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Lindsey M. Coit
University of Nebraska-Lincoln, lindsey.m.coit@gmail.com

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BEHAVIORAL PLASTICITY ACROSS NON-SOCIAL CONTEXTS IN FEMALE GREEN SWORDTAILS, *XIPHOPHORUS HELLERII*

by

Lindsey M. Coit

A THESIS

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BEHAVIORAL PLASTICITY ACROSS NON-SOCIAL CONTEXTS IN FEMALE GREEN SWORDTAILS, *XIPHOPHORUS HELLERII*

Lindsey M. Coit, M.S.

University of Nebraska, 2017

Advisor: Alexandra L. Basolo

Phenotypic plasticity is the ability of an individual to alter its phenotype in response to environmental change. Individuals that express plasticity in behavior can quickly respond to changes that occur in the environment. Therefore, individuals that exhibit behavioral plasticity can alter their behavioral expression to best match current environmental conditions. The degree and direction of behavioral plasticity may be influenced by variation in individual characteristics. Understanding how variation in individual traits affects behavioral plasticity, and, whether patterns of behavioral plasticity are consistent across behavioral contexts are important topics to explore as we try to better understand how plasticity evolves and is maintained. For the study, we tested female green swordtails (*Xiphophorus hellerii)* to determine whether individuals express behavioral plasticity in response to a predator in two non-social contexts: (i) environmental assessment and (ii) foraging. Specifically, we were interested in how behavioral plasticity varies among individuals that differ in size and maturation age, and whether individual behavioral plasticity is correlated across contexts. In both the environmental assessment context and the foraging context, females expressed predator-related behavioral plasticity in the same six behaviors. For one foraging-related behavior in the foraging context, small individuals expressed plasticity of the behavior, whereas large females did not. This

result suggests that body size may influence predator-related plasticity in foraging. Maturation age did not significantly affect the expression of plasticity in any of the behaviors measured. In the six behaviors that were significantly affected by the predator treatment, we found no evidence of correlated plasticity across the two non-social contexts. The design of this study allowed us to take a detailed look at how females adjust their behaviors in an environmental assessment context and in a foraging context in response to predator presence. Continued exploration into these topics may yield valuable information on how behavioral plasticity evolves and is maintained within and across populations.

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INTRODUCTION

Phenotypic plasticity is the ability of an individual to alter its phenotype in response to environmental change (Bradshaw 1965; West-Eberhard 1989). Selection can favor the evolution of phenotypic plasticity when it enables individuals to adaptively respond to environmental changes that occur within their lifetimes. In some cases, the expression of phenotypic plasticity may come at a cost to the individual. For example, plasticity in morphological and life-history phenotypes can be adaptive, yet induced changes may not be reversible. Thus, such traits can be maladaptive in some contexts though highly adaptive in others. In contrast, individuals that exhibit plasticity in behavioral traits can often shift behaviors in response to variable environments, thereby allowing them to respond in an adaptive manner (West-Eberhard 1989).

Behavioral plasticity has been well documented in a number of behavioral contexts (*e.g.* mating, foraging, anti-predator, *etc.*). For example, in environmental conditions that favor hybridization, female spadefoot toads (*Spea bombifrons*) will switch their mating preference from conspecific males to heterospecific males (*Spea multiplicata*) (Pfennig 2007). In a foraging context, migratory birds often shift from a preferred food source to a more abundant food source, to handle the increased energetic demands during long migrations (McLandress and Raveling 1981, Brazely 1987, Smith *et al.* 2007). Finally, in anti-predator contexts, individuals of several fish species decrease activity and increase refuge use after predator exposure, in order to decrease the likelihood of becoming prey (Sih 1992, Wooster and Sih 1995, Eklöv and Persson 1996).

For prey species, survivorship, and therefore fitness potential, is dependent on surviving encounters with predators. When the predation environment fluctuates, selection can favor plasticity in prey behavior (*i.e.* predator-related plasticity), potentially allowing individuals to reduce the risk of predation. For instance, Johnson and Basolo (2003) found that in the absence of a predator, female green swordtails (*Xiphophorus hellerii*) preferred a long-sworded male to a male lacking a sword extension (regarding the nature of swords, refer to Basolo 1996); yet in the presence of a predator, females no longer exhibited a preference for a long-sworded male. Thus, female green swordtails modulate the preference for a long sword based on the current predation environment (Johnson and Basolo 2003). Males too will adjust mating behavior in response to predation risk. For example, males from five species of coexisting birds were able to detect when predator presence increased around nest sites, and in response, reduced the rate at which they visited nest sites to feed incubating females (Ghalambor and Martin 2002). And, in Trinidadian guppies (*Poecilia reticulata*), males spend less time courting females and increase the number of sneak copulation attempts in the presence of a predatory pike cichlid (Godin 1995).

Individual traits, such as body size, can greatly influence the risk of predation an individual experiences. In some species, individuals with a larger body size may be more conspicuous and profitable to predators, thus more likely to be attacked (Zach and Fall 1978, Jones 1990, Johansson *et al.* 2004). Larger body size may also inhibit an individual’s ability to maneuver through the surrounding environment, limiting their capacity to escape a predator (Domenici and Blake 1993, Domenici 2001). In other cases, smaller individuals are expected to experience a higher risk of predation. For example, in many aquatic species, risk of predation is greater for smaller individuals due to gape limitations of sympatric predators (Stein 1977, Sogard 1997, Basolo and Wagner

2004). Because of size-related variation in susceptibility to predation, differences in body size can affect the expression of predator-related behavioral plasticity exhibited by an individual. The expression of predator-related behavioral plasticity may be further complicated by differences in the individual needs that are associated with size

Individuals differing in size also differ in energy demands. Survivorship and consequently fitness are dependent on individuals fulfilling resource demands, all while avoiding predation. Optimally, animals should express foraging behaviors that maximize food intake. However, behaviors that increase food intake (*i.e*. higher activity levels) can decrease the animal's level of safety (Stephens *et al*. 2007). Because of this, we expect the evolution of plasticity in foraging behavior when levels of predation risk fluctuate in the environment. Regardless of size, resource acquisition behaviors should decrease in situations in which predation risk is high (Lima 1998). This response, however, may be restricted for some due to individual variation in needs. Small individuals in particular generally have lower energy reserves and higher energetic demands, as their resting metabolism is faster (Clarke and Johnston 1999). As such, smaller individuals are expected to have higher levels of activity than larger individuals, even in the presence of a predator (Brown and Braithwaite 2004). Predator-related plasticity then may be constrained in small individuals with high metabolic needs. In the three-spined stickleback (*Gasterosteus aculeatus*), for example, although smaller individuals experience a higher risk of predation, they spend significantly less time hiding in refuges than larger individuals after exposure to a predator stimulus; this is likely due to a greater energetic need to resume foraging (Krause *et al.* 1998).

Understanding how variation in individual traits such as body size affects the degree of behavioral plasticity has become increasingly important as we try to better understand how plasticity evolves and is maintained within a population (Nussey *et al*. 2007, Dingemanse and Wolf 2013). In this study, we aim to determine whether size at testing or the age at which test subjects matured affects the degree and/or direction of predator-related behavioral plasticity exhibited by female green swordtails (*Xiphophorus hellerii*). Few studies have investigated how age influences the expression of behavioral plasticity (Tinghitella *et al.* 2013, Atwell and Wagner 2014), and even less is known about how age at maturation may affect behavioral plasticity.

Fish in the *Xiphophorus* genus (family Poeciliidae) are a good model for exploring how these particular individual traits affect the expression of predator-related behavioral plasticity for several reasons. First, swordtails have been extensively studied in both the lab and field (Rosen 1960, Kallman and Borkoski 1978, Rauchenberger *et al.* 1990). Second, in many *Xiphophorus spp*., body size and age at maturation are heritable traits influenced by alleles at the pituitary locus (P-locus) on the W, X, and Y-chromosomes (Kallman and Borkoski 1978, Kallman 1983, Zimmerer and Kallman 1989, Kallman 1989, Basolo 1994). In species of *Xiphophorus* that exhibit P-allele-like variation, different genotypes at the P-locus result in individuals that differ in age and size at sexual maturation. More specifically, depending on the alleles at the P-locus, individuals mature early at a small size, later at a large size, or, individuals will be of an intermediate age and size at maturation (Kallman *et al.* 1973, Basolo 2008). Lastly, in one northern swordtail species, there is evidence that P-alleles associated with body size and age at maturity may also influence male behavioral plasticity within a sexual-social context (Ryan and Causey 1989, Zimmerer and Kallman 1989).

Patterns of age at maturation and body size in *Xiphophorus hellerii* suggest that a P-allele-like system is in place for this species (Kallman 1989, Campton 1992). Male *X. hellerii* show determinate growth, thus as adults, early maturers are smaller than late maturers (Basolo 2008). Kallman (1989) reports that females mature at a similar age and size as early maturing males. However, it appears that females also express variation in age and size similar to male *X. hellerii,* at least in some populations, including the population used in this study (Basolo *in prep.*). Further, females, unlike males, continue to grow after sexual maturation (Basolo 2008). This means that by using female *X. hellerii,* we can determine whether variation in behavioral plasticity is associated with the P-allele-like system and/or is associated with body size at testing. If we find that females who are dissimilar in size, but similar in age at maturation show similar predator-related plasticity, this would suggest that the P-like-alleles influence behavioral plasticity. However, if we find that individuals similar in size, but dissimilar in age at maturation exhibit a similar degree of plasticity, current body size may be directly affecting the expression of behavioral plasticity.

In the last decade, studies investigating behavioral syndromes have gained popularity. Behavioral syndromes are cases of consistency in animal personality in which individuals express correlated behaviors across different behavioral contexts (Sih *et al.* 2004). For example, individuals who express an aggressive or bold personality will consistently exhibit aggressive or bold behaviors, even in situations in which these behaviors can be considered maladaptive (Sih *et al.* 2004, Johnson and Sih 2005).

Therefore, individuals with behavioral syndromes show little to no behavioral plasticity across contexts. A less explored question, then, is to what extent individual expression of behavioral plasticity differs across contexts or whether there is consistency of behavioral plasticity cross-contextually. By comparing changes in behavioral expression in different contexts, we can determine whether individuals that are highly plastic in behaviors expressed in one context (*i.e.* environmental assessment), are also highly plastic in the same behaviors expressed in a different context (*i.e.* foraging). Here, we explore patterns of behavioral plasticity expressed by individuals of differing phenotypes across contexts in order to better understand how natural selection can optimize behavioral plasticity. Ultimately, we stand to gain new insight into how plasticity evolves and is maintained within populations.

To our knowledge, no studies have explored individual variation in predator-related plasticity expressed by females across differing non-social contexts. Further, outside of the mate-choice context, studies that focus on female behavior are limited (Pocklington and Dill 1995, Gowaty 1997, Beery and Zucker 2011). In this study, we consider: (i) whether female green swordtails adjust their movement patterns and other behaviors in response to a predator, (ii) how inter-individual variation in behavioral plasticity can be explained by current body size and/or age at maturation (*i.e.* P-like-alleles) in different contexts, and (iii) to what extent are patterns of behavioral plasticity consistent across different non-social contexts (*i.e.* environmental assessment and foraging). We hypothesize that due to size-dependent risk of predation (Basolo and Wagner 2004) and the correlation between ages at maturation (driven by P-alleles) and behavior in another species of swordtail (Zimmerer and Kallman 1989), body size and maturation age will affect the degree of behavioral plasticity exhibited by female *X. hellerii*. We further predict, that if individual phenotype (*i.e.* body size and maturation age) does influence the degree and direction of behavioral plasticity, then individuals that exhibit a high degree of behavioral plasticity in one context will also express a high degree of behavior plasticity in the other context.

METHODS

Study System

The green swordtail, *Xiphophorus hellerii,* is a species of live bearing fish belonging to the subfamily *Poeciliinae*. These fish are found in freshwater rivers and streams throughout Mexico, Honduras and Belize (Rosen 1960). The test subjects (G1) were the female progeny of gravid females (G0) collected in March 2013 and 2014 from Little Barton Creek, 1 km NW of the Western Highway (Mile 59, N 17° 12’ 28’’/W 88° 56’ 36”) outside of Unitedville, Belize. The collection of fishes was approved by the Belize Fisheries Department (Research Permits no. 00012-13 (2013) and 00016-14 (2014)). Wild-caught (G0) green swordtails were transported back to the University of Nebraska – Lincoln and housed individually in 20.8 L glass tanks (40.6 x 20.3 x 25.4 cm). Each glass tank contained a loose gravel substrate (gravel depth = 1.5 cm), an under-gravel water filter and a handful of the freshwater plant Java moss. G0 females that were brought back to the laboratory but did not have offspring were mated in the laboratory to males collected from the same location. Potential G1 test subjects were collected randomly within 10 hours of birth and reared in individual 6.6 L plastic tanks (29.9 x 19.7 x 20.3 cm) containing 10-15 small freshwater snails, a loose gravel bottom (gravel depth = 1 cm) and a small bunch of Java moss to help maintain the quality of the aquatic environment. G1 offspring were physically, but not visually isolated from one another.

Potential test subjects were fed TetraMin® tropical flakes twice daily at 0900 h and 1400 h, and Brine Shrimp Direct live nauplii twice daily at 1200 h and 1600 h. Individuals were kept on a 12:12 light:dark cycle; temperature was controlled between 26 – 28C^o.

To track the development of G1 offspring, individuals were measured at the age of 77 +/- 7 days, and then every 17.5 +/- 5 days until sexually mature. Sexual maturity was determined based on the development of a gravidity spot. The gravid spot is located just behind the anal fin. Its development is commonly used to recognize the onset of sexual maturation in poeciliids (Melie and Basolo unpublished data). Sexually mature G1 individuals were then considered ready to be potential test fish.

Traits of Test Fish

Of the 32 female *X. hellerii* tested, females tested were either half or full siblings from 22 families. Female test fish represented a range of maturation ages (days) and sizes (mm). In our lab, as in field populations (Basolo and Wagner 2004), standard length (mm) and body mass (g) were highly correlated (Pearson's correlation coefficient r = 0.987, df = 128, p < .001). Therefore, standard length (SL) was used as a proxy of size, and is defined as the distance from the most distal point of the mouth to the midpoint of the caudal vein. For all females tested (n=32), SL ranged from 37.15 to 62.00 mm and age at maturation ranged from 112 to 279 days. Body mass ranged from 1.11 to 5.68 g. At the date of their first trial, individuals ranged in age from 179 to 570 days old.

Pre-trial Acclimations

To acquaint test subjects to the environmental conditions experienced during testing, female test fish were exposed to a series of pre-trial acclimations. Five days prior to the trial start date, fish were individually exposed to a conspecific female of a smaller size in a tank (60 x 30 x 30 cm) with a white panel bottom partitioned into a 3 x 6 grid of equally-sized squares (9 x 9 cm), and covered with a thin layer of gravel (gravel depth = 3 mm) secured with silicon aquarium sealant (American Sealant, Inc.). The tank was partitioned into two sections of equal size (30 x 30 x 30 cm) by a clear, permeable plastic divider placed in the middle. Therefore, fish were physically, but not visually or chemically isolated from one another for 20 min.

Four days prior to when trials began between 1600 and 1800 h, an empty foraging ring was placed in test subject's home tanks and left overnight. When a test subject was scheduled to complete foraging context trials, a TetraMin® Tropical Pellet was placed in the foraging ring between 0800 and 1000 h three days prior to testing. The pellet and foraging ring were removed after two hours. Additionally, three days prior to trial commencement, test fish were individually placed in a tank (60 x 30 x 30 cm) with the 3 x 6 gridded gravel panel bottom and two sides (one 60 x 30 cm and one 30 x 30 cm) covered with sky blue felt. On the other 30 x 30 cm side of the tank was a monitor (Truetech 2-in-1 17" LCD TV + DVD Model No. PVS21175S1) that played a 34-min video stimulus of a predation event by a large Jack Dempsey cichlid predator, *Rocio octofasciata* (known at various times by nine other species names; Froese and Pauly 2016), on a male conspecific (*X. hellerii*). Monitor side was randomly determined.

Experimental Design

A testing framework was developed in which each female was tested twice per week over a four-week period (with three to four days in between trials). Within each test week, a female was tested within one of four contexts in two different treatments.

For the environmental assessment context, females were placed in the experimental tank void of any other fish or any foraging opportunities. For the foraging context, females were placed in the experimental tank void of any other fish, and a foraging opportunity was presented. For a separate study discussed elsewhere, the same females were tested in two social contexts: an intrasexual social context and an intersexual social context (DiSciullo and Basolo *in prep*). Context order was randomized prior to the start of trial. Within each context, treatment order was also randomized.

The treatments for this study were two predator environments: predator-absent and predator-present. Trials were 40-min in duration and divided into four 10-min periods: acclimation (P0), pre-exposure (P1), exposure (P2), and post-exposure (P3). For the predator-absent treatment, one of 13 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-absent treatment, P1-P3 were identical to the acclimation (P0) period because in these periods, the film stimulus showed an environment devoid of a predator for the entire 40 min. For the predator-present treatment, one of 12 film stimuli was randomly designated and displayed on a monitor at one end of the test tank during a trial. In the predator-present treatment, the third 10-min period (P2) showed a large, field-collected fish, the Jack Dempsey cichlid, swimming across the monitor, in and out of view, in varying depths-of-field. P1 and P3 were identical to the acclimation (P0) period because in these periods, a film stimulus showed an environment devoid of predators. Jack Dempsey cichlids are piscivorous predators that co-occur with green swordtails in the wild (Basolo and Wagner 2004). In the laboratory Jack Dempsey cichlids consume *X. hellerii* (Coit personal observation). Some of the film stimuli used in this study were created for previous experiments with green swordtails (Melie and Basolo *in revision*).

Testing Chamber Set-up

Trials were conducted in a glass tank (60 x 30 x 30 cm) placed in one of four sound-damped testing chambers in the Basolo lab. A monitor (DELL UltraSharp 2005FPW 51.05-cm Wide Aspect Flat Panel LCD Monitor) was randomly placed at one of the two ends of the test tank (30 x 30 cm) to display a film stimulus. The glass at the other end of the tank (30 x 30 cm), opposite the monitor, was covered on the outer side with sky blue felt. One of the long sides of the tank (30 x 60 cm), *i.e.* the back of the tank relative to the front-facing camera (see below), was also covered (on the outer side) with sky blue felt.

To track the movement of subjects, a visually distinct 3 x 6 grid of 18, 9 x 9 cm squares was made by securing a thin layer of gravel across a white panel with silicon, except at the borders of the 18 squares. The grid panel was situated on the bottom of the tank during experimental trials. The six 9 x 9 cm squares closest to the monitor displaying a stimulus were designated Zone 1, the six 9 x 9 cm squares in the middle of the tank were designated Zone 2, and the six 9 x 9 cm squares farthest from the monitor displaying a stimulus were designated Zone 3 (see Figure 1). Moreover, two artificial plant refuges (Imagine Gold Ambulia Green, 18 cm in length) were suspended in the middle of the tank, at the border of Zones 1 and 2 and at the border of Zones 2 and 3. Lastly, a clear plastic foraging ring was suspended across the tank from front to back, and equidistant to the ends of the tank.

Two cameras were positioned in the chamber to record experimental trials: one 33.8 cm above the tank (top-facing; DCR-Sony SR47 Handycam or Sony DCR-SR68 Handycam; see Figure 1) and one in front of the tank, 130.8 cm away from the tank (side-facing; DCR-Sony SR47 Handycam, Sony DCR-SR68 Handycam or Panasonic 5100HS WV-PS03). In addition, trials could be viewed remotely via two monitors located outside of the test chamber. The test chamber was illuminated by two overhead lights (40W Vita light TM bulbs covered by a layer of vellum paper to decrease light reflection at the surface). Filmed sequences of trials were recorded onto Fugi Pro VHS tapes or memory cards (Sony Memory Stick PRO Duo 4 GB).

Conducting and Scoring Trials

Prior to the start of a trial, the order of testing for each fish was randomized for the four-week period. Within each week, treatment order (predator-absent or predator-present environment) was randomized. Fish were not fed for a two-hour period prior to testing. The chamber was fully prepared, including starting and pausing the film stimulus, such that the monitor displayed a predator-absent environment (P0) when test subjects were introduced into a test tank. For each trial, the test female was placed in the center of the testing tank along with a red TetraMin flake. The film stimulus was then resumed and the cameras started recording the trial. Each trial was 40-minutes in duration and all trials were run between 1000 and 1700 h (Central Time) between June 2014 and February 2015.

At the end of the 40-min test period, test subjects were returned to their home tanks. If the trial series was not yet complete, the next trial was run three to four days after the previous trial. Testing tanks were drained immediately after each trial completion, and the tank and its components were rinsed with an 80% denatured ethyl alcohol solution to eliminate any trace of the previous occupant.

Behaviors that we chose to investigate were scored from the filmed trials that were uploaded to a computer (see Table 1). Behaviors exhibited during the pre-exposure (P1) and exposure (P2) periods were scored and compared within and across treatments. A freeware program, SolomonCoder (Péter, 2016) was used to track frequency (number of times a behavior was exhibited, *i.e.* count) and duration (the time spent exhibiting the behavior from start to finish, *i.e.* duration in sec) of the behaviors scored (http://solomoncoder.com).

ANALYSES

Effect of Predator Treatment, Maturation Age and Body Size on Behavioral Plasticity

To determine whether the predator treatment and/or the covariates had an effect on the behavioral response variables within each context (*i.e.* environmental assessment and foraging), linear mixed models with a Gaussian distribution were used. Data were analyzed in R (version 0.99.893), and the lmer function of the *lme4* package (version 1.1-12; Bates *et al*. 2015) was used for the linear mixed modeling.

A global model was used to measure the effect of each predictor on all behavioral responses measured. We ran this model once for the environmental assessment context data and again for the foraging context data. The model included: the fixed effect of treatment (predator-absent or predator-present environment); the covariates of female body size at testing (mm) and age at maturation (days); and all possible two and three-way interactions. Female ID and the family to which the fish belonged were included as random effects. Female ID was nested within family ID. The random effects accounted for individuals belonging to the same family and the repeated measures design of the study.

The behavioral response variable was defined as the difference in a behavior measure from the P2 to the P1 trial periods. Behavioral plasticity was defined as the change in the response variable between the predator-present and the predator-absent environments within a context. Predator-related plasticity is indicated by an effect of treatment or an interaction between the treatment and any of the covariates.

The drop1 function was used to run a chi-squared likelihood ratio test in order to obtain p-values. This allowed us to determine the effect of all predictors on the response variables. This function compared models with and without the predictors. To validate the models, all fixed effects, random effects, and the covariates were checked for collinearity. Further, residuals of the models were checked to ensure that there were no violations of assumptions of normality or homoscedasticity.

Consistency in Plasticity Across Non-Social Contexts

To measure consistency in predator-related plasticity, a Pearson's product moment correlation was carried out for behaviors that were significantly affected by treatment (*i.e.* predator environment) within both contexts using the corr.test function in R (version 0.99.893). Correlations compared the expression of predator-related plasticity in these behaviors in the environmental assessment context with the expression of predator-related plasticity in the foraging context. Significant correlations indicate consistency in plasticity for individual females across contexts.

Alpha Correction for Multiple Tests

To account for multiple comparisons, the Benjamini-Hochberg (B-H) procedure was used to control the false discovery rate (FDR) (Benjamini and Hochberg 1995). The FDR was set at Q = 0.05 and adjusted critical p-values (P) to q-values (Q). Significance values within each analysis were ranked and adjusted per the B-H procedure.

RESULTS

Effect of Predator Treatment, Maturation Age and Body Size on Behavioral Plasticity in the Environmental Assessment Context and in the Foraging Context

There were no significant effects of the three-way interactions on any of the behaviors measured in either in the environmental assessment context, or the foraging context. Therefore, the three-way interactions were dropped from both global models (all $Q \geq 0.499$). Further, there were no significant effects of the two-way interactions on predator-related behavioral plasticity in the environmental assessment context. Therefore, all two-way interactions were dropped from the environmental assessment context global model (all $Q \geq 0.070$).

Time in Zone 1

In the environmental assessment context and in the foraging context, there were significant effects of treatment on female time spent in Zone 1 (Table 2, $Q < 0.001$). In both contexts, females decreased the amount of time in Zone 1 during the P2 trial period of the predator-present treatment (Figure 2). There were no significant effects of age at maturation or body size at testing on the amount of time females spent in the zone next to the stimulus (Zone 1) in either context.

Backward Swim Away from Stimulus

In the environmental assessment context and in the foraging context, there were significant effects of treatment on female time spent back-swimming away from the stimulus (Table 3, $Q < 0.001$). In both contexts, females increased the amount of time they spent back-swimming away from the stimulus during the P2 trial period of the predator-present treatment (Figure 3). There were no significant effects of age at maturation or body size at testing on the amount of time females spent back-swimming away from the stimulus in either context.

Swim Away from Stimulus

In the environmental assessment context and in the foraging context, there were significant effects of treatment on the number of times females swam away from the stimulus (Table 4, $Q < 0.001$). In both contexts, females increased the number of times they swam away from the stimulus during the P2 trial period of the predator-present treatment (Figure 4). There were no significant effects of age at maturation or body size at testing on the number of times females spent swimming away from the stimulus in either context.

Time at Tank Perimeter

In the environmental assessment context and in the foraging context, there were significant effects of treatment on female time spent at the tank perimeter (Table 5, $Q < 0.001$). In both contexts, females decreased the amount of time they spent at the tank perimeter during the P2 trial period of the predator-present treatment (Figure 5). There were no significant effects of age at maturation or body size at testing on the amount of time females spent at the tank perimeter in either context.

Motionless Oriented toward Stimulus

In the environmental assessment context and in the foraging context, there were significant effects of treatment on female time spent motionless oriented toward the stimulus (Table 6, $Q < 0.001$). In both contexts, females increased the amount of time they spent motionless and oriented toward the stimulus during the P2 trial period of the predator-present treatment (Figure 6). There were no significant effects of age at maturation or body size at testing on the amount of time females spent motionless oriented toward the stimulus in either context.

Watch and Approach Stimulus

In the environmental assessment context and in the foraging context, there were significant effects of treatment on female time spent watching and approaching the stimulus (Table 7, $Q < 0.001$). In both contexts, females increased the amount of time they spent watching and approaching the stimulus during the P2 trial period of the predator-present treatment (Figure 7). There were no significant effects of age at maturation or body size at testing on the amount of time females spent watching and approaching the stimulus in either context.

Dart Away from Stimulus

After controlling the FDR, there were no significant effects of treatment, age at maturation, or body size at testing on the number of times females darted away from the stimulus in the environmental assessment context or in the foraging context (all $Q \geq 0.070$).

Refuge Use

After controlling the FDR, there were no significant effects of treatment, age at maturation, or body size at testing on the time females spent in the refuge in the environmental assessment context or in the foraging context (all $Q \geq 0.700$).

Motionless under Refuge

After controlling the FDR, there were no significant effects of treatment, age at maturation, or body size at testing on the time females spent motionless under the refuge in the environmental assessment context or in the foraging context (all $Q \geq 0.365$).

Movement

After controlling the FDR, there were no significant effects of treatment, age at maturation, or body size at testing on female movement in the environmental assessment context or in the foraging context (all $Q \geq 0.280$).

Effect of Predator Treatment, Maturation Age and Body Size on Plasticity in Foraging Behaviors in the Foraging Context

Foraging Peck on Pellet

Within the foraging context, there were no significant two-way interactions or significant effects of treatment, age at maturation or body size at testing on the number of times females pecked at the food pellet (all $Q \geq 0.122$).

Non-Pellet Foraging Peck

After controlling the FDR, there was a significant effect of the interaction between body size and treatment on the number of times females pecked at the substrate and/or refuge (Table 8, $Q < 0.032$). Smaller females decreased the number of times they pecked at the substrate and/or refuge during the P2 trial period of the predator-absent treatment, while females of a larger size did not adjust this behavior across treatments (Figure 8). There was no significant effect of age at maturation on the number of bites on the substrate and/or refuge.

Consistency in Plasticity Across Non-Social Contexts

Of the six behaviors in which predator-related plasticity was significant that could be compared between each context (*i.e.* time in zone 1, time at tank perimeter, motionless oriented toward stimulus, watch and approach stimulus, backward swim away from stimulus, and swim away from stimulus) there was no evidence of significant consistency in predator-related plasticity (Table 9, $Q \geq 0.689$).

DISCUSSION

In both the environmental assessment context and the foraging context, we found that individuals exhibited predator-related plasticity in time spent in Zone 1 (*i.e.* 1/3 of tank closest to stimulus monitor). Females decreased the amount of time they spent in the zone closest to the predator stimulus (Zone 1) when the predator was present (Table 2, Figure 2). This result suggests that the test individuals are likely recognizing the predator stimulus shown in predator-present treatments as a possible predation threat. In a previous study, Melie and Basolo (*in revision*) found similar results expressed by male *X. hellerii.* Given these results, it can be expected that both male and female green swordtails, regardless of size, will attempt to increase the distance between themselves and a predator to lessen the likelihood of being attacked.

We also found that females exhibited behavioral plasticity in the overall time that they the spent backward swimming away from the stimulus, and, in the number of times they swam away from the stimulus when a predator was present in both the environmental assessment context and in the foraging context. Expression of both of these behaviors was increased when a predator was present (time spent backward swimming: Table 3, Figure 3; number of times swam away: Table 4, Figure 4). These results support our findings that when a female sees a predator, she will attempt to increase the distance between herself and the stimulus monitor, as seen with the reduction of time spent in Zone 1. The increase in backward swimming may also indicate that in certain instances, females attempted to lengthen the distance between themselves and the predator, while remaining in a state in which they could visually access the predator and signal their alertness.

Further, females expressed predator-related plasticity in the amount of time they spent at the tank perimeter in both the environmental assessment context and the foraging context. Females decreased the amount of time they exhibited this behavior (*i.e.* time at tank perimeter) when a predator was present (Table 5, Figure 5). In our study, females were displaced from their home tank and placed in an experimental tank for testing. As has been found in a number of other study organisms, individuals will explore or assess their surroundings when placed in a foreign environment (Barnett 1958, Mettke-Hofmann *et al.* 2002). Although females were preconditioned to the test tank conditions, including the presence or absence of a predator, they could be expected to explore and access the test tank environment each time. Thus, a decrease in this behavior suggests that females are decreasing explorative behavior while the predator was present. This finding supports documented evidence that individuals decrease general activity and explorative behaviors after exposure to a predator (Lima and Dill 1990, Werner and Anholt 1993).

In both contexts, to a similar degree and direction, females expressed predator-related plasticity in two other anti-predator behaviors: time spent motionless oriented toward the stimulus and amount of time they spent watching and approaching the stimulus. Females increased the amount of time they spent motionless oriented toward the stimulus while the predator was present, in both the environmental assessment context and in the foraging context (Table 6, Figure 6). Previous work suggests that freezing or remaining motionless is a common anti-predator behavior expressed across many taxa (Williams and Brown 1991). By remaining motionless, individuals may be less likely to be detected by a predator and therefore, successfully decrease the risk of becoming prey (Werner and Anholt 1993, Martel and Dill 1995). Females also increased the amount of time they watched and approached the stimulus while a predator was present, in both of the environmental assessment context and the foraging context (Table 7, Figure 7). Approaching (or inspecting) a predator from a distance is a common behavior documented in many vertebrate species (Dugatkin 1988, Godin and Davis 1995). Though this behavior might seem risky, inspection behavior (*i.e.* watch and approach stimulus) can allow individuals to gather information regarding a predator's location and motivation (Pitcher *et al.* 1986, Dugatkin and Alfieri 2003). Additionally, individual predator inspection may in fact be a signal to the predator of both the individual's alertness and its ability to escape, in turn encouraging a deterred response from the predator (Godin and Davis 1995).

Vigilance is often described as an act in which an individual suspends their current activity to scan the environment or to assess present risk in their environment (Quenette 1990). The decrease in time spent at the tank perimeter in our study, coupled with the increase in time spent motionless oriented toward the stimulus and the time spent watching and approaching the stimulus, suggests an overall increase in vigilance in response to predator presence. Individuals in this study showed significant predator-related plasticity in multiple behaviors associated with vigilance, suggesting that female green swordtails' explorative and anti-predator behavioral responses are highly plastic in the two different non-social contexts.

We also examined whether body size and age at maturation significantly affects predator-related plasticity. Of the non-foraging behaviors measured, we found no evidence of any size- or age-at-maturation-related differences in predator-related behavioral plasticity. Large and small females of all ages of maturation responded similarly to predator-presence in non-foraging behaviors. Here, individual traits like body size at testing and age at maturation appear to have little to no effect on how individuals are responding to predator environments. Melie and Basolo (*in revision*) found that body size influences the expression of predator-related behavioral plasticity in male *X. hellerii*. Our results suggest that this is not the case for female *X. hellerii* in our study population.

Of the foraging behaviors that were measured in the foraging context, we found no predator-related behavioral plasticity in number of foraging pecks at the pellet in females of any size or age at maturation. However, there was a significant interaction of current body size on the number of non-pellet foraging pecks. Specifically, we found that smaller females expressed predator-related plasticity in non-pellet foraging pecks. Small females decreased the number of non-pellet foraging pecks when a predator was present, whereas large females did not (Table 8, Figure 8). While we did not detect behavioral plasticity in the number of foraging pecks on a pellet, the interaction of body size and treatment on the number of non-pellet foraging pecks suggests that smaller individuals may be forgoing resource acquisition behaviors in response to predator presence, thereby potentially increasing vigilance and reducing predation risk.

In this study, we set out to investigate whether female green swordtails adjust their behaviors in response to a predator in two non-social contexts, and how inter-individual variation in behavioral plasticity can be explained by current body size and/or age at maturation (*i.e*. P-like-alleles). Our study indicates that predator-related plasticity is expressed by female *X. hellerii*, however, little evidence was found to suggest that variation in two traits (current body size and/or age at maturation) affect the expression of behavioral plasticity in the environmental assessment context or the foraging context.

Of the ten behaviors that were measured in both the environmental assessment context and the foraging context, individuals expressed plasticity in six: time at Zone 1, time at tank perimeter, motionless oriented toward stimulus, watch and approach stimulus, backward swim away from stimulus, and swim away from stimulus. When we compared individual plasticity across contexts, we found no evidence of individual consistency of behavioral plasticity. The lack of correlation indicates that individuals who are highly plastic in one behavior within one context (*i.e.* environmental assessment) are not similarly plastic in the same behavior within the other context (*i.e.* foraging). This result indicates that although the population-level expression of predator-related behavioral plasticity is similar in both degree and direction for each context, within individuals, the expression of behavioral plasticity varied across contexts. Therefore, we did not find consistency of behavioral plasticity across the two contexts.

This study allowed us to take a detailed look at how individual females adjust their behaviors in response to predator presence when a foraging opportunity is present, or absent. Although we found little effect of the two individual traits, current body size and age at maturation, on the expression of predator-related behavioral plasticity, it does appear an individual's expression of behavioral plasticity varies across contexts. Our results that predator-related plasticity is similar at the population level across contexts, but not at the individual level, signifies the importance of considering and testing the expression of plasticity at the individual level. Further study is needed to determine whether individual variation in traits other than current body size and maturation age can help explain inter- and intra-individual variation in patterns of predator-related behavior plasticity. Continued exploration may yield valuable information on how behavioral plasticity evolves within and across populations.

ACKNOWLEDGEMENTS

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REFERENCES

Atwell A, Wagner WE. 2014. Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. Anim. Behav. 98:177-183.

Barnett SA. 1958. Exploratory behaviour. Brit. J. Psychol. 49:289-310.

Basolo AL. 1994. The dynamics of Fisherian sex-ratio evolution: theoretical and experimental investigations. Am. Nat. 144:473-490.

Basolo AL. 1996. The phylogenetic distribution of a female preference. Syst. Biol. 45: 290-307.

Basolo AL. 2008. Evolution of pleiotropic alleles for maturation and size as a consequence of predation. Biol. Lett. 4:200–3.

Basolo AL, Wagner WE. 2004. Covariation between predation risk, body size and fin elaboration in the green swordtail, *Xiphophorus helleri*. Biol. J. Linn. Soc. 83:87–100.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Version: 1.1-12. J. Stat. Softw. 67.

Bazely DR. 1987. Snow Buntings feeding on leaves of salt-marsh grass during spring migration. The Condor. 89:190-192.

Beery AK, Zucker I. 2011. Sex Bias in Neuroscience and Biomedical Research. Neurosci. Biobehav. Rev. 35:565–572.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57:289–300.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13:115-155.

Brown C, Braithwaite VA. 2004. Size matters: a test of boldness in eight populations of the poeciliid Brachyraphis episcopi. Anim. Behav. 68:1325–1329.

Brown JS, Kotler BP, Smith RJ, Wirtz WO. 1988. The effects of owl predation on the foraging behavior of heteromyid rodents. Oecologia 76: 408-415.

Campton DE. 1992. Heritability of body size of green swordtails, Xiphophorus helleri: I. Sib analyses of males reared individually and in groups. J. Hered. 83:43–48.

Clarke A, Johnston NM. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol. 68: 893-905.

Dill LM. 1983. Adaptive flexibility in the foraging behavior of fishes. Can J of Fish Aquat Sci. 40:398-408.

Dingemanse NJ, Wolf M. 2013. Between-individual differences in behavioural plasticity within populations: causes and consequences. Anim. Behav. 85:1031–1039.

DiSciullo, RA Basolo AL. Inter-individual variation in predator-related behavioral plasticity expressed by female green swordtails (Xiphophorus hellerii). *in prep*.

Domenici PAOLO, Blake RW. 1993. Escape trajectories in angelfish (Pterophyllum eimekei). J. Exp. Biol.. 177: 253-272.

Domenici P. 2001. The scaling of locomotor performance in predator–prey encounters: from fish to killer whales. Comp Biochem Physiol A Mol Integr Physiol. 131: 169-182.

Dugatkin LA. 1988. Do guppies play TIT FOR TAT during predator inspection visits?. Behav Ecol Sociobiol. 23:395-399.

Dugatkin LA, Alfieri MS. 2003. Boldness, behavioral inhibition and learning. Ethol Ecol Evol. 15:43-49.

Eklöv P, Persson L. 1996. The response of prey to the risk of predation: proximate cues for refuging juvenile fish. Anim. Behav. 51: 105-115.

Froese R, Pauly D. 2016. FishBase. World Wide Wide Electron. Publ. http://fishbase.org
Ghalambor CK, Martin TE. 2002. Comparative manipulation of predation risk in incubating birds reveals variability in the plasticity of responses. Behav. Ecol. 13:101-108.

Godin JGJ. 1995. Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). Oecologia 103:224-229.

Godin JGJ, Davis SA. 1995. Who dares, benefits: predator approach behaviour in the guppy *(Poecilia reticulata)* deters predator pursuit.
Proc. R. Soc. Lond., B., Biol Sci. 259:193-200.

Gowaty PA. 1997. Principles of females' perspectives in avian behavioral ecology. J. Avian Biol: 95-102.

Johansson J, Turesson H, Persson A. 2004. Active selection for large guppies, Poecilia reticulata, by the pike cichlid, Crenicichla saxatilis. Oikos 105:595-605.

Johnson JB, Basolo AL. 2003. Predator exposure alters female mate choice in the green swordtail. Behav. Ecol. 14:619-625.

Johnson JC, Sih A. 2005. Precopulatory sexual cannibalism in fishing spiders (Dolomedes triton): a role for behavioral syndromes. Behav Ecol Sociobiol. 58:390-396.
Jones G. 1990. Prey selection by the greater horseshoe bat (Rhinolophus ferrumequinum): optimal foraging by echolocation?. J Anim Ecol. 587-602.

Kallman KD. 1983. The sex determining mechanism of the Poeciliid fish, Xiphophorus montezumae, and the genetic control of the sexual maturation process and adult size. Copeia 1983:755-769.

Kallman KD. 1989. Genetic control of size at maturity in Xiphophorus. In: Meffe GK, Snelson, FF, editors. Ecology and evolution of lifebearing fishes (Poeciliidae). Englewood Cliffs, NJ: Prentice Hall. p. 163–184.

Kallman KD, Borkoski V. 1978. A sex-linked gene controlling the onset of sexual maturity in female and male platyfish (Xiphophorus maculatus), fecundity in females and adult size in males. Genetics: 89:79-119.

Kallman KD, Schreibman MP, Borkoski V. 1973. Genetic control of gonadotrop differentiation in the platyfish, Xiphophorus maculatus (Poeciliidae). Science 181:678–80.

Krause J, Loader SP, McDermot J, Ruxton GD. 1998. Refuge use by fish as a function of body length–related metabolic expenditure and predation risks. Proc R Soc Lond [Biol]. 265: 2373-2379.

Lima SL. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv Study Behav. 27:215-290.

Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool. 68:619-640.

Martel G, Dill LM. 1995. Influence of movement by coho salmon (Oncorhynchus kisutch) parr on their detection by common mergansers (Mergus merganser). Ethology. 99:139-149.

McLandress MR, Raveling DG. 1981. Changes in diet and body composition of Canada geese before spring migration. The Auk. 65-79.

Melie A, Basolo AL. Plasticity in a preexisting mating bias in response to variation in predation. *in revision*.

Mettke-Hofmann C, Winkler H, Leisler B. 2002. The significance of ecological factors for exploration and neophobia in parrots. Ethology. 108:249-272.

Nussey DH, Wilson AJ, Brommer JE. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. J. Evol. Biol. 20: 831-844.

Péter A. 2016. Solomon Coder. Milano. Version: beta 16.06.26. http://solomoncoder.com

Pitcher TJ, Green DA, Magurran AE. 1986. Dicing with death: predator inspection behaviour in minnow shoals. J. Fish Biol. 28:439-448.

Pfennig KS. 2007. Facultative mate choice drives adaptive hybridization. Science. 318: 965-967.

Pocklington R, Dill LM. 1995. Predation on females or males: who pays for bright male traits?. Anim. Behav. 49:1122-1124.

Rauchenberger M. Kallman KD, Morizot DC. 1990. Monophyly and geography of the Rio Panuco basin swordtails (genus Xiphophorus) with descriptions of four new s pecies. American Museum of Natural History.

Rosen DE. 1960. Middle-American poeciliid fishes of the genus Xiphophorus (Vol. 5). University of Florida.

Ryan MJ, Causey BA. 1989. "Alternative" mating behavior in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus* (Pisces: Poeciliidae). Behav. Ecol. Sociobiol. 24:341–348.

Quenette PY. 1990. Functions of vigilance behaviour in mammals: a review. Acta Oecologica. 11:801-818.

Sih A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. The Am. Nat. 139: 1052-1069.

Sih A, Bell A, and Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evolut. 19:372-378

Smith SB, McPherson KH, Backe JM, Pierce BJ, Podlesak, DW, McWilliams SR. 2007. Fruit quality and consumption by songbirds during autumn migration. Wilson J Ornithol. 119: 419-428.

Sogard SM. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull. Mar. Sci. 60: 1129-1157.

Stein RA. 1977. Selective Predation, Optimal Foraging, and the Predator-Prey Interaction Between Fish and Crayfish. Ecology. 58:1237-1253.

Stephens DW, Brown JS, Ydenberg RC. 2007. Foraging: behavior and ecology. University of Chicago Press.

Tinghitella RM, Weigel EG, Head M, Boughman JW. 2013. Flexible mate choice when mates are rare and time is short. Ecol. Evol. 3:2820-2831.

Werner EE, Anholt BR. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. 142: 242-272.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Syst. 20:249–278.

Williams P, Brown J. 1991. Developmental changes in foraging-predator avoidance trade-offs in larval lumpfish Cyclopterus lumpus. Mar Ecol Prog Ser. 76:53-60.

Wooster D, Sih A. 1995. A review of the drift and activity responses of stream prey to predator presence. Oikos. 3-8.

Zach R, Falls JB. 1978. Prey selection by captive ovenbirds (Aves: Parulidae). J. Anim. Ecol. 929-943.

Zimmerer EJ, Kallman KD. 1989. Genetic basis for alternative reproductive tactics in the pygmy swordtail, Xiphophorus nigrensis. Evolution 43:1298–1307.

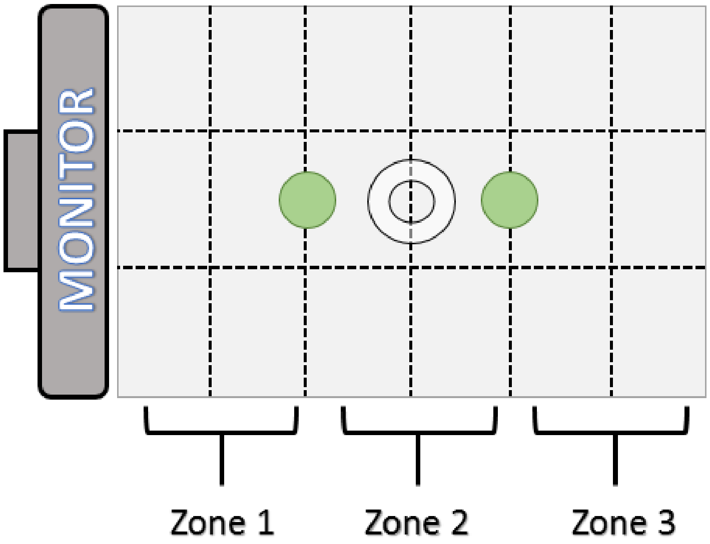

Figure 1. Experimental set-up to investigate predator-related behavioral plasticity in female *X. hellerii*. A monitor was randomly placed at one end of tank to display the treatment stimulus. Zone 1 indicates the 2 x 6 grid of squares closest to the monitor displaying the stimulus. The filled circles represent the refuges. The ring in the center of the tank represents the foraging ring.

Table 1. Behavioral responses and descriptions used to investigate predator-related behavioral plasticity in the environmental assessment context and the foraging context.

Name of Behavior	**Measurement Type**	**Type of Behavior**	**Description of Behavior**
Measured in the Environmental Assessment Context and in the Foraging Context			
Time in Zone 1	Duration (sec)	Anti-predator	Amount of time spent in 1/3 of tank closest to stimulus monitor
Time at tank perimeter	Duration (sec)	Explorative	Fish swims up and down, or back and forth alongside any of the four glass tank walls
Backward swim away from stimulus	Duration (sec)	Anti-predator	Fish swims backwards away from stimulus monitor; head faces stimulus monitor
Swim away from stimulus	Count	Anti-predator	Fish swims away from stimulus monitor at a normal pace (*i.e.* not darting); caudal fin faces stimulus monitor
Motionless oriented toward stimulus	Duration (sec)	Anti-predator	Fish remains motionless and oriented toward the stimulus monitor, regardless of whether at the surface, in the water column or near the bottom
Watch and approach stimulus	Duration (sec)	Anti-predator	Fish watches and approaches stimulus monitor
Dart away from stimulus	Count	Anti-predator	Fish swims away quickly from stimulus monitor; caudal fin faces stimulus monitor
Refuge use	Duration (sec)	Anti-predator	Fish hides within refuge, regardless of orientation
Motionless under refuge	Duration (sec)	Anti-predator	Fish remains motionless under refuge, regardless of orientation
Movement	Count	Explorative	Number of grid squares traversed
Measured in the Foraging Context			
Non-pellet foraging peck	Count	Foraging	Fish pecks at substrate and/or refuge
Foraging peck on pellet	Count	Foraging	Fish pecks food pellet regardless of whether at the surface, in the water column or near the bottom

Table 2. Linear mixed models examining the effects of the predictors on female time in Zone 1 (zone closest to stimulus monitor) for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Time in Zone 1					
Environmental Assessment Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	23.104	14.895	2.302	0.129	0.579
Treatment	**-103.642**	**22.778**	**16.070**	**< 0.001**	**< 0.001**
Age at Maturation	6.092	14.852	0.165	0.685	0.872
Random Effects	*Variance*	*SE*			
Focal Fish	2841.753	41.399			
Family	37.653	6.104			
Foraging Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	1.103	15.977	0.005	0.945	0.946
Treatment	**-87.931**	**22.679**	**12.323**	**< 0.001**	**< 0.001**
Age at Maturation	-4.065	16.024	0.064	0.800	0.946
Random Effects	*Variance*	*SE*			
Focal Fish	4026.982	45.113			
Family	0.000	0.000			

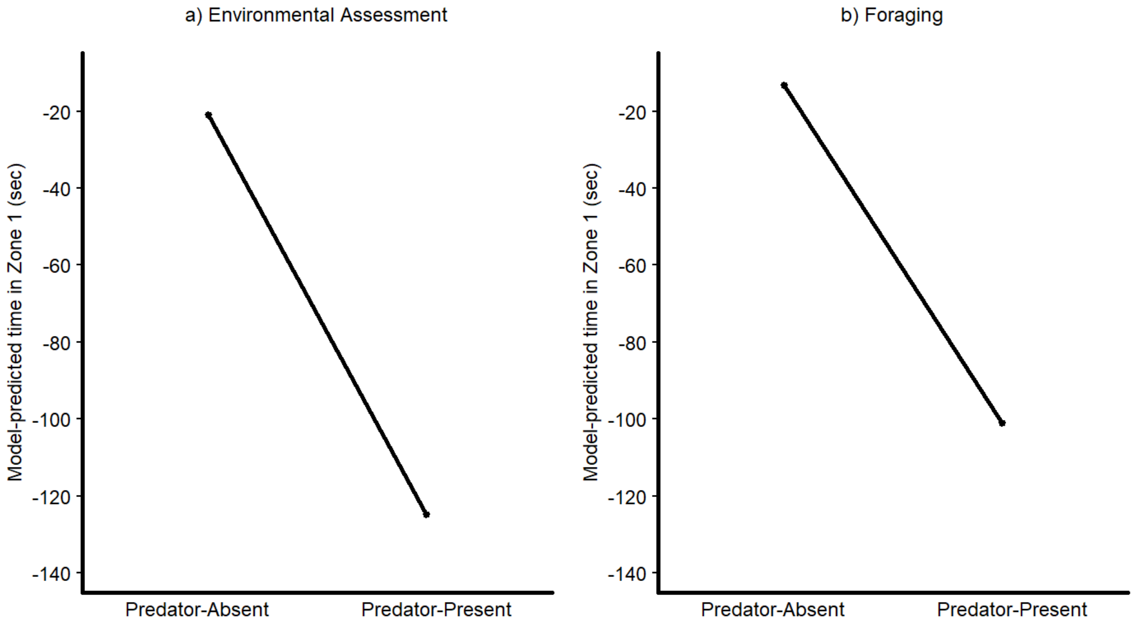

Figure 2. Model-predicted behavioral reaction norms representing the predator-related plasticity in the amount of time spent in Zone 1 for the two contexts: a) environmental assessment and b) foraging.

Table 3. Linear mixed models examining the effects of the predictors on female time spent backward swimming away from stimulus for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Backward swim away from stimulus					
Environmental Assessment Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	-2.322	1.970	1.375	0.241	0.667
Treatment	**24.970**	**3.895**	**31.956**	**< 0.001**	**< 0.001**
Age at Maturation	-0.962	1.965	0.239	0.625	0.844
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			
Foraging Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	-0.29	1.115	0.068	0.795	0.946
Treatment	8.844	2.226	14.113	**< 0.001**	**< 0.001**
Age at Maturation	1.207	1.118	1.156	0.282	0.665
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

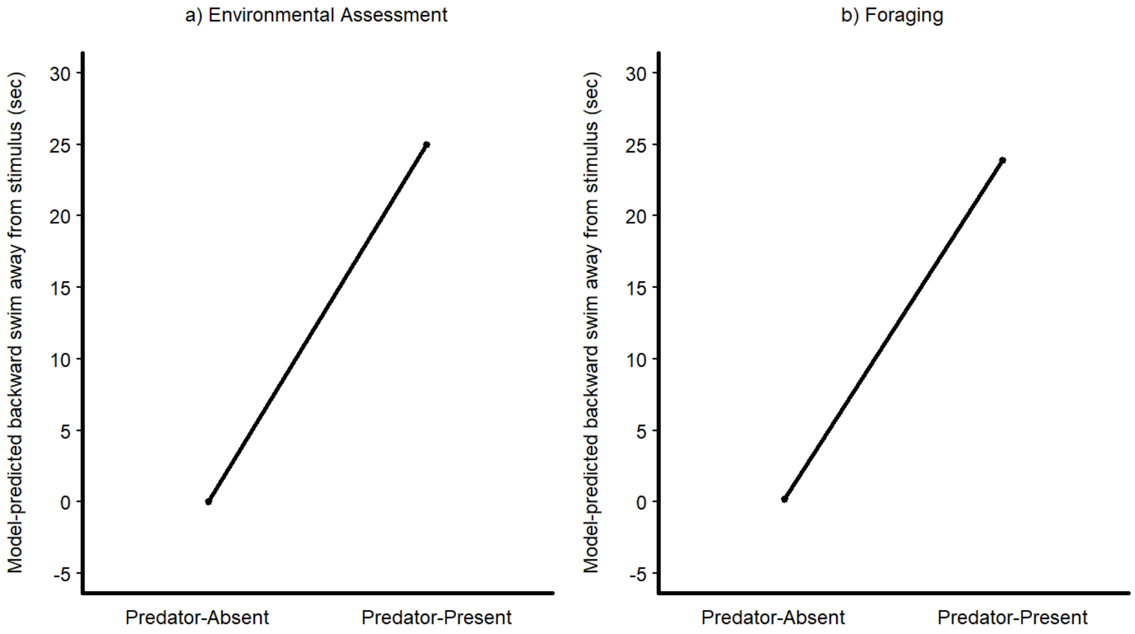

Figure 3. Model-predicted behavioral reaction norms representing the predator-related plasticity in the amount of time spent backward swimming away from stimulus for each context: a) environmental assessment and b) foraging.

Table 4. Linear mixed models examining the effects of the predictors on number of times females swam away from the stimulus for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Swim away from stimulus					
Environmental Assessment Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	-1.731	1.199	1.958	0.162	0.648
Treatment	**10.612**	**2.357**	**17.584**	**< 0.001**	**< 0.001**
Age at Maturation	0.187	1.195	0.024	0.876	0.970
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.622	0.788			
Foraging Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	-4.07	2.161	3.451	0.643	0.378
Treatment	**23.65**	**4.316**	**24.623**	**< 0.001**	**< 0.001**
Age at Maturation	-0.252	2.168	0.013	0.908	0.946
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

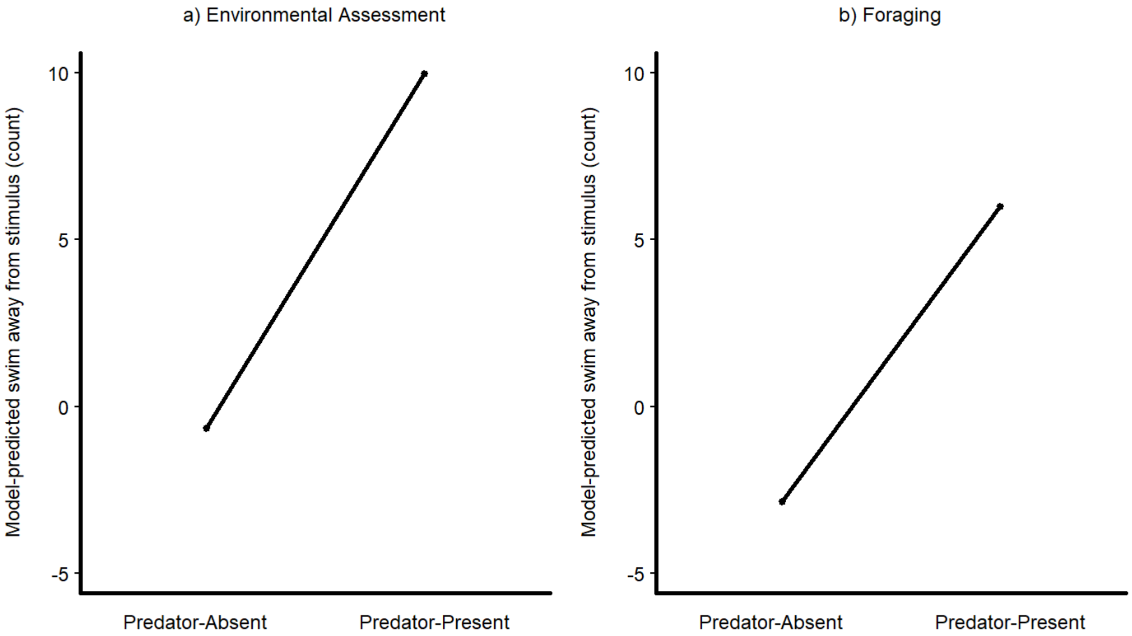

Figure 4. Model-predicted behavioral reaction norms representing the predator-related plasticity in the number of times a female swam away from the stimulus for each context: a) environmental assessment and b) foraging.

Table 5. Linear mixed models examining the effects of the predictors on female time at tank perimeter for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Time at tank perimeter					
		Environmental Assessment Context			
Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size	0.563	16.460	0.001	0.973	0.973
Treatment	**-190.218**	**32.537**	**27.542**	**< 0.001**	**< 0.001**
Age at Maturation	-28.443	16.414	2.936	0.087	0.462
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			
		Foraging Context			
Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size	-7.910	13.603	0.337	.561	0.907
Treatment	**-187.75**	**27.162**	**35.689**	**< 0.001**	**< 0.001**
Age at Maturation	-14.762	13.643	1.16	0.281	0.665
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

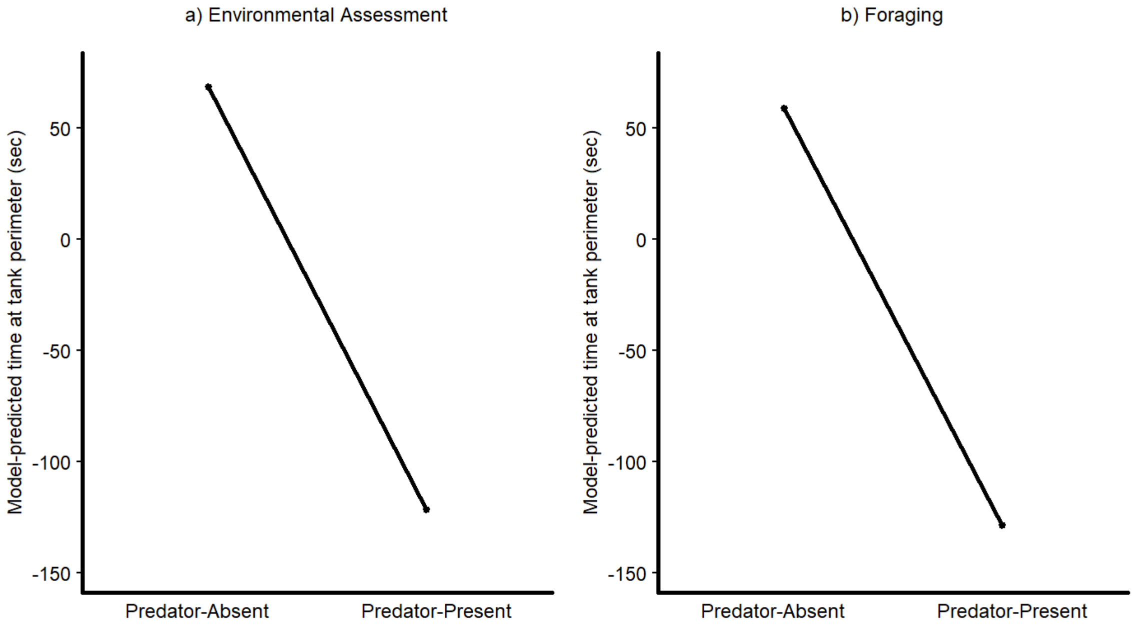

Figure 5. Model-predicted behavioral reaction norms representing the predator-related plasticity in the amount of time at tank perimeter for the two contexts: a) environmental assessment and b) foraging.

Table 6. Linear mixed models examining the effects of the predictors on female time spent motionless toward stimulus for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Motionless oriented toward stimulus					
Environmental Assessment Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	7.568	11.618	0.423	0.515	0.816
Treatment	**138.867**	**22.966**	**29.094**	**< 0.001**	**< 0.001**
Age at Maturation	-9.747	11.586	0.704	0.401	0.816
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			
Foraging Context					
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size	-0.835	12.437	0.005	0.946	0.946
Treatment	**173.256**	**24.078**	**37.079**	**< 0.001**	**< 0.001**
Age at Maturation	-12.388	12.473	0.971	0.324	0.676
Random Effects	*Variance*	*SE*			
Focal Fish	295.178	16.659			
Family	0.000	0.000			

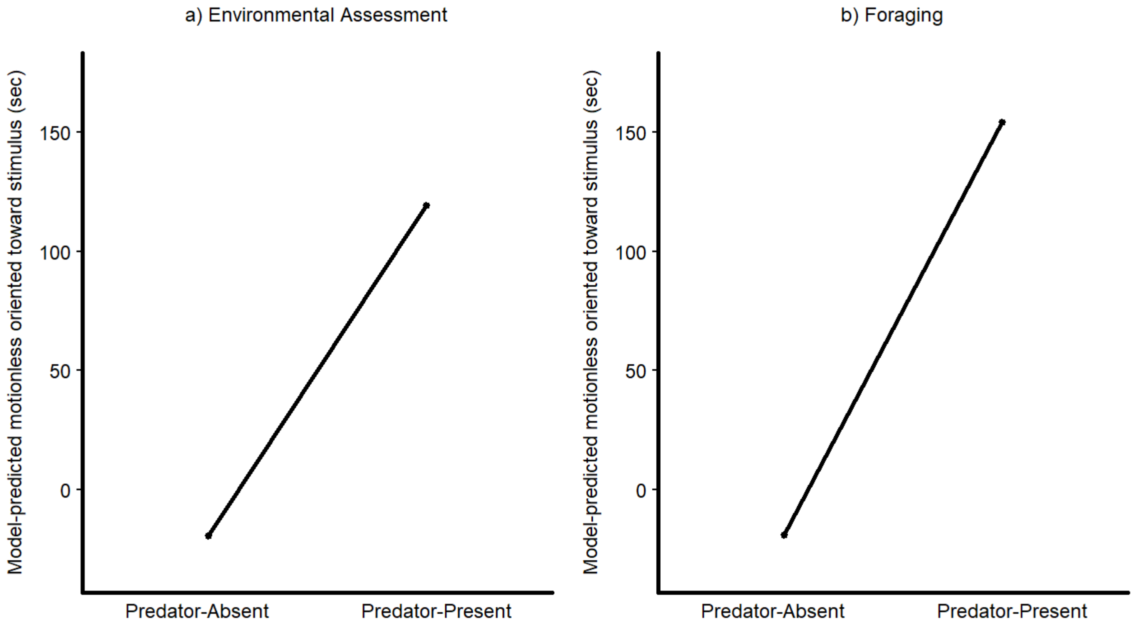

Figure 6. Model-predicted behavioral reaction norms representing the predator-related plasticity in the amount of time spent motionless toward stimulus for the two contexts: a) environmental assessment and b) foraging.

Table 7. Linear mixed models examining the effects of the predictors on female time spent watching and approaching the stimulus for the environmental assessment context and the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Watch and approach stimulus					
Environmental Assessment Context					
Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size	-2.610	4.603	0.321	0.571	0.844
Treatment	**58.533**	**9.099**	**32.127**	**< 0.001**	**< 0.001**
Age at Maturation	5.602	4.590	1.473	0.225	0.667
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			
Foraging Context					
Fixed Effects	*Coefficient*	*SE*	X^2	*P*	*Q*
Body Size	6.194	5.357	1.31	0.252	0.665
Treatment	**48.1**	**10.094**	**18.563**	**< 0.001**	**< 0.001**
Age at Maturation	6.586	5.372	1.469	0.226	0.665
Random Effects	*Variance*	*SE*			
Focal Fish	99.998	9.438			
Family	0.000	0.000			

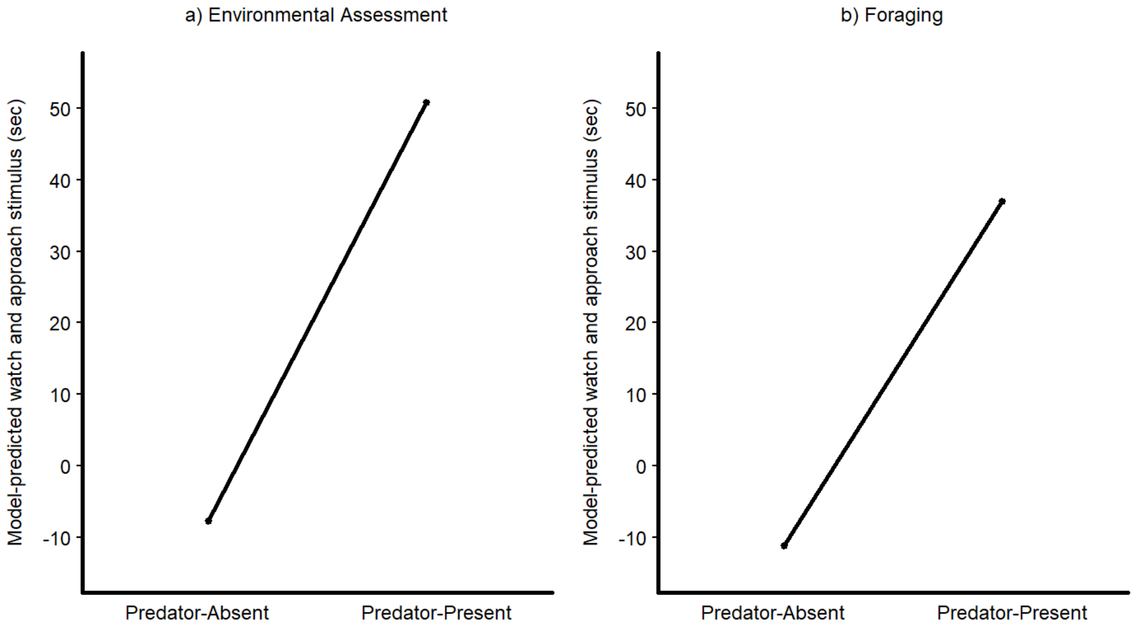

Figure 7. Model-predicted behavioral reaction norms representing the predator-related plasticity in the amount of time spent watching and approaching stimulus for the two contexts: a) environmental assessment and b) foraging.

Table 8. Linear mixed model examining the effects of the predictors on the number of non-pellet foraging pecks within the foraging context. Focal fish identity and family identity were included as random effects. Q is the Benjamini-Hochberg adjusted p-value.

Behavior Non-pellet Foraging Pecks					
		Foraging Context			
Fixed Effects	*Coefficient*	*SE*	*X^2*	*P*	*Q*
Body Size at Testing * Age at Maturation * Treatment	-0.365	1.989	0.034	0.854	0.946
Body Size at Testing * Treatment	**4.733**	**1.527**	**8.949**	**0.003**	**0.032**
Body Size at Testing * Age at Maturation	1.221	0.995	1.490	0.222	0.665
Treatment * Age at Maturation	-0.554	1.532	0.130	0.718	0.946
Body Size	-0.858	0.829	1.062	0.303	0.670
Treatment	**-5.938**	**1.655**	**11.723**	**0.001**	**0.012**
Age at Maturation	-1.210	0.831	2.083	0.149	0.626
Random Effects	*Variance*	*SE*			
Focal Fish	0.000	0.000			
Family	0.000	0.000			

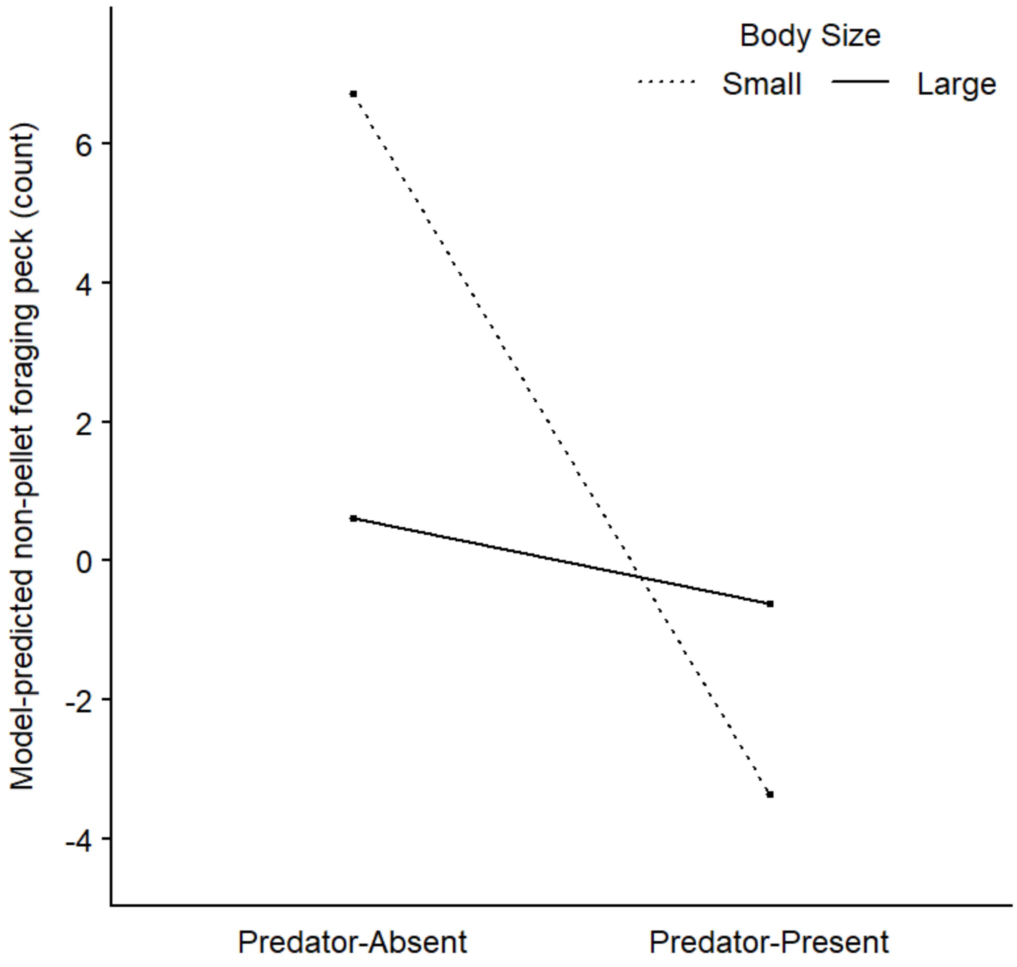

Figure 8. Model-predicted behavioral reaction norm representing differences in the predator-related plasticity in the number of non-pellet foraging pecks within the foraging context from females of differing size at testing.

Table 9. Pearson’s product-moment correlation between plasticity measures of the same behavior in the two non-social contexts. Q is the Benjamini-Hochberg adjusted p-value.

Behavioral plasticity measured	*df*	*P*	*Q*	*Correlation Coefficient*
Time spent in zone 1	30	0.607	0.721	0.095
Time at tank perimeter	30	0.242	0.711	0.213
Motionless oriented towards stimulus	30	0.172	0.689	0.247
Watch and approach stimulus	30	0.643	0.721	-0.085
Swim away from stimulus	30	0.960	0.960	-0.009
Back-swim away from stimulus	30	0.315	0.711	0.183