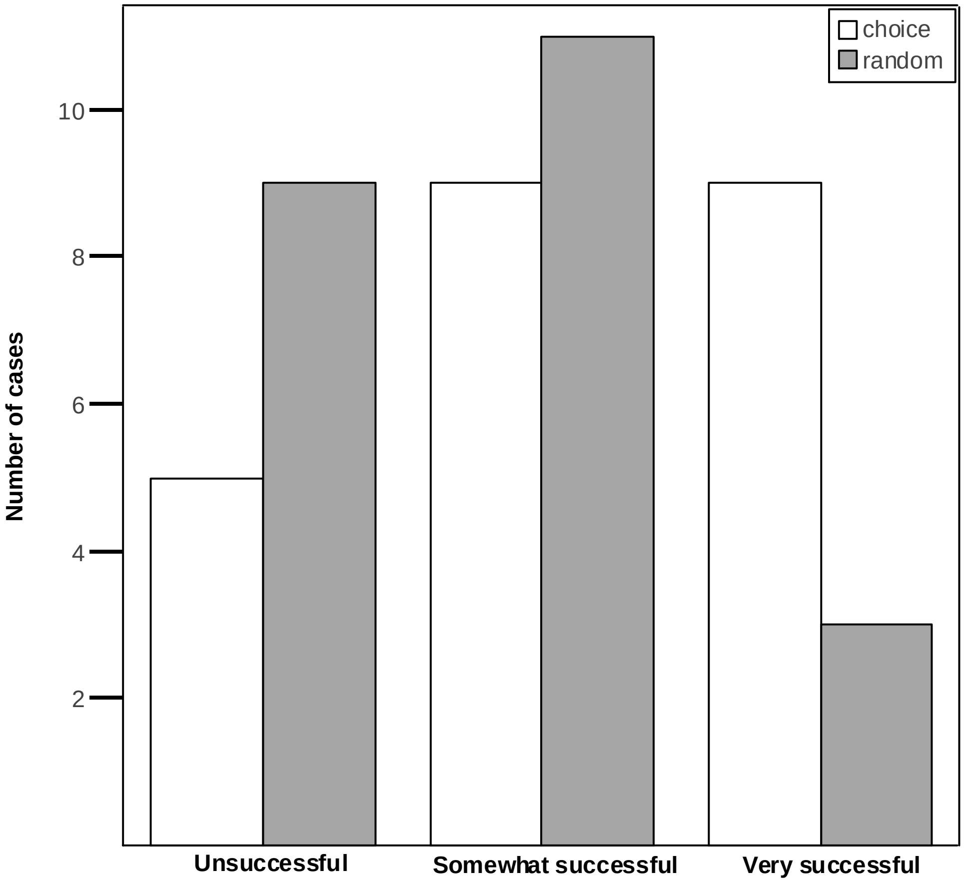

0.8
0.7
0.6
0.5
0.4
0.3
0.2
Gnathopod size (mm width)
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
Body size (mm head length)
Originally paired males
Random-sires
Choice-sires

Figure 4



Random-daughters
Choice-daughters
Fecundity
50
40
30
20
10
0
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
Body size (mm head length)

Figure 5


☐ choice
▨ random
10
8
6
4
2
Number of cases
Unsuccessful
Somewhat successful
Very successful