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Hunger-dependent and Sex-specific Antipredator Behaviour of Larvae of a Size-dimorphic Mosquito

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Abstract

1. Modification of behaviors in the presence of predators or predation cues is widespread among animals. Costs of a behavioral change in the presence of predators or predation cues depend on fitness effects of lost feeding opportunities and, especially when organisms are sexually dimorphic in size or timing of maturation, these costs are expected to differ between the sexes.

2. Larval *Aedes triseriatus* (Say) (Diptera: Culicidae) were used to test the hypothesis that behavioral responses of the sexes to predation cues have been selected differently due to different energy demands.

3. Even in the absence of water-borne predation cues, hungry females (the larger sex) spent more time browsing than did males, indicating a difference in energy needs.

4. In the presence of predation cues, well-fed larvae of both sexes reduced their activity more than did hungry larvae, and males shifted away from high-risk behaviors to a greater degree than did females, providing the first evidence of sex-specific antipredator behavior in foraging mosquito larvae.

5. Because sexual size dimorphism is common across taxa, and energetic demands are likely correlated with size dimorphism, this research demonstrates the importance of investigating sex specific behavior and behavioral responses to enemies and cautions against generalizing results between sexes.

Keywords

Antipredator behavior; *Aedes triseriatus*; *Corethrella appendiculata*; predation cues; sexual dimorphism

INTRODUCTION

Prey species often reduce their activity in the presence of predation cues so they are more difficult to capture or to kill (Lima, 1998; Benard, 2004), a behavioral flexibility that results in large direct and indirect effects of predators on populations of prey (Lima, 1998; Nelson et al., 2004).. In addition to the complete fitness loss of successful predation attempts, cues to predation risk impose nonconsumptive costs on prey animals by altering the time allotted to resource patches and eliciting predator avoidance strategies (e.g., reduced movement,

vigilance, moving into refugia) that reduce foraging activity (Brown, 1999). Animals face a trade-off between food and safety, and must assess their environment to weigh the benefits of foraging against the costs of predation risk (Brown and Kotler, 2004). Because predator avoidance is costly, prey animals should engage in these behaviors only when fitness increases relative to ignoring predation threat (Nonacs & Blumstein, 2010). A reduction in foraging effort is predicted to increase fitness when (1) the marginal value of energy is low (a product of the animal's condition and food quality), (2) the encounter rate with predators is likely to be high, (3) the avoidance strategy employed is effective in reducing predation, or (4) predators are highly lethal (Brown, 1999). So, if an animal is hungry (increasing the marginal value of food), or less susceptible to predation than other individuals (reducing predator encounter rate), the fitness increase of foraging may outweigh the benefit of predation avoidance behavior. Because aquatic systems are convenient for examining antipredator behavior, both allowing for actual predation and using water-borne predation cues, the most numerous examples of the trade-off between foraging and antipredator behavior are found in fish (Godin and Crossman, 1994; Giaquinto and Volpato, 2001), larval amphibians (Horat and Semlitsch, 1994; Anholt et al., 1996; Fraker, 2008), and aquatic arthropods (Johnson and Jakobsen, 1987; Sih, 1992; Koperski, 2003).

Further, when the marginal value of energy gained by foraging is different for the sexes because of sexual dimorphism of any sort, the tradeoff between hunger and safety is likely to impact the sexes differently due to different energy requirements for growth and reproduction (Lima and Dill, 1990). Sexual differences in susceptibility to predation from any source may also result in variation of behavioral responses by increasing the probable encounter rate with predators (Matity et al., 1994; Brown, 1999; Hedrick, 2000). Numerous examples of sex-specific antipredator behavior can be found among animals (Table 1), though the specific nature of the intersexual difference is expected to depend on the size dimorphism, ecology, and life history of the prey organism (Nonacs & Blumstein, 2010) and the hunting mode and habitat domain of the predator (Preisser et al., 2007).

There is growing evidence that predation or water-borne predation cues induce sex-specific modifications of life history traits that are correlated with behavior and feeding rate in dipteran larvae (Table 1). Physiological correlates of size dimorphism (e.g., difference in energetic needs) combined with the trade-off between hunger and safety are likely to affect differentially both the consumptive and nonconsumptive costs of predation on males and females, resulting in evolution of dimorphic antipredator behavior. In this study, we use larvae of a sexually size dimorphic species of mosquito to test the hypotheses that antipredator behavior is hunger-dependent and sex-specific. This hypothesis predicts that better-fed individuals will exhibit more pronounced predation avoidance behavior than will hungry individuals when predation cues are present. Additionally, because males (the smaller sex) have lower energetic needs for growth, males are predicted to alter their behavior in response to cues to predation to a greater degree than do females. The possibility of sex-specific antipredator behavior in mosquito larvae has been examined as a small part of a study on interspecific differences in life history effects of predation cues, though that study failed to detect intersexual behavioral differences (Costanzo et al. 2011). In this study, we use a single highly dimorphic species of *Aedes* that is well known to show strong

antipredator behavior to maximize our ability to test the hypothesis of intersexual differences in antipredator behavior associated with differential costs and benefits for the sexes.

MATERIALS AND METHODS

Study System

Aedes triseriatus is a treehole mosquito native to North America. As in most mosquitoes, females are larger, and among container-dwelling *Aedes*, *A. triseriatus* is consistently one of the most dimorphic species, with well-fed females up to 1.8× larger in dry mass at eclosion than similarly fed males (Wormington and Juliano, in review). Females also have longer development time than males. Larvae of this species display a strong facultative response to water-borne cues from the ambush predators *Toxorhynchites rutilus* (Coquillett) (Diptera: Culicidae) (Kesavaraju and Juliano, 2004; Kesavaraju et al., 2007b) and *Corethrella appendiculata* (Grabham) (Diptera: Corethrellidae) (Kesavaraju et al., 2007a, Costanzo et al., 2011), resting more at the surface of the water when predation cues are present. This antipredator response can be modified by controlled selection by predation (Juliano and Gravel 2002) and declines with a reduction in cue concentration (Kesavaraju et al., 2007b) and with increasing hunger (Juliano et al., 1993).

Corethrella appendiculata is a predatory midge common in the Southeastern United States, the 3rd and 4th instars of which feed on mosquito larvae when available, capturing them by ambush (McKeever and French, 1991). Because of the large size of their prey relative to *C. appendiculata* body size, 2nd instar mosquito larvae are more vulnerable to predation than are 3rd or 4th instars (Kesavaraju et al., 2007a).

Aedes triseriatus eggs were obtained from laboratory stock originating in Vero Beach, Florida (Indian River Hills Cemetery) and raised on a Brewer's Yeast-lactalbumin (1:1) mix. Adults were kept in colonies, allowed to mate freely, and blood fed on anaesthetized guinea pigs (Illinois State University Animal Care protocol #01-2010). *Corethrella appendiculata* larvae were provided by the Florida Medical Entomology Laboratory in Vero Beach, Florida, and raised on aquatic nematode-oatmeal slurry until large enough to consume mosquito prey (3rd instar). Adults were maintained in a free-mating colony, and females laid eggs autogenously in tap water.

Preparation of Predation Cues

A single 4th instar *C. appendiculata* larva was placed in a 10 mL plastic cup with 10 2nd instar larvae of *A. triseriatus*, and allowed to prey on these larvae. Control treatments held *A. triseriatus* larvae only. Daily, dead or eaten mosquitoes were replaced with live 2nd instar larvae. This process was repeated for 5 consecutive days. Before behaviors were observed, all larvae, prey and predator, were removed from the water, though detritus (feces, exoskeleton, uneaten pieces of larvae) remained.

Hunger manipulation

Predator-naïve 2nd instar larvae were held individually for 8-12 hours in containers with 3 levels of Difco™ Nutrient Broth (a substrate for the bacterial on which larvae feed) and water (0.1, 0.05, and 0 g/L; hereafter High, Medium, and Low, respectively). Larvae were then placed into 10 mL plastic cups holding previously prepared water containing either predation + conspecific cues (hereafter, “predation”) or conspecific cues only (hereafter, “control”).

Video Recording

Six 10 mL cups each containing one larva and representing all treatment groups (3 feeding levels × predation vs. control) were video recorded for 35 minutes. In total, 19 videos were taken, representing 114 larvae. After 5 minutes for habituation, position and activity (see Juliano and Reminger, 1992 for detailed protocol) of each larva were noted every minute for 30 minutes. Positions were: 1) Surface, with respiratory siphon in contact with the water-air interface; 2) Wall, larva less than 1 mm from the wall of the cup; 3) Bottom, larva less than 1 mm from the bottom of the cup; and 4) Middle, larva greater than 1 mm from the surface, wall, and bottom of container. When position could be scored as either surface or wall, precedence was given to surface. Larvae at the junction of the bottom and the wall were judged to be at the bottom of the container. Activities were: 1) Resting, larva not moving through the water or feeding; 2) Browsing, the larva's mouthparts in contact with a surface and movement via the mouthparts detected; 3) Filtering, the larva not in contact with a surface and driven through the water by the mouthparts with no body movement; and 4) Thrashing, larva quickly moving through the water propelled by vigorous body movements.

Statistical Analysis

After behavioral observations, larvae were reared in individual vials and sex was determined when and if individuals reached pupation or adulthood (N=83; 35 females, 48 males). Roughly the same proportion of larvae emerged as adults for the two temporal blocks. Activities and positions were converted to proportions and summarized using Principal Components Analysis (PCA) to reduce the number of variables. Our data met the assumptions of PCA, as has typically been the case for similar behavioral data in this system (Kesavaraju and Juliano, 2004; Kesavaraju et al., 2007a). Principal components (PCs) with large eigenvalues (greater than 2) associated with behaviors known either to promote (resting at the surface) or to diminish (thrashing) the chances of survival in the presence of a predator (Juliano and Reminger, 1992; Kesavaraju et al., 2007a) were subjected to ANOVA to test for differences due to predator treatment, sex, and food level. Least squares means for principal component scores significantly different from one another were identified by multiple comparisons using Tukey's test.

RESULTS

Two PCs captured 58% of the variation in position and activity of *A. triseriatus* larvae (Table 2). Based on rotated factor patterns, large positive scores on PC1 were positively associated with greater time spent resting at the surface than in other activities below the

surface (Table 3). Large positive scores on PC2 were positively associated with greater time spent thrashing than spent browsing.

ANOVA on PC1 produced three significant effects (Table 4): Predation, Food, and a Predation*Food interaction. Predation cues induced larvae in the high food treatment to change behavior significantly, shifting towards greater time resting at the surface (Fig. 1), whereas for larvae in low and medium food treatments this change in behavior was not statistically significant (Fig. 1). Increasing hunger did not yield significant differences in PC1 for the control treatment (Fig. 1, open symbols), but in the predation treatment, high food induced greater time resting at the surface, compared to low food (Fig. 1, filled symbols). The absence of any significant effects or interactions involving sex for PC1 (Table 4) indicates that both sexes showed statistically indistinguishable changes in the behaviors summarized by PC1.

In contrast to PC1, there was a significant predation*sex effect on PC2, along with significant main effects of both predation and food (Table 4). The significant main effect of food resulted from larvae in the low food group spending significantly more time browsing than thrashing compared to the high food group (Fig. 2a), and this effect appeared to be independent of both sex and predation treatment (Table 4). The significant predation*sex interaction for PC2 resulted from males responding to predation cues by thrashing less and browsing more than did males in control water, and the absence of this significant change in behavior with predation cues for females (Fig. 2b). This reduction in thrashing by males in responses to predation cues was consistent in magnitude across hunger levels (Fig. 3). Similarly, females consistently showed no strong reduction of thrashing in response to predation across all hunger levels (Fig. 3).

DISCUSSION

Our results show both sex-specific and hunger-dependent behavioral changes in response to predation cues in *Aedes triseriatus*, but we find no evidence for interactive effects of hunger and sex. *Aedes triseriatus* modified their behavior significantly in the presence of predation cues, consistent with past studies showing increased time spent resting at the surface of the water column (Kesavaraju and Juliano, 2004). In an experiment exposing *A. triseriatus* to predation by *T. rutilis*, out of four activity categories, resting (no movement) was the least likely to lead to capture, and thrashing (fast movement associated with descent from the surface and alarm; Walker and Merritt, 1991) the most likely, with browsing and filtering (feeding movements) intermediate (Juliano and Reminger, 1992). Similarly, Kesavaraju et al. (2007b) observed that all *A. triseriatus* larvae killed by *C. appendiculata* were thrashing at the time of capture. In the present study, when predation cues were present, larvae were more likely to be found browsing or resting at the surface than in control water, where thrashing or other subsurface activities were more common. This indicates a shift from risky behaviors like thrashing and feeding below the surface to safer behaviors involving less movement in predation treated water.

Poorly fed mosquitoes spent significantly more time browsing and less time thrashing or resting at the surface (Fig. 1, 2a), and as predicted, showed less response to predation cues

than did well fed larvae. This demonstrates that *A. triseriatus* larvae behave differently based on their foraging needs, and not surprisingly, more energy is devoted to gathering food as hunger increases. Antipredator response was greatest when larvae were well fed and diminished as hunger increased (Fig. 1), providing evidence that mosquito larvae trade off predation avoidance behaviors and the marginal value of energy. Poorly fed mosquitoes likely have reduced energy stores, forcing them to seek food actively and preventing them from adopting behaviors that reduce the probability of capture by an ambush predator. Well-fed larvae, on the other hand, can and do spend more time in low-risk activities when predator cues are present, and this change would seem to reduce their ability to seek food.

Analysis of PC2 yielded a significant interaction of sex and predation treatment (Fig. 2b), with male mosquitoes displaying a significantly greater shift in behavior between predation and control treatments. Both sexes showed a trend away from the risky behavior of thrashing and to browsing (Fig. 2b), but the difference between control and predation was significant only for males. This result is consistent with observations by Alto et al. (2005), wherein cohorts of *A. triseriatus* adults emerging from containers containing *C. appendiculata* were male-biased, suggesting that males are less vulnerable to predation than are females. The more pronounced behavioral shift away from risky behavior shown by *A. triseriatus* males in predation-treated water likely contributes to this differential survival and vulnerability to predation. This difference in behavioral responses to predation was evident only for PC2, and not for PC1. Past studies of antipredator responses of container *Aedes* have largely found the biggest behavioral response to be a shift away from below-surface activities and positions to resting at the surface when predator cues are present (Kesavaraju and Juliano, 2004; Kesavaraju et al., 2007a; Kesavaraju et al., 2007b, Costanzo et al., 2011). The significant intersexual difference in antipredator behavior that we observe does not involve this most obvious behavioral change, but instead involved PC2, with males showing a greater reduction in thrashing than did females. Thrashing is the behavior that entails the greatest predation risk (Kesavaraju and Juliano, 2004; Kesavaraju et al., 2007a) hence reducing it in response to predator cues seems likely to be adaptive. Thrashing is also a behavior that results in transitions from surface to subsurface and back again (Walker and Merritt, 1991), and reduced thrashing in males but not females suggests that though both sexes increase time resting at the surface in response to predator cues, males would seem to adopt lower-risk behaviors to get from surface to subsurface and back. By browsing along vertical walls of the container, a larva can move from surface to the bottom without thrashing (Walker and Merritt, 1991). Because young larvae like the 2nd instars used in our behavioral observations are positively buoyant (Chapman et al. 2013) they may rise to the water's surface passively, again avoiding thrashing.

Adult *A. triseriatus* emerge from environments with predation at lower mass, an effect that is more evident for females, and with prolonged female development (Hechtel & Juliano, 1997) unless predator-mediated competitive release mitigates this effect (Alto et al., 2005). Fecundity increases with size in most female invertebrates, as well as many endothermic vertebrates (Shine, 1988). The ways in which female mosquitoes profit reproductively from large size are well established: larger mosquitoes can better locate hosts, take larger blood meals, and produce more eggs, and more eggs per unit of blood ingested, than do smaller

mosquitoes (Briegel, 1990; Frankino & Juliano, 1999; Blackmore & Lord, 2000). Thus, even small predator-induced changes in behavior of females that result in life history changes may result in relatively large fitness costs due to reduced size and development rate. For males, greater body mass can be associated with a greater number of potential ejaculations between sugar meals and more sperm transferred (Ponlawat & Harrington, 2007; 2009; Helinski & Harrington, 2011), though the fitness effect of a change in size is likely smaller for males than for females. Thus, females and males both behaviorally trade off safety and hunger, but the fitness cost of lost foraging opportunities is likely greater for female *A. triseriatus* than for males.

Although the sex*food interaction was not significant in analysis of either PC, in the lowest food treatment, females browsed more than did males, particularly in the absence of predation cues (Fig.3), a trend that suggests a biologically relevant greater energetic need by females. The behavioral difference between the sexes in response to predation cues was also not dependent on food treatment (i.e., there was no significant three-way interaction). Despite this, means for PC2 scores show the expected trends (Fig. 3): regardless of hunger, males show a pronounced reduction in thrashing; whereas females show little evidence of change in behavior at medium or low food levels. Sexual size dimorphism often leads to or is caused by diet differences between the sexes (Shine, 1989; Blanckenhorn, 2005), and competition effects on the adult mass of females of another mosquito, *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), are more pronounced than on male mass (Bedhomme et al., 2003), suggesting a stronger response of female mosquitoes to food availability. Costanzo et al. (2011) found that cues from real or simulated predation significantly increased larval development time for *A. triseriatus* males but not for females, and this effect is consistent with the stronger, likely costly, behavioral response we observe in *A. triseriatus* males compared to females (Fig. 2b, Fig. 3). Paradoxically, Costanzo et al. (2011) reported no significant effects involving sex on behavior of their 2nd instar larvae. Their study and ours used 2nd instar larvae, which is the most relevant instar for evaluating responses to cues from the small predator *C. appendiculata* (Kesavaraju et al., 2007a; Costanzo et al., 2011). Sexual size dimorphism is likely to be modest in the 2nd instar, resulting in subtle effects of sex on behavior, which is consistent with the limited (our study) or non-significant (Costanzo et al., 2011) effects involving sex on behavior. We did find significant dimorphism in behavior (Fig. 2b) and that dimorphism seems to be more pronounced at lower food levels (Fig. 3). It seems inevitable that later instars become more size dimorphic as differences in growth accumulate over larval development, culminating in newly eclosed adult females having up to 1.8× the dry mass of newly eclosed adult males (Wormington and Juliano, in review) and therefore we predict a more dramatic intersexual difference in the shift in behavior with hunger and predation cues for later instar larvae (e.g., 4th instar larvae responding to the larger predator *Toxorhynchites*).

This study found greater antipredator response in the smaller sex. Though responses to predation threat vary widely across taxa and can include such diverse strategies as vigilance, flight, refugia use, and social behavior, it is worthwhile to compare the results of studies testing for sex-specific responses to predation cues (Table 1). In 3 other studies finding behavioral dimorphism in this direction, the males are not only larger but the females

provide parental care (Blanchard et al., 1992; Blumstein et al., 1997; Creel & Winnie Jr., 2005; Winnie Jr. & Creel, 2007), compounding the selection pressure for females to optimize fitness by enhancing offspring survival, and confounding the effect of sexual size dimorphism and its resultant difference in energy needs with sexual division of labor. It would be interesting to test this hypothesis in an organism that displays larger-sex parental care. A few species showed qualitative dimorphism in avoidance strategy rather than dimorphism in response size. For example, male grasshoppers jump away from foraging birds more often than females, who are cryptically colored and not as likely to attract the attention of predators (Schultz, 1981). Male lantern flies, the smaller sex, more frequently use a startle display rather than fleeing, as females do (Kang et al., 2011). In *Anolis* lizards, males (the larger sex) are more sensitive to an approaching human being, fleeing sooner and for longer distances than females, a difference which cannot be explained by sexual dimorphism in size or habitat use (Vanhooeydonck et al., 2007). Females, the smaller sex, tend to invest in cryptic behavior as a predator avoidance strategy, which more closely resembles the reduction in activity of male mosquito larvae. Some studies show the alternative pattern of greater antipredator behavior in the larger sex. Though not dissimilar to females in overall size, male fiddler crabs, which have conspicuously enlarged front claws, remain in their burrow for longer than females after exposure to a predator-like stimulus (Jennions et al., 2003), counter to theoretical predictions. However, males are lighter in color and more susceptible to bird predation than their darker, more cryptic sexual counterparts (Koga et al., 2001). Clearly, even when sexually dimorphic behavior is exhibited in the direction predicted by the trade-off between energy needs and predator avoidance, other factors can reinforce or oppose selection pressure for divergent behavior.

Because we have included only studies that specifically mention sexual dimorphism, Table 1 is not a comprehensive review of the available information on sex specificity in behavioral threat response. As evidence accumulates, it may be useful to statistically search for patterns governing the expression of sexually dimorphic predation response in relation to dimorphism in energy needs or other life history characteristics, effectiveness of escape/avoidance strategy, and type of predation stimulus (i.e., predator encounter or presence of predator cues).

Kesavaraju and Juliano (2004) found that *Aedes albopictus* (Skuse) (Diptera: Culicidae) did not show a behavioral response to the presence of predation cues from *T. rutilus*, but an experiment done by E. Dubrovsky (personal communication) found a significant behavioral response of *A. albopictus* males to predation cues from *C. appendiculata* but no response in females. Thus it seems quite possible that some studies failing to uncover antipredator responses did so because they pooled groups of individuals, in this case males and females, that show differential responses. Sex-specific behaviors are widespread across taxa. Despite this, behavior, with the obvious exception of reproductive behavior, is often not investigated using a sex-specific approach (Beery and Zucker, 2011). In organisms where the sexes are dimorphic in size or activity, selection is particularly likely to act on the sexes in different ways, which could lead to intersexual divergence in behavioral responses to a number of different cues, including predation. Even when study organisms are monomorphic in form or size, sexual differences in behavior can occur (e.g., Williams et al., 2001; Elliott et al., 2010).

due to differences in energy needs for gamete production, mating displays, aggression, etc. Studies that evaluate behavior but pool the sexes may fail to illuminate an important source of variation. The differences in the sexes' responses to predation or to hunger in this study suggest that ignoring the sexes of subjects could result in very misleading results and interpretations of adaptive behavior.

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Fig. 1. PC1 least squares means ($\pm$ SE) for Predation*Food combinations. Different letter designations indicate significant differences in LSmeans. In the presence of predation cues, time spent resting at the surface is interpreted as antipredator behavior. Antipredator response was greatest when larvae were well-fed.

Fig. 2.

Least squares means ($\pm$ SE) for PC2 for (A) Predation*Food combinations; Horizontal axis values associated with the same letter have food level least squares means that are not significantly different; and (B) predation*sex combinations showing differential response of the sexes to predation cues. Least squares means associated with the same letters are not significantly different. In the presence of predation cues, a shift away from time spent thrashing is interpreted as antipredator behavior. (A) As hunger increases (from high to low

food level), larvae spend more time actively engaged in foraging (browsing) than traveling (thrashing). (B) Male antipredator response is more exaggerated than the female response.

Fig. 3.

Least squares means ($\pm$ SE) for all treatment-food-sex combinations. In the presence of predation cues, a shift away from time spent thrashing is interpreted as antipredator behavior. Though this effect was non-significant, females tended to respond to predation cues to a smaller degree when hungry, while the male response was more consistent across food treatments.

Table 1

Select examples of sexual dimorphism in behavioral and life history responses to threat of predation

Taxon	Sexual Dimorphism	Dimorphism of Antipredator Response	Dimorphism of Life History Response	Reference
Red deer <i>Cervus elaphus</i> Linnaeus	Males larger	Greater female vigilance		Creel & Winnie Jr., 2005; Winnie Jr. & Creel, 2007
Norway rat <i>Rattus norvegicus</i> (Berkenhout)	Males larger	Greater female vocalizations		Blanchard et al., 1992
Yellow-bellied marmot <i>Marmota flaviventris</i> (Audubon & Bachman)	Males larger	Females alarm call more often; Greater male foraging suppression after alarm call		Blumstein et al., 1997; Lea & Blumstein, 2011
Anole lizards <i>Anolis</i> sp.	Varies across ecomorphs	Varies across ecomorphs; Lower male approach distance before flight; Greater male flight distance		Vanhooydonck et al., 2007
<i>Thamnophis sirtalis</i> Linnaeus	Females larger	Males flee more often, females strike more often		Shine et al., 2000
Teleost fish	Diverse	Diverse		Reviewed by Magurran & Garcia, 2000
<i>Uca lactea perplexa</i> (Milne-Edwards)	Enlarged male chelae, males lighter in color	Greater male return-to-forage latency		Jennions et al., 2003
<i>Astroma riojanum</i> Mello-Leitão	Female larger	Males jump away more often		Schultz, 1981
<i>Lycorma delicatula</i> White	Females larger	Females flee more; Males use more startle display		Kang et al., 2011
<i>Chironomus tentans</i> Fabricius	Females larger		Male development slowed	Ball & Baker, 1996
<i>Culex pipiens</i> Linnaeus	Females larger		Greater slowing of female development	Beketov and Leiss, 2007
<i>Culiseta longiareolata</i> Macquart	Females larger		Male development slowed	Stav et al., 2005
<i>Aedes triseriatus</i> (Say)	Females larger		Female development slowed; Sex ratio male-skewed	Hechtel and Juliano, 1997; Alto et al., 2005

Table 2

Eigen analysis for components of larval behavior.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.54	2.07	1.23	1.11	0.57	0.30
Proportion of Variance	0.32	0.26	0.15	0.14	0.07	0.04
Cumulative Proportion of Variance	0.32	0.58	0.73	0.87	0.94	0.98

PCs in bold with eigenvalues >2 were retained for behavior analysis.

Table 3

Varimax rotated factor patterns from Principal Components Analysis for PC1 and PC2.

Response Variables	PC1	PC2
Resting	88	-1
Thrashing	-13	95
Browsing	-29	-62
Filtering	-3	4
Surface	85	-9
Bottom	-14	-6
Wall	-30	11
Middle	-19	27
Interpretation	Resting, surface vs. Subsurface activities Thrashing vs. Browsing	

Values in bold indicate strong loadings on each principal component.

Table 4

ANOVA results for PC1 and PC2.

Factor	df	PC1		PC2	
		F Value	Pr>F	F Value	Pr>F
Block	1	0.14	0.7084	0.56	0.46
Predation	1	17.46	<.0001	17.79	<.0001
Food	2	8.51	0.0005	5.3	0.01
Sex	1	0.01	0.9041	0.48	0.49
Predation*Sex	1	0.24	0.6290	4.51	0.04
Food*Sex	2	1.54	0.2220	1.25	0.29
Predation*Food	2	3.73	0.0288	0.62	0.54
Predation*Food*Sex	2	0.49	0.6159	0.55	0.58
Error	70				

Significant effects are listed in bold print.