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PREDATION AND BEHAVIORAL PLASTICITY IN GREEN SWORDTAILS: MATE CHOICE IN FEMALES AND EXPLORATORY BEHAVIOR IN MALES

by

Andrew J. Melie

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PREDATION AND BEHAVIORAL PLASTICITY IN GREEN SWORDTAILS: MATE CHOICE IN FEMALES AND EXPLORATORY BEHAVIOR IN MALES

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University of Nebraska, 2013

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Two studies were carried out with green swordtails, *Xiphophorus helleri,* to investigate the effect of predation on swordtail behavior, and to determine how behavioral plasticity operates in both a mate choice and an anti-predator context. Male green swordtails vary in colorful conspicuous traits, *e.g.* the colorful dorsal fin and sword. Female swordtails have a preexisting bias for males with a sword, and prefer long-sworded males to short-sworded males, but this preference is plastic. The first study examined predator-related plasticity in the behavior of males differing in size. Smaller males showed greater behavioral plasticity; they were more active in the absence of a predator, but reduced activity in the presence of a predator, while larger males maintained lower activity levels regardless of predation environment. Males, regardless of size, entered the area nearest to where a predator had been, shortly after it had swam off. Males also utilized refuges furthest from a successful predator while the predator was visible, but did not differentially use refuges after the predator departed, regardless of male size. The second study examined whether different predation environments differ in their effects on female sword responses. Females switched their preference to short-sworded males, regardless of whether the predator was a large cichlid chasing and consuming a male swordtail with a short sword, a large cichlid alone, or a small cichlid alone. We also looked at the lasting effect of predation environment on sword response and found that the preference for short-sworded males persisted to the following day. To our knowledge, this is the first example of enduring plasticity in a receiver bias. Finally, we addressed whether females respond differently to differing predation environments in a non-mating context. Females perceived large cichlid predators alone to be as dangerous as successful predators, but not small cichlids. The results of these studies indicate that predation can have a profound influence on the expression of suites of behaviors, in both mating and non-mating contexts.

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Chapter 1. Anti-Predator Behavioral Plasticity in Small, but Not Large Male Swordtails

Abstract - A primary environmental factor that favors plasticity in behavior is predation. While numerous studies of behavioral plasticity have centered on the effect of predation on mating and foraging behaviors, few have considered size-related differences in behavioral plasticity in anti-predator behavior as it relates to general activity in a novel environment. Male green swordtails, *Xiphophorus helleri*, vary in colorful conspicuous traits, *e.g.* the colorful dorsal fin and sword, and large body size that could attract predators. We tested predator-related plasticity in the behavior of males differing in size. Males were exposed to either an environment in which a predation event on a male swordtail occurred, or the same environment devoid for fish. General activity, spatial distribution, and refuge use were quantified for each male. Smaller males showed greater behavioral plasticity; they were more active in the absence of a predator, but reduced activity in the presence of a predator, while larger males maintained lower activity levels regardless of predation environment. There are at least two non-mutually exclusive explanations for this result. First, larger males may be more cautious all of the time because of their more conspicuous size and secondary sexual traits. Second, smaller males may be more risk–adverse when they detect a predator because they are more easily consumed. Males also spent more time furthest from predators, regardless of size. This result is similar to the behavioral response to predators exhibited by female green swordtails (Ch. 2). Male swordtails, regardless of size, entered the area nearest to where a predator had recently been, possibly as a means of determining a predator’s location. Males also utilized refuges furthest from the predation

event, but did not differentially use the refuges after the departure of the predator, regardless of size. This study demonstrates that size can mediate the degree of expression of anti-predator behavior.

INTRODUCTION

Natural selection favors traits that improve an individual's viability, while sexual selection favors traits that are attractive to potential mates or improve competitive ability for access to mates (Darwin 1871). Sexual selection can favor conspicuous traits that males actively display, such as coloration, song, enlarged structures, and greater overall size (Andersson 1994), but can also increase risk due to increased conspicuousness to predators. Predation risk can be reduced by temporarily altering morphological and/or behavioral traits. For example, the conspicuous coloration preferred by female birds (Guianan cock-of-the-rock (*Rupicola rupicola*), white-throated manakin (*Corapipo gutturalis*), and white-fronted manakin (*Lepidothrix serena*)) can be temporally reduced by males moving from light patches to shaded areas (Endler & Théry 1996). In crickets, the male acoustic signal used to attract females can attract predators. Males can reduce predation costs by expressing a less attractive call when predation risk increases (Hedrick 2000). Many morphological traits, however, cannot be readily adjusted, such as feather coloration, body size (in most cases), and the sword in swordtail fishes. In contrast, behavioral traits can be adjusted in response to a temporally changing environment. For example, male fiddler crabs wave their claws less often and build fewer mud pillars, which attract females, when avian predators are present (Koga *et al.* 1998). When the predation environment is constantly changing, selection may favor such facultative modification of behavior in order to reduce predation risk, especially when conspicuous morphological traits cannot be easily adjusted. In such cases, risk-sensitive behavior may be particularly important.

Predation is often dependent on the activity of the prey. High prey activity typically exposes individuals to enhanced predation (Werner 1992; Sih 1994). Searching activity is often required for prey to locate food and mates, therefore there can be a conflict between anti-predator behavior and other behaviors important to fitness (Lima & Dill 1990). For example, there may be a trade-off between time spent watching and avoiding a predator, and time spent obtaining food. When prey use a refuge, its predation risk is lowered, but eventually it must cease anti-predator behavior, as hiding can reduce an individual’s food intake, growth, and mating opportunities (Sih 1997).

Few traits influence animal behavior as universally as size (see Alcock 2009 for examples), which is often important in competing for access to mates and food, and avoiding predation. Larger individuals in many species are generally able to outcompete smaller individuals (see Andersson 1994 for examples), yet small body size in adults persists. One explanation for the persistence of smaller size is that there can be costs to larger size. Larger individuals can be more conspicuous (Winemiller 1990), and thus more likely to be attacked by predators (Trexler *et al.* 1994). When given a choice, predators often attack the larger of several individuals (Brooks & Dodson 1965, Johansson *et al.* 2004). If larger males become wary when a predator is perceived, and respond by reducing conspicuous mating behavior, less preferred or less competitive males that would otherwise be excluded from mating may have greater access to females and thus a greater chance to mate (Basolo & Nootz in revision). While both large and small individuals are expected to use refuges to reduce predation risk, size can influence the degree of use. For example, large sticklebacks emerge from a refuge later than small individuals, and spend less time outside of a refuge (Krause *et al.* 1998). One explanation for this behavior is that smaller fish have relatively higher energetic costs than larger fish (Brett 1979), even when resting, thus they need to leave a refuge sooner to forage. Because size is negatively correlated with metabolism (Brett & Glass 1973), large individuals can energetically afford to remain in a refuge longer. There is also a trade-off between size and maneuverability (Domenici & Blake 1993). Larger individuals turn more slowly (Domenici 2001), meaning that larger males may not be able to maneuver as well as small males to escape from a predator.

In the poeciliid fish genus, *Xiphophorus*, male size can vary greatly in all but two of the species in which size has been investigated, and much of this variation has been shown to be influenced by a sex-linked genetic polymorphism at the P-locus (Kallman 1989). Females prefer larger to smaller males in several species, including *X. helleri*, green swordtails (Basolo 1998). Competition for mates is common within *Xiphophorus*, with larger males generally out-competing smaller males (Beaugrand & Zayan 1985, Zimmerer & Kallman 1988, Moretz 2003, Benson & Basolo 2006), as well as gaining greater access to females (Zimmerman & Kallman 1988, 1989, Morris *et al.* 1992). And, for one swordtail species, males of the largest of four P-genotypes have higher reproductive success than smaller males (Zimmerer & Kallman 1989). There is evidence that males differ in the degree of mating behavior plasticity expressed depending on the predation environment. Larger male *X. montezuma* are more risk adverse than smaller males; they reduce time spent near females when a predator is detected, while small males do not (Basolo & Nootz in revision). Male green swordtails can vary in size up to five-fold, and express colorful conspicuous traits (enlarged dorsal fins, elongated caudal fins, coloration, *etc.*) that could attract predators. The sword is a structure comprised of colored ventral caudal rays that can grow beyond the caudal margin (Basolo 1995, 1996). In *X. helleri*, males with longer swords win more contests (Benson & Basolo 2006), and post-maturation growth in body size and sword length can be food-dependent (Basolo 1998). Size is often correlated with male sword length in green swordtails; within wild populations, larger males have longer relative swords than smaller males in non-predation populations. However, relative sword length to body length varies with sympatric predators (Basolo & Wagner 2004).

While numerous studies of behavioral plasticity have centered on the effect of predation on mating behavior, few have considered differences in behavioral plasticity in anti-predator behavior in a non-social context. In this study, we consider (i) whether male body size affects general activity, both during and after a predation event on another individual. We also consider (ii) whether there are differences in male refuge use during a predation event on another individual. Finally, we consider (iii) where males are likely to be situated relative to a predation event on another individual.

METHODS

Study System

The green swordtail, *Xiphophorus helleri* is a freshwater, live-bearing fish in the subfamily Poeciliinae (Parenti & Rauchenberger 1989). Subjects in this study were third and fourth generation male descendants of fish caught from Savannah Stream near mileage marker 28 on the Western Highway, Belize (refer to Basolo and Wagner 2004). Male green swordtails from this population can vary in body size up to five-fold. Males for this study were reared individually in 5.68l tanks on a 12:12 h light-dark cycle at 24^0C, and were fed live brine shrimp nauplii in the morning and flakes in the late afternoon ad libitum. Test subjects had not experienced predators prior to testing, and all were sexually mature.

Experimental Protocol

Tests were conducted in a 60 x 30 x 30 cm tank (Fig. 1.1) with a removable white plastic barrier positioned widthwise 8 cm from one end of the tank creating a space of 52 x 30 x 30 cm in which test-trials were conducted. The back of the tank was covered in blue felt. At the bottom of the tank, there was a white board with a thin layer of gravel (height of gravel = 3 mm) that was positioned with silicon to form 12 equally sized rectangles (each 17 cm L x 7.5 cm.), which were distinct from one another to the tester. The grid was classified into three equal zones for scoring purposes: the third of the grid nearest the monitor (zone 1), the third of grid in the middle of the tank (zone 2), and the third of the grid furthest from the monitor (zone 3). The grid was used to record general male activity (movement from one rectangle on the grid to another), and the time within refuges. Artificial plastic plants (Imagine Gold Ambulia Green) that acted as refuges were located at six points in the tank: one at each left corner of zone 1, one at the center front and back in zone 2, and one at each right corner of zone 3 (Fig. 1.1). The refuges were suspended

15.24 cm from the top of the tank, providing cover for the males at six points in the testing space.

A monitor (DELL UltraSharp 2005FPW 20.1-inch Wide Aspect Flat Panel LCD Monitor) was centered at one side of the tank to play video stimuli to the males. The monitor was switched between sides after each trial in order to control for potential side effects. Two video cameras were used to record the trials: one camera (DCR-SR47 Handycam) was centered overhead (height = 33.8cm) to capture a male's movement in the tank and one camera (Panasonic 5100HS WV-PS03) was centered in front of the tank (distance to tank = 130.8cm) to capture a male's position and refuge-use. Feeds from the two cameras simultaneously went to a video editor (Videonics MX-1), which was used to produce a split-screen image that was viewed by an observer remotely (but in real-time) on a monitor from outside the test chamber. The test chamber was illuminated from above by two 40W Vita light^{TM} bulbs covered by a layer of vellum paper lit the tank from above.

Cichlid Stimuli Construction

Predatory cichlids collected from Belize field locations in which they co-occur with green swordtails were used for this study. These included large Jack Dempsey, *Cichlasoma octofasciatum* (currently there are multiple specie names for this cichlid including *Rocio octofasciata*), standard length: 141–176 mm; n = 4) and a large bay snook, *Petenia splendida*, (standard length: 201 mm; n=1). Both of these cichlids are predators of smaller fishes (Konings, 1989; Conkel, 1993; Greenfield & Thomerson, 1997), and easily capture and consume adult *X. helleri* in the lab (personal obs.).

Two types of predator stimuli were constructed for this study: (1) a large cichlid swimming in a tank and capturing and consuming a swordtail = predation; (2) the same tank devoid of fish = no predation.

To construct a predation event stimulus, a large predatory cichlid was removed from its home tank and placed in a filming tank (61 x 30.5 x 40.5 cm) to acclimate for a five-day period. The filming tank was divided such that one-fourth was a temporary predator “home” area, and three-fourths a recording area. An opaque divider separated the two areas. This divider had an entry/exit hole that allowed the cichlid to pass between the two areas, and a partition was in front of the hole in order to sequester the cichlid in the home area. The filming tank was surrounded with blue felt and contained a short length of pipe (like that in the cichlid’s home tank from which it was temporarily moved for filming). (In their home tanks, cichlids often remain in their pipe when they are not actively foraging.) Two 60cm Vitalites illuminated the filming tank from above. Up to 2 cm of natural gravel (varied shades of tan) covered the bottom of the tank. On filming day, the cichlid was sequestered in the home area of the filming tank, and a male swordtail (standard length = 43 mm +/- 4.2; sword length = 28.5 mm) was introduced into the recording area of the filming tank. The partition was removed which allowed the cichlid to move into the recording area and attack the swordtail. The fish were filmed with a Canon Vixia HG20 camera positioned in front of the tank. The entry/exit hole allowed the cichlid access to both the home area and the recording area during this period, allowing subsequent edits of filmed video footage to be seamless.

Video footage was edited using iMovie ’09 (v. 8.0.6) to create a 10-min predation event sequence. This sequence showed: (1) a swordtail swimming and then moving out of view; (2) the swordtail re-appearing; (4) a cichlid appearing and then moving out of view; (5) the cichlid re-appearing, pursuing and capturing the swordtail; and (5) the cichlid swimming out of view. All of the attacks on the swordtails were successful, but differed slightly in the approach behavior of the cichlid and to a greater degree the attack behavior of the cichlids. Strikes were exhibited on the caudal fin and the flank, but not the head. Two of the cichlids (the *P. splendida* and the largest *C. octofasciatum)* struck the male and swallowed it whole, without noticeable handling after capture, or, escape of the male from the mouth of the cichlid. Handling time for the remaining cichlids included repositioning the male within the mouth. One *C. octofasciatum* (standard length = 153mm) made multiple strikes at the elongation component of the sword, damaging or tearing off the sword, and eventually severing the caudal peduncle before capturing the male. Using iMovie, the behavior sequence for each of the five cichlid film stimuli was flipped on the vertical axis so that the cichlid exited and re-entered the recording field from both the left and the right side. This processing resulted in predation event stimuli with smooth transitions between clips, and with all predatory behaviors presented an equal number of times on each side. The five resulting stimuli will hereafter be referred to as the predation stimuli. Five predation exemplars of predator sequences were created to represent potential phenotypic variation in wild populations.

The no predation sequences were also shot in the filming tank; the steps were identical to those followed to film the predation sequences, except no fish were present in the tank. Using iMovie, the video footage was linked to construct five 10-min, no predation stimuli. The five resulting stimuli will hereafter be referred to as no predation stimuli.

Each of the 10 stimuli was looped to produce 20-min presentation stimuli. Treatment 1, the predation presentation stimuli, consisted of a 10-min large cichlid-swordtail encounter sequence culminating in capture of the swordtail, followed by a 10-min predator absent sequence. The five presentation stimuli constructed in this way were used for Treatment 1 – predation environment.

For Treatment 2, the no predation treatment, each no predation presentation stimulus consisted of a 20-min sequence of a tank devoid of fish. The five presentation stimuli constructed in this way were used for Treatment 2 – no predation environment.

Effect of Predation on Male Activity

Before each trial, a male was placed in the center of the test tank, given a red Tetramin flake (to ensure that males were not hungry), and allowed 20 minutes to acclimate. The males had access to the entire testing area during this time. During the acclimation period, the monitor in the test chamber displayed an aquatic environment devoid of fish. After the 20 min acclimation period, the trial was initiated when the male entered the half of the testing area nearest the monitor. One of two presentation stimuli (predation environment or no predation environment) was then displayed on the monitor (either a no predation stimulus or a predation stimulus) for 10 minutes (exposure period). Following this 10-min presentation stimulus sequence, a 10-min sequence of a tank devoid of fish (post-exposure period) was presented. Males that spent greater than 80% of a trial in one end of the tank were considered to have a side bias. Of the 50 males tested, four showed a side bias (no predation n = 3, predation n = 1) and were not included in the analysis.

The amount of time that males spent in each of the three zones, both during the exposure period and during the post-exposure period, was quantified. The number of rectangles on the grid that the male moved between was also quantified for both during and post-exposure time periods. In addition, the amount of time the males spent in the refuges in the three zones was quantified. Males were determined to have used a refuge if the eye of the male was within the plant material for greater than three seconds. Refuge time constitutes time spent in a zone of the tank as well, thus is included in the analyses of zone time.

In the predation treatment, stimuli was constructed so that the male swordtail was alone on screen for a period of time, the cichlid was on screen for a period of time, and both the male and the cichlid were on screen together for a period of time. In this treatment, the proportion of time males spent in the area of the scoring grid nearest the predator was quantified and compared for two sequences: 1) the time a cichlid, prior to the appearance of the swordtail (cichlid-alone sequence), was displayed on the monitor; 2) the time an actively attacking predator sequence (in which the cichlid attacked, caught and consumed a swordtail) was displayed on the monitor. The proportion of time males spent in the area of the scoring grid furthest from the predator was also quantified for the same two sequences. All time and movement data was quantified using the Basolo lab computer program Udon 2.0. Males were only tested on one of the two treatments and were never tested twice. At the completion of a trial, the test male was returned to his home tank, and the test tank was drained, sprayed with ethyl alcohol (200 proof, denatured), wiped down and refilled for the next trial.

Males were randomly assigned to treatment (n=26 for No predation; n = 24 for Predation). Standard length of the males did not differ between the two treatments (Predation: $\bar{x} = 45.10$, range 35.1 – 54.3 mm; No predation: $\bar{x} = 44.27$, range = 35 – 57.8 mm; $t_{48} = -0.948$, $p = 0.348$). Trials were run between 1000 and 1700 hrs.

ANALYSES

Effect of Predation on Male Activity

A generalized linear model with maximum likelihood estimation and negative binomial errors was used to determine if treatment and time period had an effect on general activity (male activity being measured as the number of unique movements between the 12 rectangles delineated at the bottom of the tank). The fixed factors were treatment (no predation vs. predation), time period (during exposure vs. post-exposure) and standard length. All possible interactions were included in the model. Male identity was included as a random factor.

A generalized linear model with maximum likelihood estimation and negative binomial errors was used to determine if treatment had an effect on the amount of time males spent in the area of the scoring grid nearest the monitor (zone 1). The fixed factors were treatment (no predation vs. predation), time period (during exposure vs. post-exposure) and standard length. All possible interactions were included in the model. Male identity was included as a random factor. We also used a generalized linear model with maximum likelihood estimation and negative binomial errors to determine if treatment and time period had an effect on the amount of time males spent in the area of the scoring grid furthest from the monitor (zone 3). The fixed factors were treatment (no predation vs. predation), time period (during exposure vs. post-exposure) and standard length. All possible interactions were included in the model. Male identity was included as a random factor.

In order to determine the latency for a male to approach the area of the scoring grid next to monitor following the exposure period, a one-way between subjects ANOVA was used to compare the differences in time between the two treatments.

One-way between subjects ANOVAs were used to look at the difference in the amount of time males spent in the refuges within a treatment during the exposure period and the post-exposure period. Between treatments independent *t*-tests were used to look at the difference in the amount of time males spent in the refuges in the three zones.

All statistical analyses were performed using the software package SPSS v.19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

RESULTS

Effect of Predation on Male Activity

A generalized linear model with negative binomial errors was used to analyze the activity of large and small males. The three-way interaction between treatment, time period, and standard length was not significant ($F = 0.089$, $p = 0.765$) and was dropped from the model. There was a significant two-way interaction between treatment and standard length (Table 1.1). For the no predation treatment, smaller males showed more movement than larger males (Fig. 1.2A). However, for the predation treatment, there was no difference in the amount of movement exhibited by large and small males (Fig. 1.2B).

The effect of predation on the time spent in the area of the scoring grid nearest the predator (zone 1) was examined using a generalized linear model with negative binomial errors with treatment, time period, and standard length as the fixed factors. The three-way interaction between treatment, time period, and standard length was not significant (F = 0.774, p = 0.381) and was dropped from the model. There was a significant two-way interaction between treatment and time period (Table 1.2). During the exposure period, the males in the no predation treatment spent more time near the video stimuli than the males in the predation treatment (Fig. 1.3). During the post-exposure period, there was no difference between the treatments in the amount of time spent in the area of the scoring grid nearest the monitor (Fig. 1.3).

The effect of predation on the time spent in the area of the scoring grid furthest from the predator (zone 3) was examined using a generalized linear model with negative binomial errors with treatment, time period, and standard length as the fixed factors. The three-way interaction between treatment, time period, and standard length was not significant (F = 0.074, p = 0.786) and was dropped from the model. There was a significant two-way interaction between treatment and time period (Table 1.3). During the exposure period, the males in the predation treatment spent more time in the area of the scoring grid furthest from the monitor than males in the no predation treatment (Fig. 1.4). However, this effect was not permanent, as the males in the predation treatment recovered quickly, no longer remaining in the area of the scoring grid furthest from the predator.

During the predation treatment, the time males spent in the zone of the scoring grid nearest to a cichlid prior to the appearance of the video swordtail that is eventually preyed upon (the portion of the predation stimuli in which only the cichlid has been displayed, not the swordtail that will be attacked and eaten) compared to the portion of the stimuli showing an actively attacking predator (the portion of the predation stimuli in which the cichlid attacks, catches and consumes a swordtail) did not differ (independent-$t_{44} = -0.142$, $p = 0.888$). During both sequences, males spent a greater proportion of time in the area of the tank furthest from the monitor (cichlid alone one-way ANOVA $F_{2,66} = 36.133$, $p < 0.001$; predation event one-way ANOVA $F_{2,66} = 48.453$, $p < 0.001$). Similarly, there was no difference in the amount of time spent in the zone of the scoring grid furthest away predator (zone 3) by males between the portion of the stimuli of a cichlid prior to the appearance of the swordtail that is eventually preyed upon (cichlid-alone sequence) compared to the portion of the stimuli showing an actively attacking predator (independent-$t_{44} = -0.910$, $p = 0.368$).

Following the exposure time period, the latency to enter the area of the scoring grid nearest the monitor was compared between the two treatments. There was no significant difference between the treatments in the latency to enter zone 1 (One-way ANOVA $F_{1,41} = 1.347$, $p = 0.252$). Three males did not move following the exposure treatment and thus were excluded from the analysis (n = 2 predation males, n = 1 no predation male). Following a predation event, males were not hesitant to approach the monitor, suggesting that males are cautious when a predator in nearby, but exhibit exploratory behavior in the area nearest to which a predator was recently present.

During the exposure period, males in the predation treatment spent significantly more time in the refuges in the third of the tank furthest from the monitor (zone 3) than males in the no predation treatment (independent-t_{46} = -2.278, p = 0.027). There was no difference between the treatments in the amount of time spent by males in the refuges nearest the monitor (independent-t_{45} = 1.167, p = 0.249) (Fig. 1.5) (although, as males that did not see a predator utilized refuges very rarely, this may not be biologically relevant). Thus, when a predator is present, males use the refuges that provide the greatest distance between the male and the predator. There was no effect of size on refuge use ($F_{1,46}$ = 0.983, p = 0.327).

In the no predation treatment, there was not a significant difference in the time males spent in the refuges between the three zones during the exposure time period (One-way ANOVA: $F_{2,135}$ = 1.218, p = 0.299) or the post-exposure time period (One-way ANOVA: $F_{2,135}$ = 0.368, p = 0.693). In the predation treatment, there was a significant difference in the time males spent in the refuges between the three zones during the *exposure period* (One-way ANOVA: $F_{2,135}$ = 3.89, p = 0.023); males spent significantly more time in the refuges furthest from the monitor. However, during the *post-exposure period* there was no difference in the time spent in the refuges between the zones (One-way ANOVA: $F_{2,135}$ = 1.003, p = 0.370). Once the predator was no longer visible (had swum off screen), males did not differ in the time spent in the refuges in the three zones. This would suggest that once the predators have departed males do not feel threatened, or gaining information on the presence of the predator is more beneficial to the male then remaining in a refuge. There was no effect of size ($F_{1,44} = 0.975$, $p = 0.329$).

DISCUSSION

In the absence of a predator, smaller male swordtails were more active than larger males, but reduced their activity in the presence of a predator. Larger males maintained lower activity levels, regardless of predation environment. These results suggests that in a novel environment, larger males are more risk-sensitive overall than smaller males. There are multiple explanations for this result. First, larger males may be more cautious all of the time because their more conspicuous size and secondary sexual traits, *e.g*. the colorful male sword, likely makes detection of them by cichlid predators easier. Second, cichlids may disproportionately attack larger males because they provide more energy gained per capture (assuming that a given predator is large enough to handle and consume a large swordtail) (Emlen, 1966). Finally, as larger fish are less maneuverable than smaller fish (Domenici 2001), larger swordtails may generally be less active because if detected, they may not be able to escape as well as smaller males. In addition, the results of the study also suggest that small males are more behaviorally plastic than larger males. First, smaller males have higher relative energetic costs relative to their body size than larger males (Brett 1979, Bennett & Harvey 1987), thus in order to meet their energetic needs, they may need to seek foraging opportunities earlier than larger males. In this study smaller males showed greater movement than larger male, possibly due to smaller males requiring a foraging opportunity to deal with the energetic costs of being smaller. Second, smaller males may be more motivated to find females in the absence of predators. In a natural setting, male green swordtails will defend feeding areas or areas with females (Beaugrand *et.al*. 1984). Smaller males may not be as able to defend these areas as well as larger males, therefore they may search for areas in which females are present but not guarded by larger males. Smaller males are not preferred by females when compared to larger males, and larger males can defend females, so smaller males must search more for females in order to gain mating opportunities. This study adds to the body of evidence (Werner *et. al*. 1983; Dewitt *et. al*. 1999) that body size can mediate the expression of anti-predator behavioral plasticity. However, this is one of the few studies, which examines body size related expression of anti-predator behavioral plasticity in spontaneous activity levels.

The spatial distribution of males who witnessed a predation event differed from that of males that did not; males who witnessed a predation event spent more time in the zone furthest from an actively hunting predator compared to those males who did not witness an actively hunting predator. Once a predator had departed (swam off-screen for the final time), however, there was no difference between the treatments in terms of which zone males occupied. One explanation for this result is that males that were exposed to a predator were aware of the higher threat of predation when a predator was present, and therefore exhibited anti-predator behavior by avoiding the area in closest proximity to the predator. However, the avoidance of this area was not permanent; within 30 seconds of the final departure of the predator, males resumed active movement throughout the experimental environment. Although no foraging opportunities were available during the testing period in our swordtail study, this does not necessarily mean that the males stopped looking for foraging opportunities during the trials, particularly as they successfully foraged in the area when they were introduced to the tank (the food was exhausted during the acclimation period prior to the start of a trial). Habitat use by individuals can be strongly influenced by predation (Lima & Dill 1990). When the habitats that offer the best foraging opportunities are also the most dangerous, foragers must make a trade-off between avoiding predators and foraging. For example, tiger salamander larva, *Ambystoma tigrinum nebulosum*, are influenced by the presence of predatory diving beetles (Holomuzki 1986). In the absence of beetles, the salamander larva forage in vegetative shallows both day and night. In the presence of beetles, which forage only at night in the shallows, salamanders shift their activity to deep pelagic water, which is less energetically profitable, but presumably are safer in terms of predatory beetles. In our swordtail study, male swordtails also made a shift; males altered their habitat use when predation risk was high in order to maintain the greatest distance from the predator. But, after a predator had departed, the males actively utilized the area that had been nearest the predator. Males might also have entered the area nearest where the predator had been to seek information to determine whether the predator was still nearby. The swordtail males in the current study had never observed a predator before, thus the predation event was a novel, rare occurrence for them. When the presence of a predator is brief and infrequent, individuals are expected to exhibit the greatest amount of anti-predator behavior when predator is present (Lima & Bednekoff 1999) because the cost of not performing other behaviors (foraging, mating, *etc*.) can be made up in the periods of time when the predation risk is lower. The novelty and infrequence of predation events for the male swordtails in this study could explain the reduction of spatial distribution by males to the area of the tank furthest from a predator.

Male swordtails showed a similar response to a predation event as female swordtails in regards to their spatial distribution during the event (Thesis Ch. 2 *The effect of the presence of a predator on female behavior*) in that they both occupied the area of the tank furthest from a predation event. This suggests that both sexes recognized the danger of a successful hunting predator, and that putting distance between self and a predator would allow swordtails to gain information about the predator without having to get close to it and being attacked.

Within each of the three zones of the test tank, two refuges were available to males. Therefore, we also assessed spatial distribution of males in regards to refuges and their distance to the monitor. We found that males who witnessed a predation event used refuges that were furthest from the predator, but expressed differential use of refuges after the predator had departed. Animals balance the costs of remaining in refuges, *e.g.* loss of foraging and mating opportunities, with the benefits associated with staying in a refuge, *e.g.* predator avoidance (Sih 1997). Some prey emerge from their refuge within minutes after exposure to a predator (Waite & Grubb 1987), while others may take hours or even days to resume normal activity (Rahel & Stein 1988). In our study, males used the refuges furthest from the predator, but if in a refuge when a predator departed, they left it within 20 seconds. This suggests that there could be trade-offs between staying in a refuge at some distance from a predator, and leaving the refuge to gather more information about the predator's location (Frommen *et al.* 2009). For example, males may leave a refuge in order to assess the current motivational state of a predator; because male swordtails have witnessed the predator eating a swordtail, they may exit the refuge in order to determine whether the predator is still actively hunting.

Prey activity patterns have long been a topic of interest (Darwin 1871), and there have been an increasing number of studies on size specific differences in behavioral plasticity. Here we conducted a study to investigate the potential for predator-induced size-specific behavioral plasticity in the green swordtail. We found that smaller male swordtails show greater plasticity in general activity than larger males. We did not, however, find that there was size-specific use of refuges or of the distance a male from a predator while the predator was visibly present. In fact, males used refuges very little in the absence of a predator. We also investigated the spatial distribution of the males in the presence of a successful predator. In a result that was similar to female swordtails, males occupied the area of the tank that gave them the greatest distance from the predator. Predation may influence many aspects of an individual's life including morphology and behavioral responses (Lima & Dill 1990). Prey often have limited information on a predator's location or motivational state, and behavioral plasticity allows prey to quickly respond to changes in predation risk.

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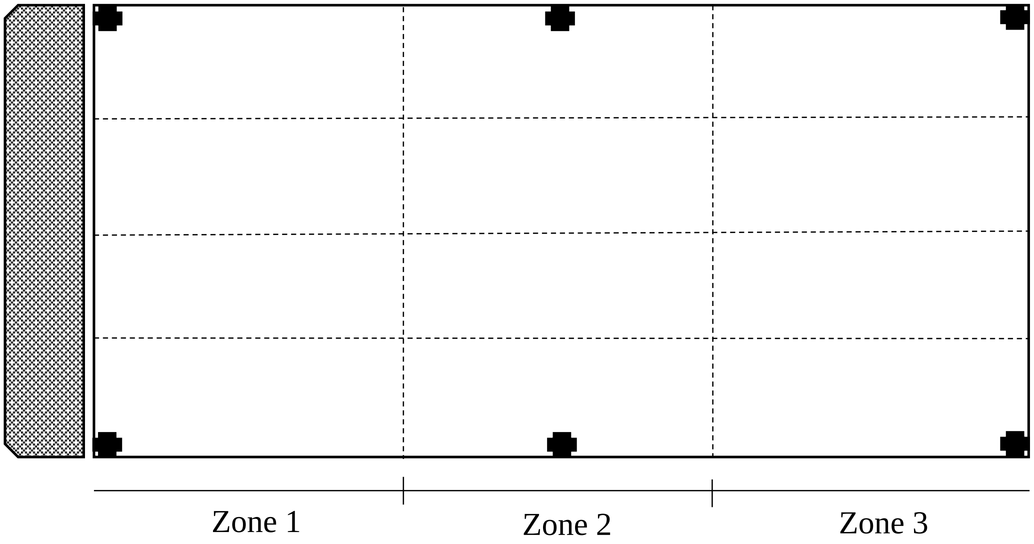

Fig. 1.1. Experimental set-up to investigate male activity. A monitor (shaded gray) was positioned at one end of the tank. During a trial, the monitor displayed one of the two treatment video stimuli. The black symbols represent position of the refuges.

Table 1.1. Linear mixed model examining the effects of the fixed factors treatment (predation vs. no predation), time period (during exposure vs. post exposure), and male standard length on male movement. Male identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	-363.06	130.74	7.712	0.007
Time Period	51.55	126.55	0.166	0.685
Standard length	-9.94	5.78	2.957	0.089
Treatment * Time Period	16.20	33.53	0.233	0.630
Treatment * Standard length	**6.39**	**2.67**	**5.703**	**0.019**
Time Period * Standard length	-1.08	2.65	0.167	0.684
Random Effect	*Estimate*	*SE*		
Male	5.43	8.21		

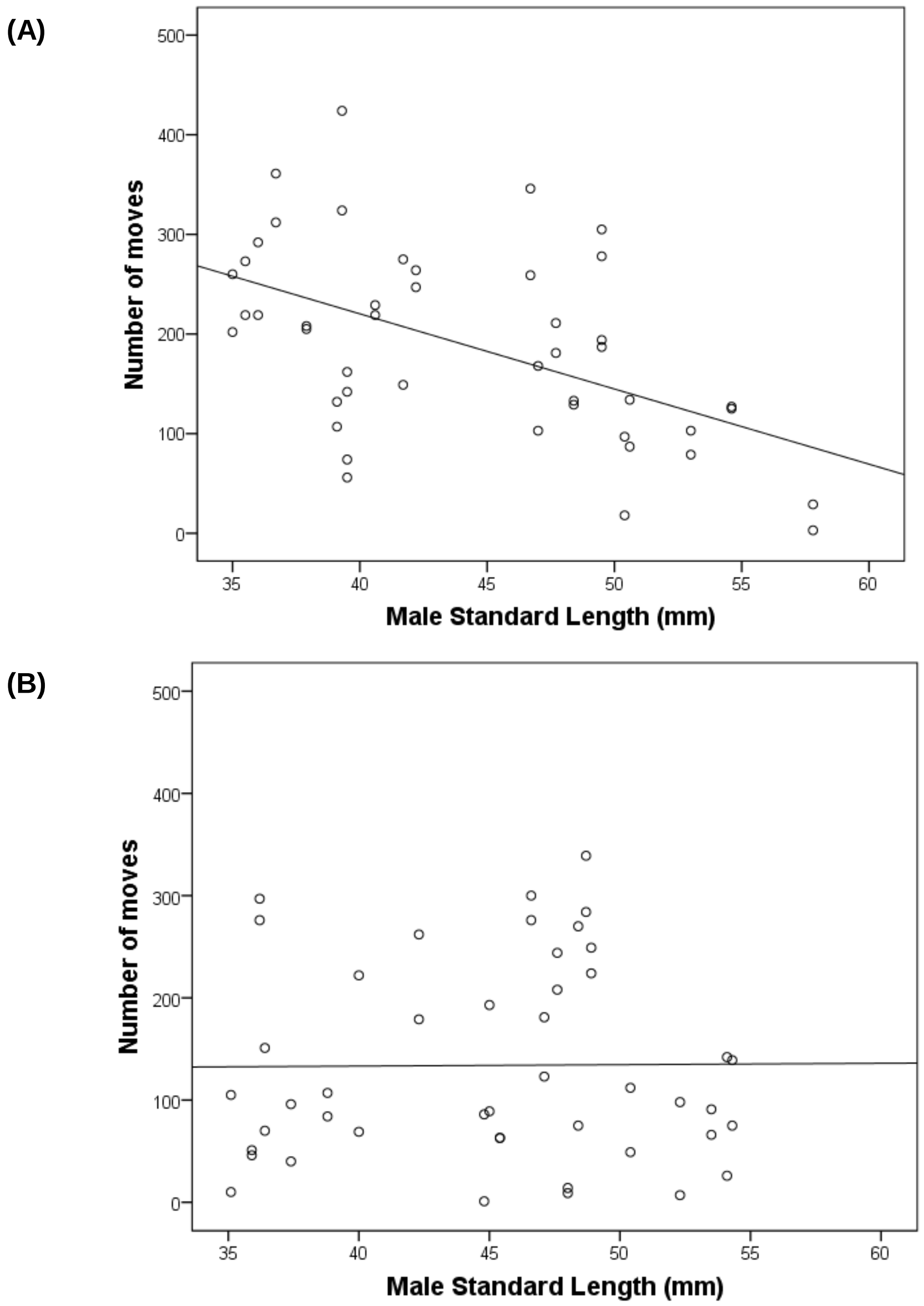

Fig. 1.2. The effect of standard length on the movement of males for the two treatments. (A) No predation treatment ($R^2 = 0.273$, $F_{1,44} = 16.56$, $p < 0.001$). (B) Predation treatment ($R^2 = 7.697E\text{-}5$, $F_{1,44} = 0.003$, $p = 0.954$).

Table 1.2. Linear mixed model examining the effects of the fixed factors treatment (predation vs. no predation), time period (during exposure vs. post exposure), and male standard length on time spent in zone 1 by males. Male identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	-111.73	221.23	0.255	0.615
Time Period	6.27	214.27	0.001	0.977
Standard length	-6.70	9.77	0.470	0.495
Treatment * Time Period	**138.01**	**56.76**	**5.915**	**0.017**
Treatment * Standard length	-3.44	4.52	0.577	0.450
Time Period * Standard length	-2.79	4.48	0.388	0.535
Random Effect	*Estimate*	*SE*		
Male	5.57	9.45		

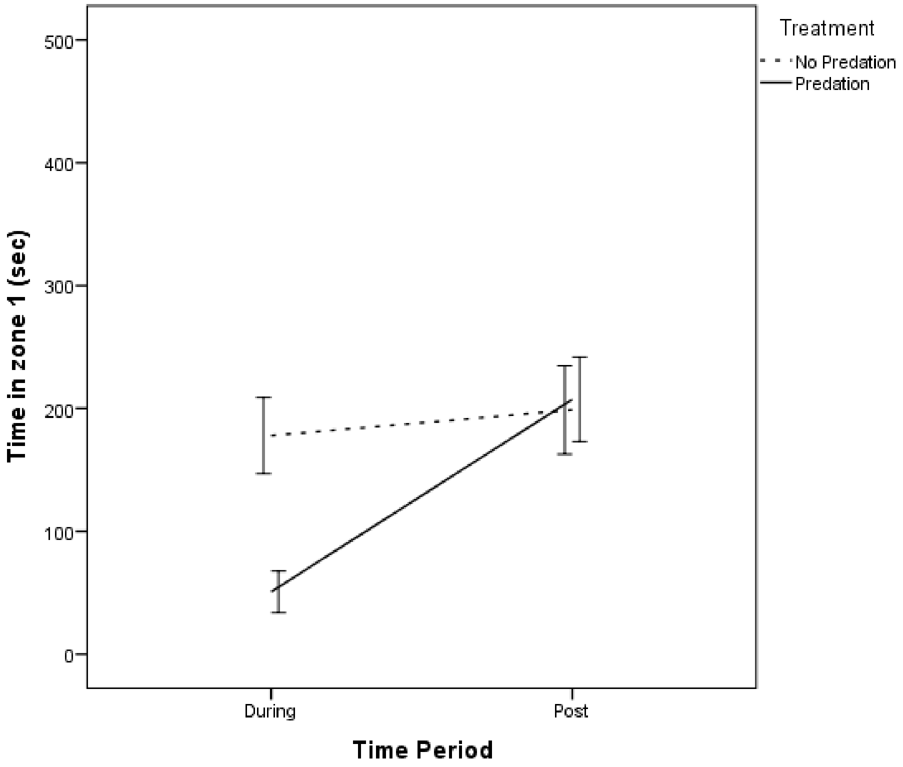

Fig. 1.3. Effect of treatment and time period on the amount of time males spent in zone nearest the predator (zone 1). Values are means +/- SE.

Table 1.3. Linear mixed model examining the effects of the fixed factors treatment (predation vs. no predation), time period (during exposure vs. post exposure), and male standard length on time spent in zone 3 by males. Male identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	796.11	245.88	10.483	0.002
Time Period	177.37	239.37	0.549	0.461
Standard Length	10.55	10.79	0.957	0.331
Treatment*Time Period	**-236.49**	**63.43**	**13.901**	**<0.001**
Treatment* Standard Length	-7.76	5.02	2.392	0.125
Time Period* Standard Length	1.59	5.01	0.101	0.751
Random Effect	*Estimate*	*SE*		
Male	0.000*	0.000		

*This covariance parameter is redundant

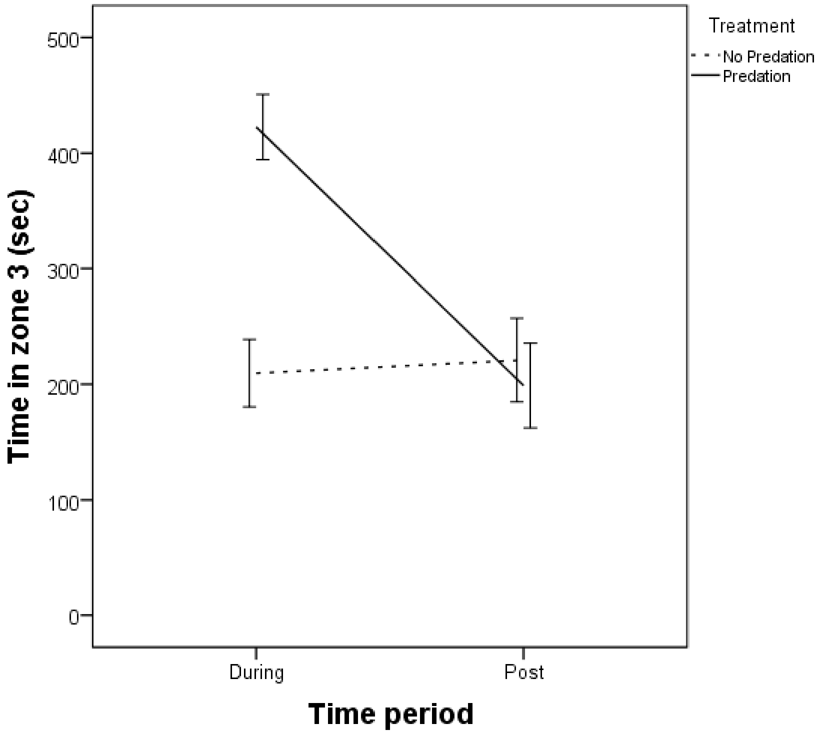

Fig. 1.4. Effect of treatment and time period on the amount of time males spent in zone 3.

Values are means +/- SE.

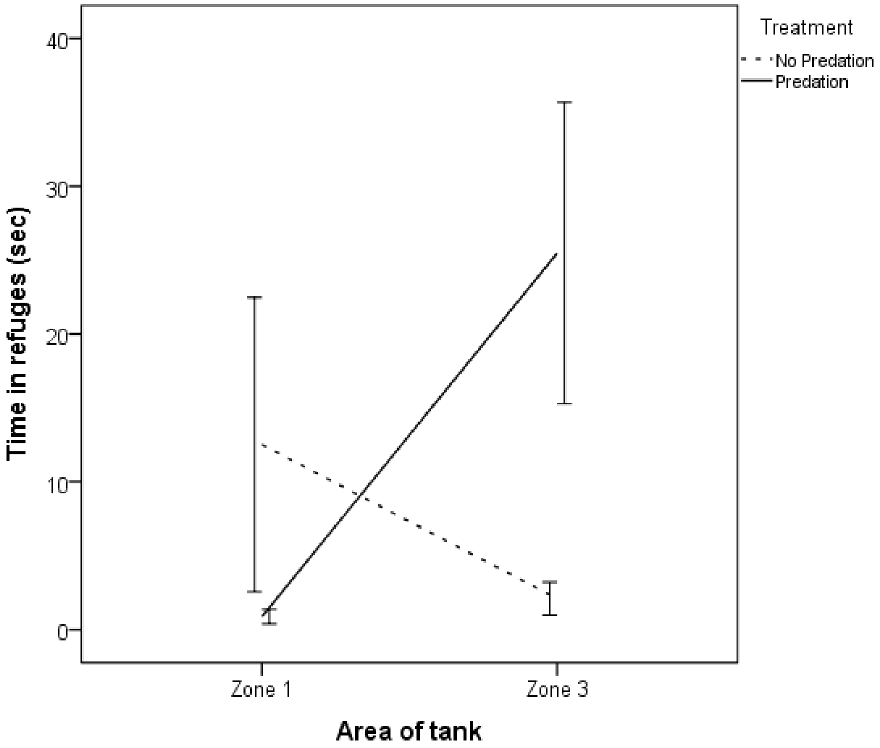

Fig. 1.5. Effect of treatment on refuge use by males during the exposure period. Values are means +/- SE.

Chapter 2. Plasticity in a Preexisting Mating Bias in Response to Variation in the Predation Environment

Abstract - Female green swordtails, *Xiphophorus helleri*, prefer males with long swords to males with short swords. Phylogenetic information suggests that this preference arose from a preexisting bias favoring a sword, yet the preference is plastic; when a predator is not present, females prefer longer swords, but after witnessing a predation event on a long-sworded male, they do not prefer longer swords. In this study, we examined whether different predation environments differ in their effect on female responses to the sword. Unlike Johnson & Basolo 2003, each stimuli present had a predator present. We used a video playback experiment to evaluate female mate choice between two simultaneously displaying males differing in sword length. We recorded female responses both prior to and after exposure to either large cichlid predators chasing and consuming a male swordtail with a short sword, large cichlid predators with no predation event, or small cichlid predators with no predation event. We found that not only did females reduce their mating response to a long-sworded male, they actually switched their preference to a short-sworded male, regardless of which type of predator was shown. This result likely represents a trade-off made by females between reducing predation risk while still finding a male with which to mate. We also looked at the lasting effect of predation environment on the response to the sword by recording the responses of females to males one day later. We found that the preference for the short-sworded male persisted to the following day, suggesting that not only is there plasticity in the sword bias, but also that the memory of past predation can have a residual effect on the bias. Finally, we addressed whether females

respond differently to predation environments, which differ in perceived predation risk, in a non-mating context. Females did not differ in their anti-predator response to large cichlid predators, regardless of whether the cichlid was consuming a conspecific or not, but differed in their response to small cichlids. Females perceived large cichlid predators alone to be just as dangerous as successful predators, while small cichlids may be too small to represent an immediate threat. To our knowledge, this study is the first to demonstrate enduring plasticity in a receiver bias.

INTRODUCTION

Darwin observed that males in many species have conspicuous characteristics that appear to reduce their survival (1859). His solution to the problem of why males have such traits was that the traits that reduce an organism’s survival can evolve if they increase reproductive success enough to outweigh the cost of having them. He proposed two mechanisms for sexual selection: intersexual selection (mate choice) and intrasexual selection (members of one sex compete for access to other sex). Four primary models have been proposed to explain the evolution of male traits via female mate choice (Andersson & Simmons 2006), but this paper will focus on the preexisting bias model, which proposes that the sensory system (or brain) of females has biases that result in preferences for a particular male trait or set of traits. Thus, male traits currently under sexual selection may have evolved due to innate biases in females (Basolo 1990, Endler & Basolo 1998).

Although females may have preferences for specific male traits, current environmental conditions may influence whether or not a preference is expressed, as well as the degree of expression. Such plasticity in female behavior may evolve when there are higher costs of expressing a preference under some environmental conditions compared to others. When organisms experience fluctuating environmental conditions, selection may favor plasticity in a trait(s), *i.e.*, change in form, state, movement, or rate of activity (West-Eberhard 2003). Within related animal taxa, there can be variation in the degree of plasticity in traits. For example, male bird song is fixed in some birds, but shows plasticity in song development and learning in others, in particular the passeriformes (Beecher & Brenowitz 2005). White crowned sparrows are fixed for song vocalization, even when experimentally deafened early in life prior to the onset of song practice, or are not permitted to hear a song tutor (Kroodsma & Konishi 1991). Conversely, male great tits are capable of learning the songs of neighbors throughout their lifetime (McGregor & Krebs 1989). Plastic behavioral responses may allow organisms to adaptively shift the expression of behavior as environmental conditions change. Plasticity can operate in non-sexual selection contexts, but has also been found to operate in sexual selection contexts. With sexual selection in particular, females may benefit by modulating the expression of mating preferences based on differing predation conditions (Pfennig 2007; Johnson & Basolo 2003; Willis *et al*. 2012). When the predation environment is constantly changing, selection may favor plasticity in the expression of pre-existing biases affecting mate choice, thereby reducing predation risk.

When males have sexually selected traits that increase their conspicuousness to predators, female risk of predation may also increase due to association with males. If so, females may modulate their response to males with conspicuous traits, based on the current predation environment. That is, if predators are currently active, a female may not choose to associate with males that are conspicuous as this might attract a predator's attention, which could then lead to a greater chance of the female being attacked (Pocklington & Dill 1995). Males with more elaborate and costly displays have been found to experience greater predation risk than those with less elaborate displays (Endler 1980; Hedrick 2000). For example, in chuckwalla populations with high levels of predation, males exhibit less conspicuous, sexually selected coloration than males in populations with low levels of predation (Kwiatkowski 2003), thus conspicuous coloration appears to be negatively associated with predation. Traits favored by female mate choice that increase male conspicuousness to predators could increase female predation risk as well, due to costs of associating with conspicuous males (Hedrick & Dill 1993; Pocklington & Dill 1995; Martin & Wagner 2010). However, if a female mating response is plastic, females may not exhibit preferences (or exhibit them to a lesser degree) when risk is high (Forsgren 1992). Other studies investigating the effect of predation on mate choice have also found facultative decreases in receiver responses with an increase in predation risk (Forsgren 1992, Gong & Gibson 1996). When predation risk is great, females may facultatively shift which male they prefer from a more conspicuous to less conspicuous male.

Examining the effects of predation on female responses is a rising area of interest, yet we know little about how individuals adjust their responses to varying levels of predation. Evans *et al.* (2002) noted that female guppies change their behavior after seeing a predator, either becoming sexually unresponsive or reducing expression of preferences. In another study of guppies, predatory pike cichlids were given mixed-sex pairs of guppies to determine which sex was targeted first. Males are the more brightly colored sex, yet females were more likely to be attacked first, even though the capture rates and handling time of the sexes were equivalent (Pocklington & Dill 1995). Therefore, in the face of predation, female guppies would be expected to associate with the less conspicuous male to decrease the risk of an attack by a predator, as less conspicuous males would be less likely to attract the attention of a predator. In Atlantic mollies, females reversed their initial preference for larger males to smaller males in the presence of a predator (Bierbach *et al*. 2011). As predation risk can vary temporally, we might expect female responses that increase predation risk to also vary temporally.

Female green swordtails, *Xiphophorus helleri*, show a preference for males with conspicuous swords; they prefer longer to shorter swords (Basolo 1990a, 1998). The sword preference appears to be at least partially based on a pre-existing bias (Basolo 1990b). Males in populations that are sympatric with predatory fish have relatively shorter swords than males in populations without predatory fish (Basolo & Wagner 2004). This could suggest that, all other things being equal, there is a higher relative cost of predation for longer sworded males. The expression of the female sword preference in *X. helleri*, however, can be modulated, based on a change in the predation environment; after viewing a predation event on a long-sworded male, females no longer exhibited a preference for a long sword (Johnson & Basolo 2003). This result could be morph specific alteration in the preference with the female adjusting the expression of the sword preference due to the length of the sword of the male being consumed. The length of the sword being consumed could be a cue to the female to avoid males with similar sword lengths. The preference for a sword shows plasticity, depending on whether a female has witnessed a predation event or not, however, it is unclear as to whether predation environments differing in perceived predation risk result in different female responses, *i.e.*, females may respond differently to a small predator compared to a large predator. In Johnson & Basolo (2003), one stimuli contained a predator while one stimuli did not. In the current study, all of the stimuli had a predator present. It is also unclear as to whether the modulation of the bias is permanent, *i.e.*, the preference is extinguished, never again to be exhibited by the female, or the change is only temporary. Here we address three *main* questions regarding female plasticity in the sword response. First, do different predation environments differ in their effect on the sword response? Second, can a reversal in the sword preference (change from preferring males with long swords to males with short swords) occur after females have experienced a predator? Third, when there is a change in the sword preference in response to predation, is it lasting? Finally, in a non-mating context, do females respond differently to different types of predators?

METHODS

Study System

The green swordtail, *Xiphophorus helleri*, is a freshwater, live-bearing fish in the subfamily Poeciliinae (Parenti & Rauchenberger 1989). Subjects in this study were third generation female descendants of fish caught from Savannah Stream near mile marker 28 on the Western Highway, Belize, C.A. Female test subjects had been separated from their brothers at birth and reared individually in 5.68l tanks to maturation. From birth until testing, females were maintained on a 12:12 h light-dark cycle at 24^0C, and were fed ad libitum daily (live brine shrimp nauplii in the morning and flakes in the late afternoon). All females were naïve in relation to predators.

Predatory cichlids, *Cichlasoma octofasciatum* and *Petenia splendida,* were collected from field locations in which they are sympatric with green swordtails, to use for this study. Both of these cichlids are predators of smaller fishes (Konings, 1989; Conkel, 1993; Greenfield & Thomerson, 1997), and easily capable of capturing and consuming adult *X. helleri* in the lab (personal obs.).

Experimental Protocol

Many researchers have successfully used video in behavioral studies (fish: Rosenthal & Evans 1998; Basolo & Trainor 2002; Johnson & Basolo 2003; spiders: Clark & Uetz 1990; lizards: Clark *et al.* 1997). Previous studies with adult female green swordtails have shown that video playback of courting males elicits female mating responses like those elicited towards live swordtail males (Trainor & Basolo 2000). In this study, we used male video stimuli per Basolo & Trainor (2002) to test the effects of different predation environments on the female preference for males with long swords (Basolo 1990a, 1998). Additionally, we investigated whether there are lingering effects on the female sword response as a result of exposure to a predator. Specifically, females were presented with videos of two males differing in sword length (see Trainor & Basolo 2000 for full description of stimuli construction). Video stimuli consisted of males exhibiting mating behavior with a sword that had been digitally elongated to 133% of its original length (sword length = 54.00mm), and the same courting male with a sword that had been digitally altered so that it did not extend beyond the caudal margin (sword coloration components only-no elongation component). Footage of each courting male was looped to create a 20-minute video sequence. The females were presented with a male pair differing in sword length both prior to and directly following exposure to a predator (described below). For predator exposure, we created three types of 10-minute video sequences designed to expose test females to differing levels of perceived predation risk: (1) a high risk predator sequence in which a large cichlid predator (SL = 141 mm – 201 mm) chased and captured a male *X. helleri* with a short sword (sword length = 28.5mm) (hereafter referred to as the predation treatment); (2) an intermediate risk predator sequence in which a large cichlid predator (range in SL = 141 mm – 201 mm) swam in an area devoid of other fish (hereafter referred to the large cichlid treatment); and (3) a low risk predator sequence in which a small cichlid predator (range in SL = 92 mm – 98 mm) swam in an area devoid of other fish (hereafter referred to the small cichlid treatment). (Note: The body length measure that we used was standard length (SL), measured from the anterior tip of the mouth along the lateral line to the point immediately posterior to the caudal vein per the original experiment testing for female preference for the sword (Basolo 1990a).

Cichlid Stimuli Construction

Cichlids used in making the video stimuli included large *C. octofasciatum* (SL: 141–176 mm; n = 4), small *C. octofasciatum* (SL: 92–98 mm; n = 5) and a large *P. splendida* (SL: 201 mm; n =1).

Three types of predator stimuli were constructed for this study: (1) a large cichlid swimming, and then capturing and consuming a swordtail = predation; (2) a large cichlid swimming in the absence of a swordtail = no predation; and (3) a small cichlid swimming in the absence of a swordtail = no predation.

To construct a predation event stimulus, a large predatory cichlid was removed from its home tank and placed in a filming tank (61 x 30.5 x 40.5 cm) to acclimate for a five-day period. The filming tank was divided such that one-fourth was a temporary predator "home" area, and three-fourths a recording area. An opaque divider separated the two areas. This divider had an entry/exit hole that allowed the cichlid to pass between the two areas. The filming tank was surrounded with blue felt and contained a short length of pipe (like that in the cichlid's home tank from which it was temporarily moved for filming). (In their home tanks, cichlids often remain in their pipe when they are not actively foraging.) Two 60cm Vitalites illuminated the filming tank from above. Up to 2 cm of natural gravel (varied shades of tan) covered the bottom of the tank. On a given filming day, the cichlid was sequestered in the home area of the filming tank. A male swordtail (standard length = 43 mm +/- 4.2; sword length = 28.5 mm) was then introduced into the recording area of the filming tank. The partition was removed which allowed the cichlid to move into the recording area and attack the swordtail. The fish were filmed with a Canon Vixia HG20 camera positioned in front of the tank. The entry/exit hole allowed the cichlid access to both the home area and the recording area during this period, allowing subsequent edits of filmed video footage to be seamless.

Video footage was edited using iMovie ’09 (v. 8.0.6) to create a 10-min predation event sequence. This sequence showed the swordtail swimming, moving out of view and then re-appearing, followed by the cichlid swimming, moving out of view and re-appearing, and finally the cichlid pursuing and capturing the swordtail. Using iMovie, the behavior sequence for each of the five cichlid film stimuli was flipped on the vertical axis so that the cichlid could be viewed as exiting and re-entering the recording field from both the left and the right side. This processing resulted in predation event stimuli with smooth transitions between clips, and with all predatory behaviors presented an equal number of times on each side. The five resulting stimuli will hereafter be referred to as high predation risk stimuli. It is interesting to note that attacks on the swordtails were ultimately successful, but differed slightly among cichlids. Two of the cichlids (the *P. splendida* and the largest *C. octofasciatum)* struck the male and swallowed it whole, without exhibiting handling time after capture, or escape of the male from the mouth of the cichlid. Handling time for the remaining cichlids included repositioning the male within the mouth. One *C. octofasciatum* (SL = 153mm) made multiple strikes at the elongation component of the sword, eventually severing the caudal peduncle before capturing the male. However, strikes of all cichlids were aimed at the caudal fin and flank, but not the head.

Sequences in which no predation occurred were also shot in the filming tank; the steps were identical to those followed to film the predation sequences, except that a swordtail was not present. For the large predator-no predation stimuli, the five large cichlids used for the predation sequences were filmed in the absence of a swordtail. These filmed sequences included a cichlid swimming around the tank, and moving out of view and reappearing on both sides of the tank. Using iMovie, video footage was linked to construct five 10-min, large predator-no predation stimuli, one for each cichlid, in which a predator moved about the tank, and swam on and off screen an equal number of times on each side. The five resulting stimuli will hereafter be referred to as large predator-no predation stimuli. For the small predator-no predation stimuli, the five small cichlids were filmed in the absence of a swordtail. These filmed sequences included a small cichlid swimming around the tank, and, moving out of view and reappearing on both sides of the tank. Using iMovie, sequences were linked to construct five, 10-min, small predator-no predation stimuli in which a predator moved about the tank, and swam on and off screen an equal number of times on each side. The five resulting stimuli will hereafter be referred to as small predator-no predation stimuli. Five exemplars of each type of predator stimuli were created to represent potential phenotypic variation in wild populations.

Each of the 15 stimuli were looped to produce three 50-min stimulus types. For Treatment 1, the high predation risk stimuli, consisted of a 20-min predator absent sequence followed by a 10-min large cichlid-swordtail encounter sequence culminating in capture of the swordtail, followed by a 20-min predator absent sequence. For Treatment 2, each large predator-no predation stimulus consisted of a 20-min predator absent sequence followed by a 10-min sequence of a large cichlid swimming around the tank and on and off screen, followed by a 20-min ending with a 20-min predator absent sequence. For Treatment 3, each small predator-no predation stimulus consisted of a 20-min predator absent sequence followed by a 10-min sequence of a small cichlid swimming around the tank and on and off screen, followed by a 20-min predator absent sequence. All stimuli were created under the same environmental conditions in which the female mate choice trials were conducted (as described below).

The test setup was similar to that in Johnson & Basolo with minor differences in the execution of the trials (see page 55 for details). The test tank (Fig. 2.1A) was divided into three equal sections (10 x 30 x 30 cm) by vertical lines made with strips of white tape on the front of the tank glass. The two side monitors (DELL UltraSharp 2005FPW 20.1-inch Wide Aspect Flat Panel LCD Monitor) were used to display the courting males, one with a long sword and one with a short sword, while a rear monitor displayed one of the three predation treatment stimuli. Water in the tank was maintained at 24^0C. Two 40W Vita light^{TM} bulbs covered by a layer of vellum paper lit the tank from above. Three video cameras were used to record female behavior during each trial: one video camera (Panasonic 5100HS WV-PS03) positioned 1.5m in front of the tank to record the female's movement and behavior; one flex camera (Videolabs FlexCam iCam) positioned above the left monitor to record the position of the female in relation to a courting male stimulus when she was on the right side of the tank; and one flex camera was positioned above the right monitor to record the position of the female in relation to the other courting male stimulus when she was on the left side of the tank. A video mixer (Videonics MX-1) and switch box (which allowed a viewer outside of the test chamber to switch the filmed view between the FlexCams to capture the behavior of a test female when she was within 10 cm (equivalent to two to three female standard lengths) of each of the male swordtails differing in sword length) were used to integrate the view of the front camera and one of the side cameras at all times. The experimental setup was housed in a sound damped chamber, and monitoring of the trials was done remotely from a room outside the testing chamber. To prevent chemical cues from a previous test trial affecting the response of the female tested subsequently, the test tank was drained, sprayed with ethanol alcohol (200 proof, denatured), wiped down, and then refilled between trials.

Effect of Predation on Female Mating Behavior

Experimental trials were conducted between 0700 and 1300 h. Each trial included the following steps. First, the three monitors were turned on and set to display an aquatic environment devoid of fishes, and then a female was introduced into the experimental tank for a pre-trial acclimation period. The acclimation period consisted of 34 minutes with no other fish present. This time allowed the female to settle down and explore her new surroundings. This was followed by 10 min with the males present; during this time the female was simultaneously shown the video males (as described above), while the rear monitor displayed a tank environment devoid of fish. If a female exhibited interest in both males during the 10-min of acclimation time in which the males were onscreen, the trial was begun and the female's behavior was recorded for a 10-in period. If a female did not show interest in the males during acclimation, the trial was considered invalid. After the 10-min mate choice period, the side monitors displayed identical tanks devoid of fish, and the rear monitor displayed one of the three treatment stimuli described above for a 10-min period. This was followed by a second 10-min mate choice period.

A total of 34 females were tested: predation video n = 10 (SL x̄ = 43.69 mm; range = 35.5 – 47.3mm); large predator video n=11(SL x̄ = 44.26 mm; range = 39.6 - 48.4mm); small cichlid video n=14 (SL x̄ = 44.96 mm; range = 40.3 – 52.6). The SL of the females did not differ between the treatments (One-way ANOVA: $F_{2,31} = 0.515$, $p = 0.603$).

For both the pre-predator exposure period and the post-predator exposure period, we scored female position: (1) time spent* within 10 cm of the short-sworded male and (2) time spent* within 10 cm of the long-sworded male. *Note: Our measure of female mating behavior was *time* spent by a female exhibiting one or more of the behaviors in the following paragraph towards a male within two body lengths of and attending to that male. Other researchers studying female preferences in poeciliids have used the total amount of time spent by the female in the 1/3 section adjacent to the male as the measure of preference, which usually did not equate to close proximity to the male (Bischoff et al. 1985; Basolo 1995a; MacLaren *et al.* 2004).

We also scored female mating behaviors (Basolo 1990b, 1995b, 2002b) exhibited towards each males as time spent: (1) in close proximity, oriented towards and watching male; (2) approaching male; (3) swimming in unison and parallel with male; (4) darting towards (female quickly swims towards male); (5) quivering (female exhibits a whole-body, rapid shiver); (6) body jerk (female exhibits an isolated spasm); (7) tail flick (quick flick of tail propels female a short distance <2 body lengths from male, resulting in a different orientation from her original position); (8) flank presentation (female presents flank to male); (9) backing (female slowly swims backwards towards male with caudal fin leading body); (10) back away swim (while facing male, female slowly swims backwards, away from him, (trying to get male to follow); and (11) circle back (female facing male swims in a circle away from male and back to facing him). Note: Females responded to male stimuli with mating behavior that is exhibited towards *live* males. A previous paper (Gabor 1999) criticized the use of proximity of a females to a male as difficult to separate from schooling behavior. In our study, however, the responses exhibited by female swordtails towards male stimuli included behaviors not exhibited during schooling.

To determine whether there were lasting effects of encountering a predator, we tested each female the next day (24 +/- 3 hrs later). These Day 2 trials were run in the same test chamber and in the same test tank as the previous day. The female was placed in the tank and given a 34-min acclimation period. Then the behavior towards and amount of time the female spent with each male was recorded for a 10-min period, per the previous day, except the sides on which the males were presented were.

Effect of Predation on Female Anti-Predator Behavior in the Absence of Males

In addition to quantifying female sword responses, we investigated female anti-predator behavior when male stimuli were not present (10-min exposure period). We quantified the amount of time females spent: (1) close to the predator (the 30 by 10 cm area at the rear of the tank (Fig. 2.1B), and therefore nearest to the monitor displaying a predator stimulus); and (2) furthest from the predator (the 30 by 10 cm area at the front of the tank (Fig. 2.1B), and therefore furthest from the monitor displaying a predator stimulus). In addition to this time data, we scored anti-predator event data as the number of following behaviors exhibited towards the predator: (1) approaching a predator; (2) following a predator (female swims parallel with the predator or trails behind the predator within two female standard body lengths); (3) in close proximity, oriented towards and watching a predator; and (4) backing away from a predator (female starts movement away from predator while in the third of the tank nearest the predator).

Note: As briefly discussed in the introduction our Day 1 protocol was similar to that used in a study by Johnson and Basolo (2003), but differed in the following ways: (1) the male swordtails seen consumed in the predation stimuli had a 28.5 mm sword, rather than 54.0 mm sword; (2) three predator stimulus types were used instead of a single predation event stimulus (in order to determine whether it is the actual predation event that alters the females' preference for the sword, or just the presence of a predator); (3) we used pre- and post-exposure times of 10 minutes rather than 20 minutes; (4) the monitors differed; (5) the acclimation period was longer; (6) the male presentations were not switched between sides for the two male presentation periods, thus females were not re-acclimated for the second 10-min mate choice period; instead, we controlled for the possibility of side bias by switching the side on which the male stimuli were presented to females across females (Basolo 1998a); and (7) a Day 2 mate choice trial was included to investigate whether there are lasting effects of different predation environments on female preference that carried over to the second day (see below). Finally, the female response to the predation treatments was examined, *i.e.*, the location of the female in relation to a predator during the exposure period was quantified.

ANALYSES

Effect of Predation on Female Mating Behavior

We first looked at the effect of predation on the female sword preference. We used a generalized linear model with maximum likelihood estimation and negative binomial errors to analyze the data. The fixed effects were time period (pre-exposure, post-exposure), male type, (long-sworded, short-sworded), predation treatment (small predator, large predator, large predator with predation event), all two-way and three-way interactions between these factors, and female standard length. We included female as a random effect to account for the repeated measurement of each female's response (pre-exposure and post-exposure, long-sworded and short-sworded male).

We used a generalized linear model with maximum likelihood estimation and negative binomial errors to look at the effect of experiencing a predator on the female preference for sword length on the second day. The fixed effects were day (pre-exposure day 1, pre-exposure day 2), male type, (long-sworded, short-sworded), predation treatment (small predator, large predator, predation event), all two-way and three-way interactions between these factors, and female standard length. We included female as a random effect to account for the repeated measurement of each female's response (pre-exposure day 1 and pre-exposure day 2, long sworded and short sworded male).

We used a generalized linear model with maximum likelihood estimation and negative binomial errors to look at the *consistency of the effect* of experiencing a predator on the female preference for sword length on the second day. The fixed effects were day (post-exposure day 1, pre-exposure day 2), male type, (long-sworded, short-sworded), predation treatment (small predator, large predator, predation event), all two-way and three-way interactions between these factors, and female standard length. We included female as a random effect to account for the repeated measurement of each female's response (post-exposure day 1 and pre-exposure day 2, long sworded and short sworded male).

We used a generalized linear model with maximum likelihood estimation and negative binomial errors was used to look at the amount of time females spent in close proximity, oriented towards and watching males between the treatments. The fixed effects were time period (pre-exposure, post-exposure), male type, (long-sworded, short-sworded), predation treatment (small predator, large predator, predation event), all two-way and three-way interactions between these factors, and female standard length. Female was included as a random effect.

A between treatments independent *t*-test was used to look at the total amount of time the females spent with males between the time prior to- and post-exposure to a predator.

Effect of Predation on Female Anti-Predator Behavior in the Absence of Males

We used a one-way between subjects ANOVA to determine whether the position of the females differed across predation environments. A one-way between subjects ANOVA was used to compare the time spent in the zone nearest a predator across the treatments. A one-way between subjects ANOVA was also used to look at the time spent in the zone furthest from the predator across the treatments.

A one-way between subjects ANOVA was used to compare the time spent in close proximity, oriented towards and watching a predator between the treatments. A one-way between subjects ANOVA was also used to compare the time spent backing away from a predator between the treatments. A one-way between subjects ANOVA was also used to compare the time spent approaching the predators between the treatments. A one-way between subjects ANOVA was used to compare the time spent following a predator between the treatments. Since three ANOVAs were run, the critical *P* for each ANOVA was adjusted to 0.017.

All statistical analyses were performed using the software package SPSS v.19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

RESULTS

Effect of Predation on Female Mating Behavior

The effect of predation on sword preference was examined using a generalized linear model with time period, male type, and treatment as the fixed effects and female identity as a random effect. The three-way interaction between time period (pre-exposure, post-exposure), male type (long-sworded, short-sworded), and predator treatment (small predator, large predator, predation event) was not significant (F = 0.032, p = 0.859) and thus dropped from the model. This indicates that the effect of the exposure on the female sword preferences did not vary among the treatments. There was a significant interaction between time period (pre-exposure, post-exposure) and male type (long-sworded, short-sworded) (Table 2.1); females decreased the amount of time spent with the long-sworded male after seeing a predator nearby (Fig. 2.2A, 2.2B). There was also a significant interaction between treatment and male type (Table 2.1); females placed on the three treatments initially had differing mating preferences. There was not a significant effect of female standard length on the behavior of females.

The effect of experiencing a predator on the female preference for sword length on Day 2 was examined using a generalized linear model with day (day 1 pre exposure vs. day 2 pre-exposure), male type, and treatment as the fixed effects and female identity as a random effect. The three-way interaction between day, male type, and predation treatment was not significant (F = 1.770, p = 0.186) and thus dropped from the model. There was a significant interaction between day and male type (Table 2.2). The data suggest that the predator effect on the female sword response persists to the next day (Fig. 2.2C). That is, females show a weaker preference for the longer-sworded male the day after seeing a predator than they did prior to seeing a predator, regardless of whether the memory was of a small cichlid, a large cichlid or a predation event.

The consistency of the effect of experiencing a predator on the female preference for sword length on the second day was examined using a generalized linear model with day (day 1 post exposure vs. day 2 pre-exposure), male type, and treatment as the fixed effects and female identity as a random effect. The three-way interaction between day, male type, and predation treatment was not significant (F = 1.737, p = 0.190) and thus dropped from the model. None of the two-way interactions were significant (Table 2.3). The data indicate that the reduction in the long-sword preference, and the switch to a preference for a short-sword as a result of experiencing a predator persisted to Day 2.

The effect of predation on the amount of time females spent in close proximity, oriented towards and watching males was examined using a generalized linear model with time period, male type, and treatment as the fixed effects and female identity as a random effect. The three-way interaction between time period, male type, and treatment was not significant ($F = 0.385$, $p = 0.536$) and thus dropped from the model. There was a strong trend ($p = 0.056$) for an effect of the interaction between treatment and time period (Table 2.4). The data, although not significant, could indicate that females in the predation treatment spent less time in close proximity, orienting towards and watching males after being exposed to a predation event than after seeing a large cichlid-no predation event or a small cichlid (Fig. 2.3).

Females exhibited approach ($F = 0.493$, $p = 0.484$), swimming in unison and parallel with male ($F = 0.083$, $p = 0.774$), dart toward ($F = 1.54$, $p = 0.217$), quiver ($F = 1.889$, $p = 0.172$), jerky swim ($F = 1.432$, $p = 0.234$), tail flick ($F = 1.486$, $p = 0.225$), flank presentation ($F = 1.89$, $p = 0.172$), backing ($F = 1.012$, $p = 0.317$), back away swim ($F = 1.433$, $p = 0.234$), and circle back ($F = 1.433$, $p = 0.234$), behaviors during the trials, however there were no significant interactions.

There was no overall change in the total amount of time the females spent with males between the time prior to- and post-exposure to a predator (Prior to-exposure: $\bar{x} = 248.97$ sec., SD = 155.60; Post-exposure: $\bar{x} = 218.40$ sec., SD = 185.98) independent $t_{35} =$

0.746, $p = 0.458$). This indicates that overall female mating interest was not affected by exposure to a predator, but how females allocated their time to males was affected.

Effect of Predation on Female Anti-Predator Behavior in the Absence of Males

We also examined female activity during the 10-min stimulus presentation period, during which one of the three predator stimuli was displayed. Female position in the tank differed for the three treatments for this time period (Fig. 2.4, Fig. 2.5). A one-way between subjects ANOVA revealed that there was significant variation among the treatments in the time females spent in the section nearest to a predator ($N = 35$, $F_{2, 32} = 7.46$, $p = 0.002$). Post-hoc comparisons using the Tukey HSD test indicated that the time spent near the small cichlid ($\bar{x} = 250.07$, $SD = 128.97$) was significantly different (Tukey's HSD: $p = 0.01$) than the time spent near a large cichlid capturing and consuming a male (predation event) ($\bar{x} = 121.70$, $SD = 81.90$) and the time spent near the large cichlid ($\bar{x} = 115.18$, $SD = 60.79$) (Tukey's HSD: $p = 0.005$). However, the time females spent nearest to large cichlids capturing and consuming males did not differ significantly from the response of females observing large cichlids (Tukey's HSD: $p = 0.988$). A one-way between subjects ANOVA also revealed that there was significant variation among the treatments in the time females spent in the section furthest from a predator ($N = 35$, $F_{2, 32} = 3.901$, $p = 0.030$). Post-hoc comparisons using the Tukey HSD test indicated that the time spent furthest from a small cichlid ($\bar{x} = 269.07$, $SD = 121.55$) was significantly different (Tukey's HSD: $p = 0.05$) than the time spent near a large cichlid-no predation ($\bar{x} = 376.72$, $SD = 95.82$). There was strong trend (Tukey's HSD: $p = 0.072$) for a difference of time furthest from a small cichlid and a predator capturing and consuming a male (predation event) ($\bar{x} = 373.40$, $SD = 107.48$).

The time females spent furthest from large cichlids capturing and consuming males did not differ significantly from the response of females observing large cichlids (Tukey's HSD: $p = 0.997$).

We examined the amount of time females watched the predators during the 10-min predation stimulus presentation period. Females differed in the time spent watching the predators in the three treatments for this time period (Fig. 2.6). A one-way between subjects ANOVA revealed that there is significant variation among the treatments in the time females spent watching a predator; ($N = 33$, $F_{2,30} = 6.49$, $p = 0.005$). Post-hoc comparisons using the Tukey HSD test indicated that the mean time spent watching the small cichlid ($\bar{x} = 4.54$, $SD = 7.92$) was significantly shorter (Tukey's HSD: $p = 0.007$) than the time spent watching a large cichlid capturing and consuming a male (predation event) ($\bar{x} = 121.70$, $SD = 81.90$) and the time spent watching the large cichlid ($\bar{x} = 115.18$, $SD = 60.79$) (Tukey's HSD: $p = 0.024$). However, the response of females watching large cichlids capturing and consuming males did not differ significantly from the time females spent watching large cichlids-no predation (Tukey's HSD: $p = 0.816$).

We examined the amount of time females spent backing away from the predators during the 10-min predation stimulus presentation period. Females differed in the time spent backing away from the predators in the three treatments for this time period (Fig. 2.7). A one-way between subjects ANOVA revealed that there was significant variation among the treatments in the time females spent watching a predator; ($N = 33$, $F_{2,30} = 5.17$, $p = 0.012$). Post-hoc comparisons using the Tukey HSD test indicated that the mean time spent backing away from the predation event ($\bar{x} = 3.00$, $SD = 1.27$) was significantly longer (p = 0.009) than the time spent backing away from a small cichlid ($\bar{x}$ = 0.00, SD = 0.00). However, the time spent backing away from the large cichlid ($\bar{x}$ = 0.91, SD = 1.58) did not differ from the time spent backing away from the small cichlid (p = 0.568) or a predation event (p = 0.097).

The time spent approaching the predators did not differ between the treatments (one-way ANOVA $F_{2,30}$ = 1.16, p = 0.326). There was also no difference in the amount of time females spent following the predators (one-way ANOVA $F_{2,30}$ = 2.43, p = 0.105).

DISCUSSION

Effect of Predation on Female Mating Behavior

Female green swordtails have been found to have a preexisting bias for long-sworded males (Basolo 1990a,b), however, in this study we found that predation resulted in a reversal of the female preference to a preference for the shorter-sword male. The general pattern that emerges from this study then is that females spend relatively less time with a male with a longer-sword compared to a short-sworded male after encountering a predator (Fig. 2.2A, 2.2B, 2.2C). This result is similar to a previous finding whereby females witnessing predation on a *long-sworded* male no longer preferentially associated with the longer-sworded male (Johnson & Basolo 2003), however in our study, the predation treatment showed a *short-sworded* male being eaten. The Johnson and Basolo (2003) results could be interpreted as a morph specific response; females were associating with the short-sworded male because they had seen a long-sworded male consumed. However, the results of this study show a more general response, in which a female witnessing a male being consumed, regardless of whether it had a long or short sword will result in a switch of the sword preference. Other studies have shown that females respond to the presence of a predator by not associating with a preferred male (Forsgren 1992; Hedrick & Dill 1992; Johnson & Basolo 2003; but see Kim *et al*. 2009). Female swordtails prefer longer sworded males, but likely increase the risk of attracting the attention of predators by associating with these more conspicuous males, which may lead to the predator attacking the female (Pocklington & Dill 1995). For those studies that have assessed the relative danger of different predators, females alter their preference for the preferred male according to their perceived danger. Bierbach *et al.* (2011) looked at female mating preference of Atlantic mollies in the presence of both piscivorous cichlids and non-piscivorous cichlids. They found that when confronted with a piscivorous predator, lab-reared females no longer associated with the preferred, larger male and instead associated more with the smaller male, which are similar results to the current swordtail study. It is evident that preexisting receiver biases can be modified (Basolo 1998a, 2002), and that current costs associated with the expression of a bias can modulate the strength of the bias across time (Basolo 1996, 1998a).

In addition to the preference decreasing after exposure to a predator, we found that this decrease persists to the second day. We do not, however, know the duration of this change, whether it would persist indefinitely or at some point revert to a preference for a long-sworded male. A follow-up study would be needed to address this. One explanation for the persistence of the reversal of the sword preference is that the females were predator naïve, and were unsure of the frequency with which a predator would appear, therefore, females maintained the preference for less conspicuous, short-sworded male to the next day.

Our females were virgin adults with developing eggs. Female green swordtails produce eggs that are reabsorbed if they are not fertilized (Bailey 1933; Tavolga 1949). This process of producing and then reabsorbing unfertilized eggs may be motivation for females to find a mate as production of eggs and reabsorption can be lead to a delay in a female's reproduction time. Sperm storage is an adaptation in live-bearing fish (Constanz 1989), which could minimize the costs of not associating with preferred, more conspicuous males (preferred males may offer more sperm, greater resistance to parasites, *etc.*) when a predator is nearby. Females could mate with the preferred males when predators are absent, and then use that stored sperm to fertilize the next batch of eggs if seeking a mate exposes one to a high predation risk. Our females were highly motivated to mate as they had not stored sperm. A follow-up study could determine whether females with stored sperm behave as the virgins used in this study.

If being with a male with a long sword is costly because it increases a female's predation risk, why has the bias been maintained in a mate choice context? Potential benefits accrued to the female may be as simple as long swords can make males more easily detected by females, thus reducing the costs of searching for a mate search. There may be other benefits of preferring a long sword; males with longer swords may have more viable sperm, may have a greater number of sperm to fertilize eggs, or may have higher fitness alleles.

In terms of specific mating behaviors, females exhibited a decrease in the time spent exhibiting one behavior, orienting towards and watching a male, and only for the predation event treatment; after exposure to the large cichlid capturing and consuming a male, females spent significantly more time exhibiting this behavior compared to the other two treatments (Fig. 2.6). One explanation for this is that females needed to focus more time to monitoring the predator and less time to mating behaviors. In guppies, after witnessing a predator females were relatively less active and concentrated on monitoring the area in which the predator had previously been located. During this time, male guppies increase the number of coercive mating attempts, perhaps due to the lower degree of attention by females towards males (Evans *et al.* 2002). Although female swordtails exhibited all the other behaviors discussed in the Methods section (indicating mating interest of females), there was no difference between the treatments in the expression of any of the other mating behaviors. This could be because females exhibited the initial mating response to males, orienting towards and watching a male in close proximity, but did not receive the reciprocal behavior from males, which may explain why the overall expression of these behaviors is lower than that which would be expressed to a live male.

Effect of Predation on Female Anti-Predator Behavior in the Absence of Males

We also assessed female anti-predator behavior. During the 10-min predation sequences when males were not present, females spent less time near the predator when it was a large cichlid or a large successful cichlid compared to a small cichlid (Fig. 2.4). It appears then that small predators were perceived as less dangerous than either large cichlids or large successful cichlids. Females also spent more time in the area furthest from large cichlids and large, successful cichlids compared to small cichlids (Fig. 2.5). In doing so, females placed themselves as far from a predator as possible, which suggests that the predators were recognized as dangerous, so the females were more wary. Predator inspection can be a risky behavior (Milinski *et al*. 1997), but often necessary because prey can gather information on the predator's location and motivational state through inspection. Small predators could have been perceived as predators that were far away, so the females may have been closer the smaller predators to assess the potential threat. Alternatively, inspecting females could have been demonstrating their alertness or escape abilities to the predator (Hansson 1991). This seems unlikely though as females would have been expected to be near large cichlids or successful large cichlids if that were the case. Females exhibited two types of wary behavior; they spent more time watching large cichlids and large successful cichlids (Fig. 2.6), and staying further away from large cichlids and successful large cichlids. Females also spent more time backing away from large *successful* cichlids, compared to large cichlids or small cichlids (Fig. 2.7). The small cichlids appear to pose less of a threat to females, possibly because their more limited gape width, which would prevent it from consuming a female (Hambright 1991, Luczkovich *et al.* 1995). In addition, small cichlids could have been perceived as larger cichlids, but farther away, thus they didn't represent as immediate a danger. Fish have laterally placed eyes and therefore monocular vision (Sovrano *et al.* 1999) so in order to interpret the distance between themselves and other objects they need to have depth clues. Spatial distance with monocular vision can be determined by the presence or absence multiple monocular cues including occlusion and relative size (Zeil 2000, Cavoto & Cook 2006). The females did not have a point of reference in which to judge the size of the small cichlid, so the females could not determine if the small cichlid was small in size or far away. The females may have moved to the area nearest the small cichlid video stimuli in an effort to inspect the size or distance of the small cichlid.

The findings of this study are consistent with studies with other animals in which females alter their mating preferences in the face of predation. There is a trade-off for females between associating with more conspicuous males and increasing one's predation risk by associating with conspicuous males. This study not only shows modulation of the pre-existing sword bias, but also that the preference for a short-sworded males persists at least to the next day. One question remaining to be addressed is whether the change in the preference is permanent? A second question is if the change is not permanent, how long does the reversal in preference endure? When the preference for the sword is costly, females show plasticity in the preference in order to reduce predation risk related to associating with a long-sworded male. Thus when plasticity in a bias evolves, biases can be maintained without being highly costly to females. The presence of a modified bias in the swordtails suggests that the bias is sometimes beneficial in a mating context. To our knowledge, this is the first example of enduring plasticity in a receiver bias.

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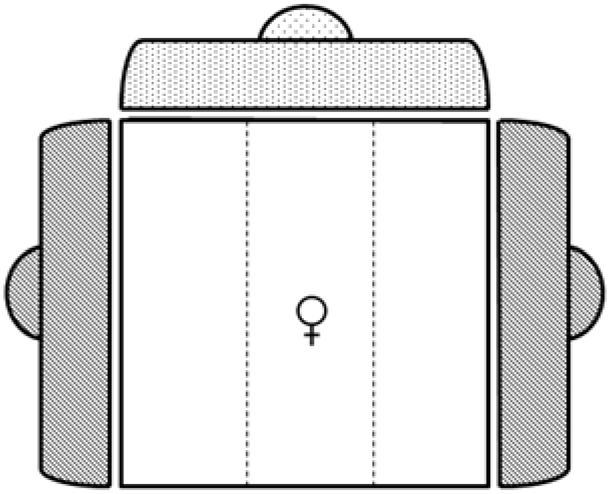

Fig. 2.1A: Experimental setup used to investigate female sword preference. A monitor was positioned next to each side of the tank, which displayed courting males with differing sword lengths before and after a predation video (grey lines). A monitor (gray dots) positioned at the back of the tank displayed either a tank devoid of fish, or one of the three predator stimuli.

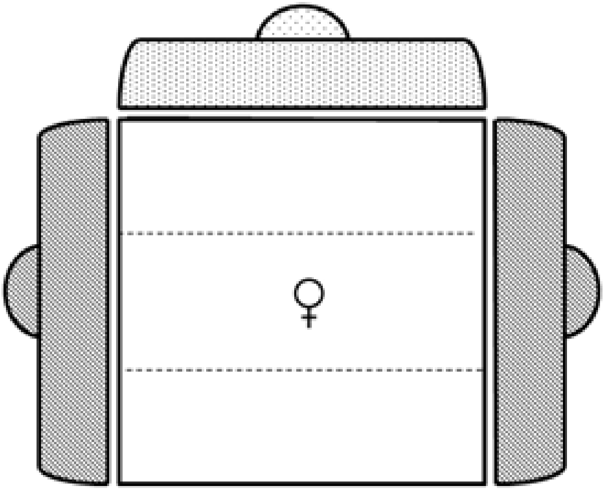

Fig. 2.1B: Experimental setup used to investigate female anti-predator behavior. A monitor was positioned next to each side of the tank, which each displayed a tank devoid of fish (grey lines). A monitor (gray dots) positioned at the back of the tank displayed one of the three predator stimuli.

Table 2.1: Linear mixed model examining the effects of the fixed factors treatment (predation vs. large cichlid vs. small cichlid), male type (short-sworded vs. long-sworded), time period (during exposure vs. post exposure), and female standard length on female sword response. Female identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	28.72	57.67	0.248	0.619
Time Period	119.84	74.3	2.602	0.109
Male Type	17.79	74.3	0.057	0.811
Female Standard Length	4.23	3.36	1.588	0.21
Treatment * Time Period	-4.41	22.0	0.04	0.841
Treatment * Male Type	**51.25**	**22.0**	**5.427**	**0.021**
Time Period * Male Type	**-83.53**	**36.6**	**5.207**	**0.024**
Random Effect	*Estimate*	*SE*		
Female	72.72	114.97		

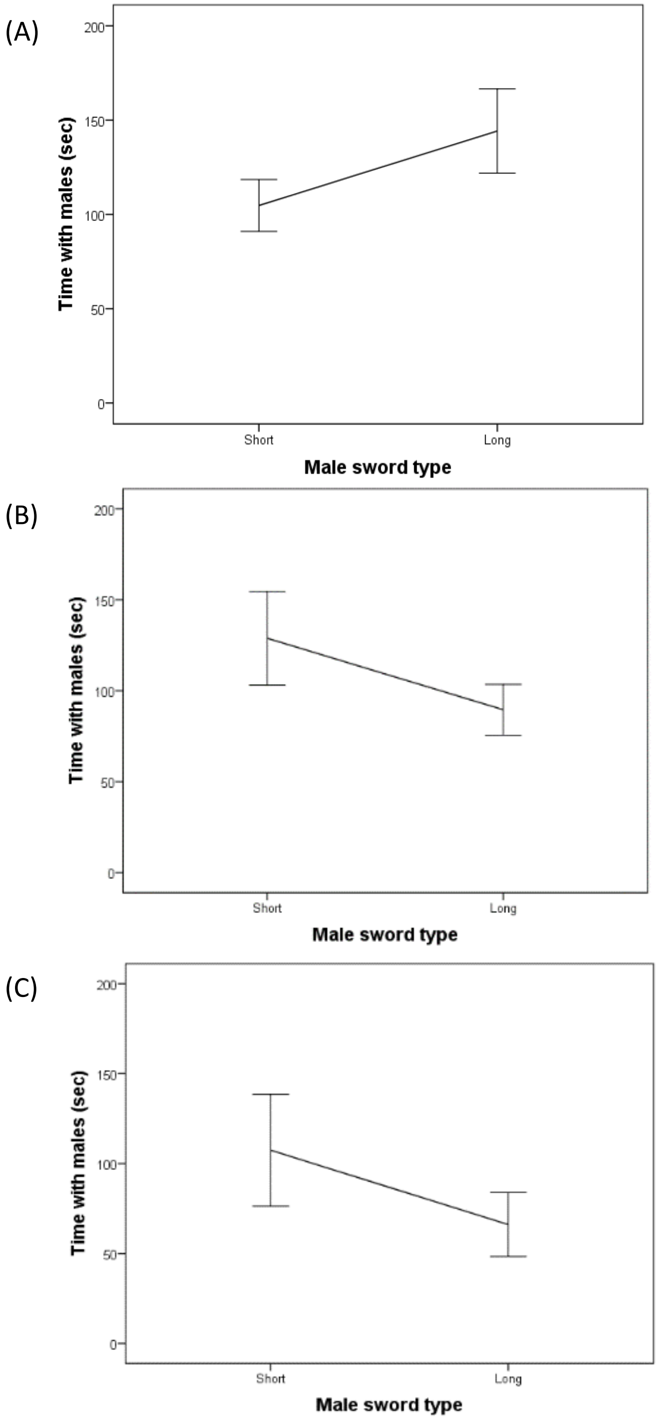

Fig. 2.2: Time spent by females on Day 1 and Day 2 with longer- and shorter-sworded males across treatments. (A) Pre-exposure period day 1; (B) Post-exposure period day 1; (C) Pre-exposure period day 2.

Table 2.2: Linear mixed model examining the effects of the fixed factors treatment (predation vs. large cichlid vs. small cichlid), male type (short-sworded vs. long-sworded), day (day 1 pre-exposure vs. day 2 pre-exposure), and female standard length on female sword response. Female identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	7.41	49.83	0.022	0.882
Day	64.49	71.0	0.825	0.365
Male Type	79.40	71.0	1.251	0.266
Female Standard Length	0.55	3.52	0.024	0.876
Treatment * Day	13.29	21.32	0.389	0.534
Treatment * Male Type	23.76	21.32	1.242	0.267
Day * Male Type	**-84.88**	**35.25**	**5.796**	**0.018**
Random Effect	*Estimate*	*SE*		
Female	26.91	42.31		

Table 2.3: Linear mixed model examining the effects of the fixed factors treatment (predation vs. large cichlid vs. small cichlid), male type (short-sworded vs. long-sworded), day (day 1 post-exposure vs. day 2 pre-exposure), and female standard length on female sword response. Female identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	69.12	56.51	1.496	0.223
Day	-47.81	72.41	0.436	0.510
Male Type	-99.92	72.41	1.904	0.170
Female Standard Length	2.34	3.28	0.510	0.476
*Treatment * Day*	8.48	21.44	0.156	0.693
*Treatment * Male Type*	27.09	21.44	1.596	0.209
*Day * Male Type*	1.82	35.68	0.003	0.959
Random Effect	*Estimate*	*SE*		
Female	105.56	160.57		

Table 2.4: Linear mixed model examining the effects of the fixed factors treatment (predation vs. large cichlid vs. small cichlid), male type (short-sworded vs. long-sworded), day (day 1 pre-exposure vs. day 2 pre-exposure), and female standard length on time spent orienting towards and watching males. Female identity was included as a random factor.

Fixed Effects	*Coefficient*	*SE*	*F*	*p*
Treatment	-10.01	6.93	2.086	0.151
Time Period	-14.54	10.50	1.919	0.169
Male Type	-1.43	10.50	0.018	0.892
Female Standard Length	1.12	0.59	3.628	0.059
Treatment * Time Period	**6.15**	**3.18**	**3.737**	**0.056**
Treatment * Male Type	-0.17	3.18	0.052	0.821
Time Period * Male Type	0.72	5.18	0.001	0.974
Random Effect	*Estimate*	*SE*		
Female	0.00*	0.00		

*This covariance parameter is redundant

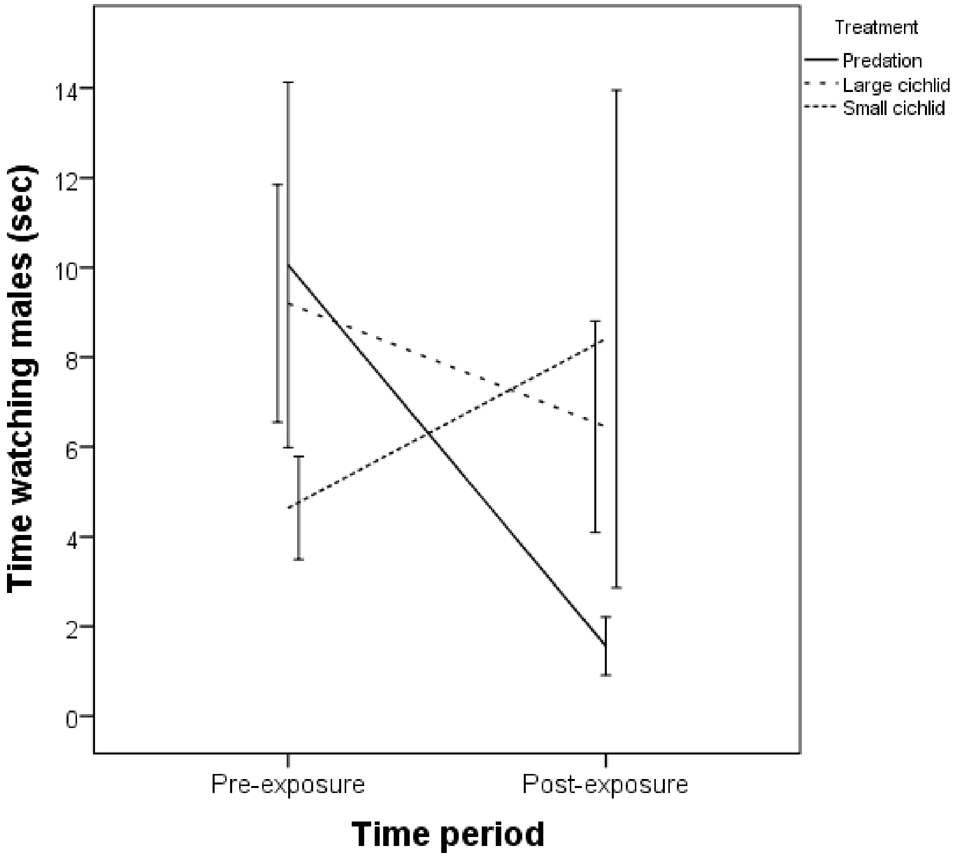

Fig. 2.3: Time spent by females in close proximity, orienting towards and watching males differing in sword length between the three treatments. Values are means +/- SE.

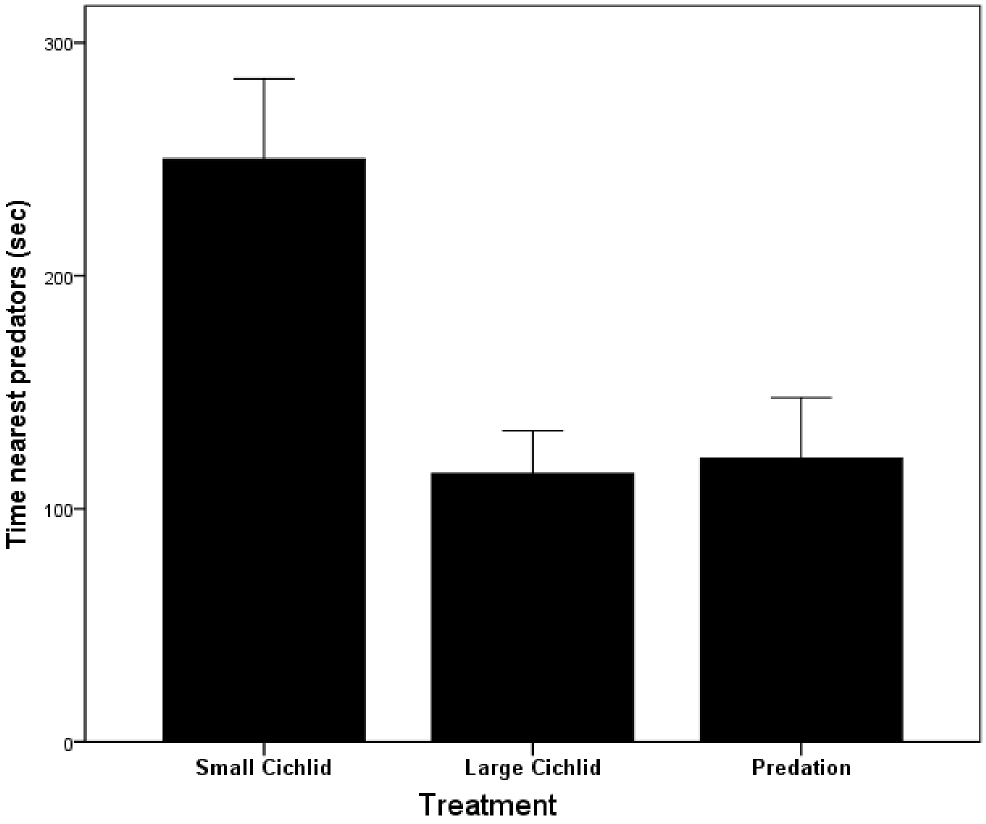

Fig. 2.4: Time females spent nearest to the predators during the exposure time period between the three treatments. Values are means +/- SE.

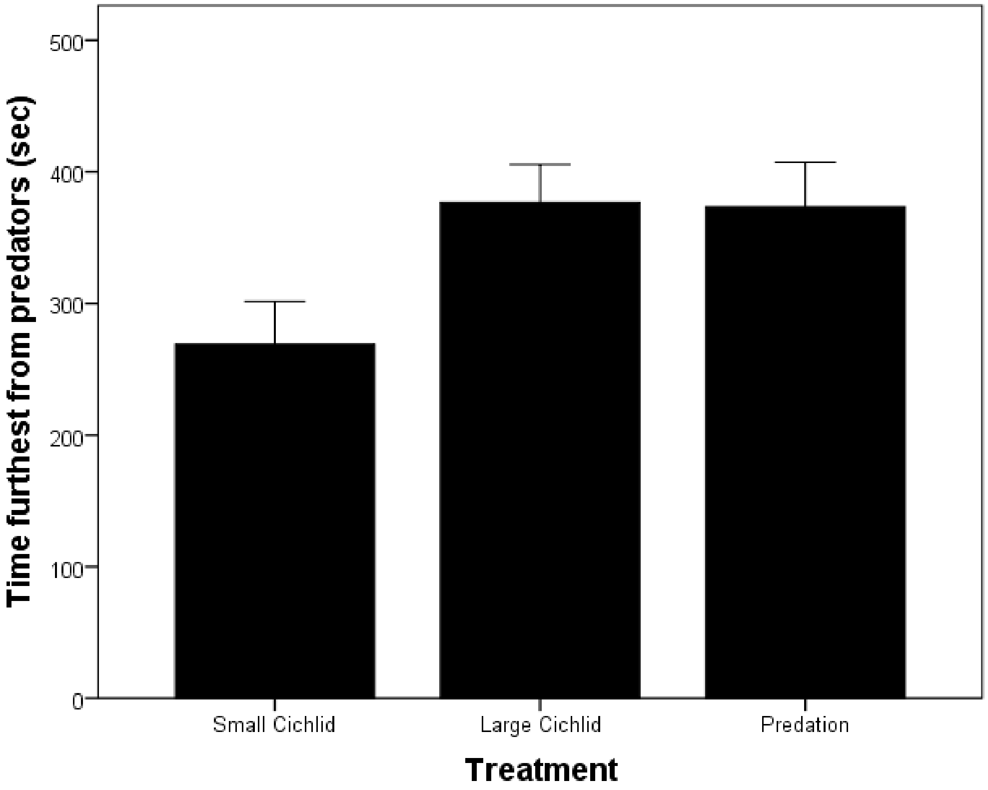

Fig. 2.5: Time females spent furthest from the predators during the exposure time period for the three treatments. Values are means +/- SE.

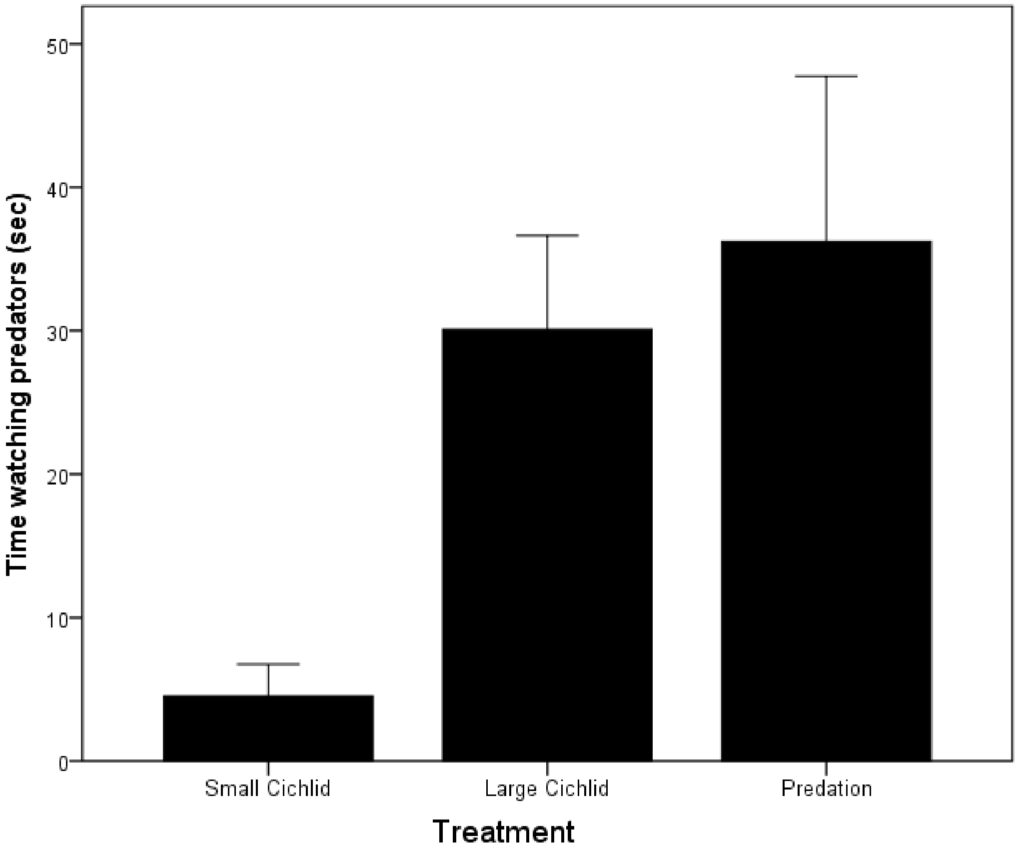

Fig. 2.6: Time spent by females watching the predators during the exposure time period for the three treatments. Values are means +/- SE.

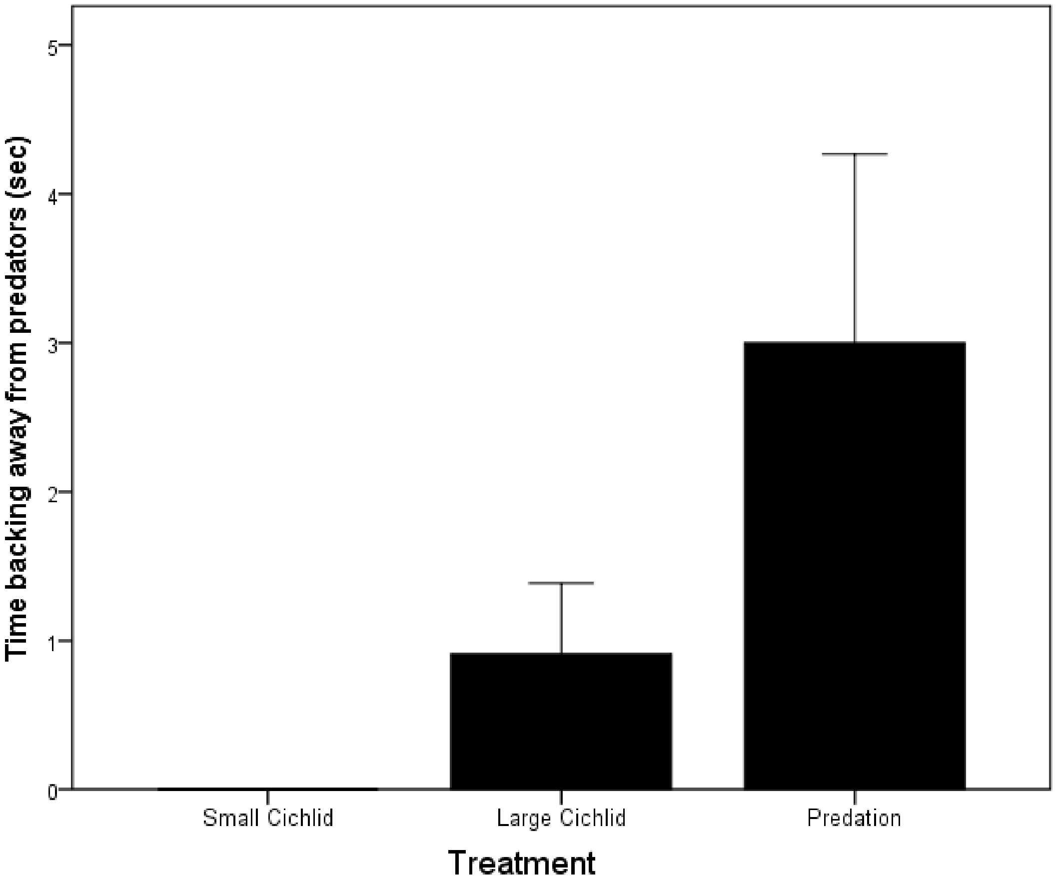

Fig. 2.7: Time spent by females backing away from the predators during the exposure time period for the three treatments. Values are means +/- SE.