Original Article

Effect of prey personality depends on predator species

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The ability of prey to respond to predators is especially important in human-altered habitats, where prey are faced with changing predator regimes. A growing body of research has shown the important ecological impacts of intraspecific, individual differences in behavior, but few studies have shown direct fitness consequences of behavioral types in a predation context. We examined behavioral syndromes in the endemic Largespring mosquitofish (*Gambusia geiseri)* and how the behavioral type of individual *G. geiseri* affected their behavior and survival when exposed to 3 different species of predators. We measured latency to emerge from a container, time spent moving, and tank area used for individual *G. geiseri* in the presence and absence of predators. We then measured behavior and survival of these same individuals in one-on-one predation trials. We found that behavioral types and correlations between latency to emerge, time spent moving, and area used were consistent regardless of predator presence. Behavioral type did not predict survival of the predation trial. However, higher behavioral scores correlated with more escapes from predatory *Fundulus grandis*. We argue that this result indicates that active/exploratory fish have a greater ability than their conspecifics to escape this species, which is a novel predator. Our results illustrate the potential importance of considering individual differences in behavior in studying the impacts of introduced predator species.

Key words: **antipredator behavior, behavioral syndrome, boldness, invasive predator, novel predator.**

INTRODUCTION

Individual differences in behavior among conspecifics can have far-reaching ecological impacts. These impacts include contributing to enhanced invasion success by pest species (Cote et al. 2010, 2011), modifying the magnitude and nature of species interactions (Pruitt and Ferrari 2011; Pruitt, Cote, et al. 2012), and limiting the presence and abundance of species in different habitats (reviewed in Sih et al. 2012). Behavioral syndromes are individual differences in behavior that are consistent within an individual across environmental situations or life history contexts (Sih et al. 2004a). Behavioral syndromes often encompass multiple correlated traits, such that an individual may display a behavioral type that is more bold, active, and exploratory relative to its conspecifics (Cote et al. 2010, 2011). Behavioral syndromes can act as a constraint on behavioral responses and cause behaviors that are advantageous in one situation to carry over into situations in which they are maladaptive (Sih et al. 2003, 2004a; Johnson and Sih 2005). However, behavioral syndromes need not preclude plasticity completely. For example, individuals' rank ordered behavioral tendencies could be maintained even in instances where they exhibit a high degree of behavioral plasticity (Sih et al. 2004a,b). Indeed, behavioral plasticity itself can be an important trait that varies among individuals (Nussey et al. 2007; Dingemanse et al. 2010; Ensminger and Westneat 2012).

Ecological consequences of behavioral syndromes are easily characterized in predator–prey interactions. For example, when shoals of guppies, *Poecilia reticulata*, are exposed to a predator, individual fish with an active/bold/exploratory behavioral type survive longer than their shy shoal mates (Smith and Blumstein 2010). Further, prey from high predation environments often exhibit stronger correlations between behavioral traits (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007), and in some instances, they display more bold, active, exploratory behavior than prey from low predation environments (Reale and Festa-Bianchet 2003; Brown et al. 2005; Archard and Braithwaite 2011). In contrast, in other systems, high predation environments are associated with reduced boldness, activity, and exploration (Hedrick and Riechert 1989; Riechert and Hedrick 1990; Biro et al. 2004). Although population-level differences in behavioral syndromes between environments with different predator regimes imply predator-induced selection on behavioral types, experimental demonstrations that predators differentially consume particular behavioral types are comparatively rare (Bell and Sih 2007, Smith and Blumstein 2010; Pruitt, Stachowicz, et al. 2012).

Individual variation in behavior is especially relevant in habitats that have been invaded by novel predators. Prey are severely

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impacted by invasion when they are not able to respond effectively to a novel predator (Courtenay and Moyle 1992; Rehage et al. 2005, 2009; Sih et al. 2010). The study of human-induced rapid environmental change must begin to consider behavioral syndromes, because of the potential for behavioral syndromes to affect an animal's ability to respond rapidly to novel selection pressures (Sih 2013). The introduction of a novel predator can create a situation where prey must learn to recognize novel cues and/or adopt new escape strategies to respond successfully to the predator (Kelley and Magurran 2003; Rehage et al. 2009; Sih et al. 2012). Previous research suggests that bold fish may fare better in these circumstances, because they are more likely to perform predator inspections, allowing them to gather information about predation risk (Brosnan et al. 2003; Dugatkin and Alfieri 2003; Pellegrini et al. 2012). Thus, there may be differences in the ability of prey of different behavioral types to gather information and develop behaviors to cope with a novel predator.

Here, we examine behavioral syndromes in a prey species, the Largespring Mosquitofish (*Gambusia geiseri*), and how exposure to 3 different species of predators affect prey behavior and survival. We examine individuals' behavior in a novel tank environment, including latency to emerge, time spent moving, and area of the tank used, because these behaviors are relevant to a predation context, and because these behaviors have been used to examine correlations among boldness, activity, and exploration in many fish species (Bell et al. 2009; Wilson and Godin 2009; Cote et al. 2010; Smith and Blumstein 2010; Cote et al. 2011). Additionally, as mentioned above, risk-taking behavior and general activity have been linked with prey survivorship in a variety of predator–prey systems (Smith and Blumstein 2010; Pruitt, Stachowicz, et al. 2012). Specifically, we test the following hypotheses: 1) *G. geiseri* exhibit correlated behaviors, 2) behavioral syndromes in *G. geiseri* are consistent regardless of predator presence, and 3) the behavioral type of an individual can predict survival during direct exposure to predators. We can then assess whether these different predator species could increase the prevalence of some prey behavioral types, either through plastic changes in individual behavior, or through differential consumption of certain behavioral types. Further, understanding the effects of novel predator species on prey within the context of intraspecific behavioral variation is increasingly important as human-altered habitats present new predation pressures that threaten the persistence of native prey species.

MATERIALS AND METHODS
Study species

G. geiseri is endemic to the headwaters of the San Marcos and Comal Rivers in Central Texas and is exposed to variety of predators in these habitats. The Green Sunfish (*Lepomis cyanellus*) is a native piscivorous predator in Central Texas (Hubbs et al. 1991) and thus shares an evolutionary history with *G. geiseri*. The Rio Grande Cichlid (*Herichthys cyanoguttatus*) is an invasive predator found in the San Marcos Spring. Originally native to the Rio Grande River and Northeastern Mexico, *H. cyanoguttatus* has spread through deliberate introductions by the US Fish and Wildlife Service and accidental aquarium releases throughout Texas and the southern United States since 1928 (Hubbs et al. 1978). Invasive cichlids have been present in San Marcos Spring for several decades, and it is possible that rapid evolution of the prey species may already have occurred in response to this predator (Strauss et al. 2006). Therefore, we also included a novel, allopatric predator in this study, the Gulf killifish (*Fundulus grandis*). *F. grandis* is native to fresh and brackish

waters along the coasts of Northeastern Florida and the Gulf of Mexico. *F. grandis* can tolerate a range of salinity and has been introduced into many freshwater environments in Texas and New Mexico through bait-bucket releases (Hubbs et al. 1991). In its current distribution, *F. grandis* co-occurs with the Western mosquitofish (*Gambusia affinis*) but is novel to our focal species, *G. geiseri* (Hillis et al. 1980; Thomas et al. 2007).

Collection and laboratory maintenance

We used adult, wild-caught *G. geiseri* (17–31 mm) collected with dip and seine nets from Spring Lake, San Marcos River, Hays County, TX (29–53′41″ N, 097-55′49″ W) and Comal Springs, Comal County, TX (29–42′37″ N, 098-07′49″ W) in September 2011, January 2012, and January 2013. We transported fish back to the laboratory in insulated 5 gallon buckets. We used only female fish for this study because their larger body size makes them easier to inject with elastomer tags, and because we found no difference in male and female behavior in *G. geiseri* in a similar study (Blake C, unpublished data). We began testing fish after they had acclimated to the lab for between 5 and 12 weeks in isolated female tanks. Once we began testing, we maintained mosquitofish in groups of 4 in 19-L tanks on a 14:10 h light cycle at 25–27 °C and fed flake food (Ocean Star International) ad libitum once a day. We collected sympatric *H. cyanoguttatus* and *L. cyanellus* from Spring Lake and collected allopatric *F. grandis* from Galveston Bay, Galveston County, TX (29-12′42″ N, 94-57′06″ W). We used juveniles (5–8 cm SL) of *L. cyanellus* and *H. cyanoguttatus* because adults of these 2 species grow to be much larger than *F. grandis*, and we wanted to ensure that we used similar-sized predators for all treatments. We maintained 3 predators of each species in individual tanks on a 14:10 h light cycle and fed them pellet food (Purina Aqua Max 200) ad libitum once a day.

Experimental protocol

We tested the same fish throughout all 3 experiments, although control group fish were not a part of the final predation trials. We tested a total of 60 treatment group fish and 70 control group fish. Some fish had to be eliminated from certain analyses due to missing data from video recording errors. At least 1 week before testing, we injected individuals with 1 of 4 colors of elastomer (Northwest Marine Technology, WA). Mortality from elastomer tagging was less than 10% and generally occurred within the first 48 h after injections. We housed fish in tanks with 3 other individuals of different elastomer colors to provide a natural shoaling environment, while still being able to distinguish individuals throughout the duration of all 3 experiments. We conducted initial experiments between February and June 2012 and tested additional control fish (50 of the total 70) in April to May 2013.

Experiment one: Behavioral correlations in *G. geiseri*

We conducted behavioral trials in a novel tank environment. The terminology and methodology of testing boldness, activity, and exploration varies throughout the literature on behavioral syndromes, and behavior in a novel environment is sometimes included in a wider definition of an exploratory syndrome (Réale et al. 2007). Here, we follow the methods of Cote et al. (2010) but will use terminology specific to the variables measured in our behavioral assay rather than general trait terms. We tested fish in a shallow, white plastic tank (52 × 35 cm). We filled the tank with 8 cm of water, so that movement was primarily horizontal. Each of these tanks also contained a clear plastic enclosure (9 × 9 × 18 cm) that remained

empty for experiment one but was placed in the tank to maintain a consistent set up between the first and second experiment. Water temperature ranged from 25 to 27 °C. We placed a mosquitofish in an opaque container and allowed the fish to acclimate for 5 min before we opened the door of the container. We calculated *latency to emerge* as the log of the maximum time allowed to exit (10 min) minus the log of the time until the fish exited the container into the novel tank environment (Cote et al. 2010). Thus, a higher value for latency to emerge indicates that the fish entered the novel tank environment sooner. We calculated *time spent moving* as the proportion of the 5-min observation period the individual spent moving and *area used* as the proportion of the tank area explored (detailed below).

We recorded behavioral assays in EvoCam at 1 frame per second, using Dynex 1.3 megapixel webcams mounted above the experiment tanks. We processed videos as virtual stacks in Image J to obtain (x, y) coordinates of the individuals' position in the tank during each second of the observation period. We defined "movement" as any change in position greater than 1 cm from the previous frame and calculated time spent moving as the proportion of time during which movement occurred. We divided the tank area into 2×2 cm grid sections and defined exploration as the proportion of these grids the fish swam through during the 5-min trial. Although exploration and time spent moving are related metrics, it is possible for an individual to obtain a high value for time spent moving and a low value for area used by moving back and forth in a small area of the tank.

Experiment two: Behavioral correlations in the presence of predators

Using the same focal individuals, we performed a second behavioral assay with a predator present in the experimental tank. We randomly assigned the 60 treatment group fish to 1 of 3 predator treatments: 1) native Green Sunfish (*L. cyanellus*), 2) invasive Rio Grande Cichlid (*H. cyanoguttatus*), or 3) novel Gulf Killifish (*F. grandis*). We also retested the 70 control group fish, without any predators present, in order to measure repeatability of behavior between consistent trial conditions. During the behavioral assays of treatment group fish, we placed the predator in the experimental tank inside a clear, water-permeable enclosure ($9 \times 9 \times 18$ cm), which provided the focal individual with both visual and chemical predator cues during the trial. We changed the arrangement of the opaque acclimation container and predator enclosure inside the testing tank between the first and second experiment to maintain a similar degree of novelty of the tank during the second trial. We tested all individuals between 4 and 6 weeks after experiment one.

Experiment three: Behavioral correlations and predation

The final stage of the study was a one-on-one predation trial using the same focal individuals. The treatment group fish were exposed to the same predator treatment they had been assigned during the behavioral assays with predators present. We filled 189.3-L predation tanks ($45 \times 90 \times 40$ cm) with 25 cm of water and added gravel substrate, 2 sponge filters, 1 artificial plant, and 1 flower pot (15 cm diameter) to provide hiding places and structure. To standardize hunger levels, we did not feed predators for 24 h preceding testing and used each predator individual only once per day. Before the start of each trial, we placed a clear, water-permeable, plexiglass divider in the middle of the tank and placed one focal mosquitofish on the empty side of the tank to allow 5 min of acclimation before

direct exposure to the predator. We began the trial by removing the barrier and allowed the predator individual 20 min to interact with the focal individual. We recorded exact survival time and also recorded survival as a binomial variable based on whether the focal individual was still alive at the end of this 20-min trial. A single consistent observer watched trials through a mirror to prevent the observer from affecting the behavior of the test subjects, and we recorded the vertical position (top third of the tank, middle, or bottom) of the focal fish every 30 s. We counted the number of escapes from predator attacks before the fish was either consumed or 20 min had passed. We defined an escape as any biting attack by the predator that did not result in the death of the focal individual. Predation trials took place between 4 and 6 weeks following experiment two.

Statistical analyses

We used Spearman rank correlations to determine if the measured behaviors were correlated and principal components analysis (PCA: on the correlation matrix) to calculate behavioral scores for individual fish. We used the random skewers method to compare the correlation matrices from the PCA of the first and second experiments (Cheverud et al. 1993). We calculated consistency of behavioral score between the first 2 experiments using Spearman rank correlations (Cote et al. 2010, 2011). We calculated repeatability of behavioral scores using a linear mixed-effects model method (Nakagawa and Schielzeth 2010). Because of the problems caused by missing values in this method, we only included individuals who exited the chamber within 10 min during both the first and second experiments ($N_{Treatment} = 46$, $N_{Control} = 53$). For the third experiment, we used exact logistic regression to test if behavioral scores or individual behaviors affected the individual's likelihood of survival. Additionally, we used a Cox proportional hazards model to asses the effect of prey behavioral score on survival time (Cox 1972). Finally, we used a generalized linear model (GLM) with a Poisson distribution to discern the fixed effects of behavioral scores and predator type on the number of escapes observed during the predation experiment. For the GLM, we included only individual prey that were consumed during the predation trial. Analyses were conducted in R2.15.0.

Ethical note

We followed ASB/ABS (2012) guidelines in designing and conducting this experiment. We chose to include direct predator exposure for this study because we were interested in fitness consequences of behavioral syndromes that are specifically the result of predator–prey interactions. We allowed prey individuals to visually and chemically sense the presence of the predator through a water-permeable barrier during the 5-min acclimation period and provided several potential refuges within the predation tank. After the 20-min trial, individuals not consumed were immediately removed, returned to group tanks, and kept at the lab for the duration of their lives. Our care and use of the fish in these experiments was approved by the Institutional Animal Care and Use Committee of Texas State University (protocol number 1111_0907_09).

RESULTS
Experiment one: behavioral correlations in *G. geiseri*

Latency to emerge, time spent moving, and area used were positively correlated (Table 1). Latency to emerge, time spent moving,

and area used loaded positively onto the first principal component, which explained 73.49% of the variance (Table 2). Subsequent components explained 20% or less of the variance and had eigenvalues less than 1. However, because latency to emerge also loaded highly on the second principal component, this suggests this behavior is not as strongly associated with the behavioral syndrome. Therefore, we performed subsequent analyzes using latency to emerge as a single predictor in addition to our analyses using behavioral score (PC1) as a predictor.

Experiment two: behavioral correlations in the presence of predators

The behavioral correlations observed in the absence of a predator (experiment one) between latency to emerge, time spent moving, and area used were also present when the fish were tested in the presence of predators (Table 2). There were no significant differences among predator treatments in mean latency to emerge ($F_{136,139} = 0.93$, $P = 0.42$), time spent moving ($F_{133,136} = 1.56$, $P = 0.20$), or area used ($F_{133,136} = 1.24$, $P = 0.30$). There was also no mean-level difference between behavioral assays with and without predators for latency to emerge ($F_{138,139} = 0.14$, $P = 0.71$), time spent moving ($F_{135,136} = 0.11$, $P = 0.37$), or area used ($F_{135,136} = 2.899$, $P = 0.09$). Further, the structure of the correlations matrices from the PCA of experiments one and two were very similar (Random skewers correlation = 0.97). We used component scores from individuals' behavior with predators present (experiment two) to calculate a behavioral score for each individual as they were the most recent measure of the individual's behavior, and because results from the first 2 experiments were so similar. Individual behaviors were non-normal, but the subsequent behavioral scores were normally distributed. Individuals were consistent and repeatable in their behavioral score across the 2 experiments for both the treatment and control groups (Table 3).

Experiment three: behavioral correlations and predation

Predators consumed 39 of the 60 treatment fish (native sunfish: 12, invasive cichlid: 9, novel killifish: 18). Behavioral score was

not a significant predictor of an individual's survival in the predation trial ($\chi^2 = 0.40$, $P = 0.53$), and neither was standard length ($\chi^2 = 0.51$, $P = 0.48$). Additionally, a Cox proportional hazards model showed no effect of behavioral score on survival time, though survival time did differ among the predator species (predator: $\chi^2 = 17.66$, $P = 0.0001$, predator × behavioral score: $\chi^2 = 2.88$, $P = 0.23$, behavioral score: $\chi^2 = 1.49$, $P = 0.22$). We also found that the single behavior latency to emerge was not a significant predictor of survival time (predator: $\chi^2 = 14.4$, $P = 0.0007$, predator × boldness: $\chi^2 = 0.97$, $P = 0.61$, boldness: $\chi^2 = 0.95$, $P = 0.33$). However, proportion of time an individual spent at the bottom of the tank during the predation trial was a significant predictor of survival ($\chi^2 = 12.74$, $P < 0.001$). There was no significant interaction between predator treatment and time at the bottom on survival ($\chi^2 = 0.94$, $P = 0.63$).

We also examined the relationship between behavioral score and the number of attacks escaped during the predation trial. The model that included behavioral score, predator type (fixed effects), and an interaction term was significant ($\chi^2 = 17.9$, df = 5, $P = 0.003$). There was a significant interaction between predator type and behavioral type (Figure 1; $\chi^2 = 9.24$, df = 2, $P = 0.01$). Individual parameter tests from the GLM showed that all terms were significant except for the interaction of behavioral score with the invasive cichlid predator (see Table 4). We also ran a GLM using latency to emerge as a single predictor and found a nonsignificant trend that was similar to our analyzes using behavioral score as a predictor, with the interaction of boldness and predator type approaching significance ($\chi^2 = 5.69$, df = 2, $P = 0.058$).

DISCUSSION

Although much work has been done on behavioral syndromes (Sih et al. 2004b), surprisingly few studies have illustrated a direct relationship between variation in individuals' behavioral tendencies and their ability to escape predators (Bell and Sih 2007; Smith and Blumstein 2010; Pruitt, Stachowicz, et al. 2012). Our findings show that latency to emerge, time spent moving, and area used are correlated behaviors in *G. geiseri*, across different situations (with and without predators present). Individual *G. geiseri* were also consistent in their behavior across situations. Further, we found that individuals with higher behavioral scores escaped more attacks from *F. grandis*, a novel predator species. Taken together, our results suggest that some behavioral types in this population may have a greater ability to escape this novel predator.

Experiment one: behavioral correlations in *G. geiseri*

Similar to previous studies on *Gambusia* (Cote et al. 2010, 2011), we found that latency to emerge, time spent moving, and area

Table 1

Spearman correlations for behaviors tested in behavioral assays without predators present ($N = 72$)

	Time spent moving	Area used
Latency to emerge	0.425 $P < 0.001$	0.389 $P < 0.001$
Time spent moving		0.701 $P < 0.001$

Table 2

Principal components analysis of behavioral assays without predators and with predators present ($N = 72$)

	Without predators		Predators present	
	Component loading (PCI)	Component loading (PCII)	Component loading (PCI)	Component loading (PCII)
Latency to emerge	0.495	0.865	0.508	0.845
Time spent moving	0.621	−0.291	0.594	−0.486
Area used	0.607	−0.410	0.624	−0.224
Variance explained	73.49%	20.39%	79.48%	17.92%
Total variance explained		93.89%		97.40%

Table 3

Rank order consistency and repeatability of behavioral score measured in behavioral assays without predators and with predators present ($N_{Treatment}$ = 46, $N_{Control}$ = 53)

Behavioral score	Rank order consistency	Repeatability
Treatment groups	$R_s = 0.402$, $P = 0.006$	$R_M = 0.324 \pm 0.134$ $P = 0.031$
Control group	$R_s = 0.313$, $P = 0.009$	$R_M = 0.404 \pm 0.123$, $P = 0.010$

Behavioral score is from the first principal component representing latency to emerge, time spent moving, and area used. For the control group, no predator was present in either of the 2 trials. Consistency was calculated from Spearman rank correlations, and repeatability was calculated using a linear mixed-effects model.
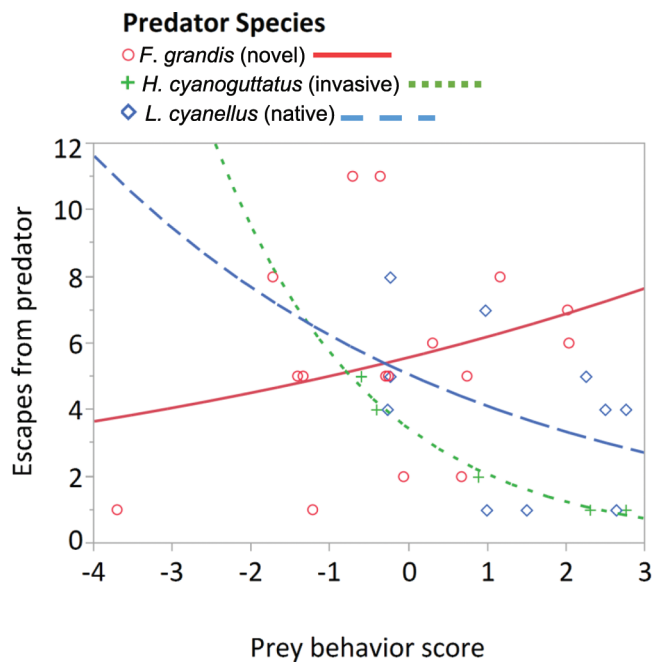

Figure 1

Relationship between behavioral score of prey individual and escapes from predator attacks, from a generalized linear model using a Poisson distribution ($N = 39$).

used are positively correlated in *G. geiseri* (Table 1). Whether this behavioral syndrome is a shared feature of the entire *Gambusia* genus remains an intriguing notion for future comparative studies. Latency to emerge also loaded highly on the second principal component in our analysis, suggesting some variance in this behavior is not explained by the behavioral syndrome. Further testing could explore other variables that affect individual's latency to emerge.

Experiment two: behavioral correlations in the presence of predators

Individuals showed rank order consistency in behavior over time, and behavioral correlations were present regardless of predator presence (Tables 2 and 3). In addition to individual consistency, we also saw no mean-level differences in behavior with and without predators. These results could indicate that subjects failed to perceive the predator as a threat; however, we do not think this is the case for several reasons. First, our preliminary work on predator

recognition in *G. geiseri* shows that they recognize and move away from visual and chemical cues of *L. cyanellus* in a similar laboratory set up (Blake C, et al., unpublished data). Here, our experimental design focused only on latency to emerge, time spent moving, and area used, and our experiment was not designed to capture other antipredator responses such as distance from cues, location in the water column, or shoaling behavior (Smith and Belk 2001; Rehage et al. 2009). Thus, a lack of change in the 3 behaviors we measured does not necessarily indicate a lack of predator recognition by our prey. Further, our findings agree with Rehage et al. (2005), who found *G. geiseri* did not reduce their activity in the presence of a predator. We conclude that the correlations we detected among latency to emerge, time spent moving, and area used constitute a behavioral syndrome that is consistent within individuals across different situations.

Our consistency and repeatability values are similar to earlier studies on latency to emerge, time spent moving, and area used in *G. affinis* over a period of several months (Cote et al. 2011). Our findings also agree with Sih et al. (2003), who found behavior to be consistent within individual salamander larvae across predator situations. Our results showing consistency of behavior within individuals regardless of predator presence is contrary to the hypothesis that predators could induce a change in behavioral type through behavioral plasticity of individuals. Rather, our results are consistent with the hypothesis that *G. geiseri* exhibit behavioral syndromes that may limit their ability to change their behavior in the presence of predators.

Experiment three: behavioral correlations and predation

We found that behavioral type influenced the number of predator attacks that a prey individual escaped. Individuals with high scores on the behavioral type axis exhibited more escapes during predation trials with our novel predator, *F. grandis*. Our analyzes using latency to emerge as a predictor showed a similar nonsignificant trend, with individuals who emerged sooner escaping more from *F. grandis*. In contrast to the results for *F. grandis*, individuals with *low* behavioral scores on the behavioral type axis escaped more attacks by *L. cyanellus* (Figure 1). Although the trend for prey exposed to *H. cyanoguttatus* predators was similar to that for *L. cyanellus*, we hesitate to make any conclusive claims about the relationship of behavioral score to escapes from *H. cyanoguttatus* due to fewer overall numbers of prey consumed by *H. cyanoguttatus* during the study. Nevertheless the finding that predator species influences the direction of the relationship between behavioral score and escapes supports our hypothesis that these different predators interact in distinct ways with prey behavioral syndromes.

Despite behavioral type affecting the number of attacks prey escaped from, we saw no affect of behavioral type on likelihood of survival of the trial or on survival time. Although our results may at first seem counterintuitive, behavioral type need not predict survival time in order for behavioral type to predict ability to escape. We argue that number of escapes is a better representation of the ability to evade predators than survival time, because survival time can be affected by many factors, including variation between trials in a predator's latency to detect or attack prey. Previous research has pointed out the shortcomings of measuring only survival time, and recording the number of attacks survived provides more information on the role of escape ability in predator–prey interactions (Smith and Blumstein 2010).

Table 4

Effects of prey behavioral score on escapes from predator attacks

Parameter estimate		χ^2	P	Lower confidence limit	Upper confidence limit
Intercept	1.53	79.05	**<0.001**	1.27	1.75
Behavioral score	−0.20	4.83	**0.03**	−0.42	−0.02
Predator (invasive *H. cyanoguttatus*)	−0.43	5.22	**0.02**	−0.86	−0.06
Predator (novel *F. grandis*)	0.33	6.44	**0.01**	0.07	0.61
Predator (invasive *H. cyanoguttatus*) × behavioral score	−0.30	3.53	0.06	−0.69	0.01
Predator (novel *F. grandis*) × behavioral score	0.31	9.24	**0.002**	0.11	0.54

Parameter estimates from a generalized linear model using a Poisson distribution, with fixed effects for behavioral score, predator type, and behavioral score × predator type ($N = 39$). P values less than 0.05 are in bold.

An alternative interpretation of our findings is that bold individuals were more likely to be attacked by *F. grandis* than shy individuals. It is possible that bold individuals may have had a higher encounter rate and/or approached predators sooner, and future studies could examine these variables. However, our finding that fish with high behavioral scores were able to escape more attacks still stands. We used only individuals that were eventually consumed in our final analysis, thus, a low number of escapes indicates that these individuals were consumed after only a few interactions with the predator, while individuals with a high number of escapes were able to successfully elude predators many times before being consumed. Thus, we interpret a positive relationship between behavioral score and number of escapes from *F. grandis* to mean that fish with higher behavioral scores were better able to escape when they were attacked.

Although the interaction of predator species and behavioral type of prey is clear in our findings, further work is needed to determine whether the degree of novelty of the predator is responsible for this effect, rather than differences in hunting strategy of the predators. We observed differences in behavior between the different predator types, including differences in latency to attack. The novel predator, *F. grandis*, was most likely to attack quickly and was the most active even when prey were not present. In contrast, we observed that the native *L. cyanellus* and invasive *H. cyanoguttatus* spent more time hiding and performed more ambush attacks. *L. cyanellus* is generally considered a sit-and wait predator, while cichlids, especially the genus *Herichthys*, are known for having a great degree of variety and plasticity in their foraging behaviors (Werner and Hall 1977; Swanson et al. 2003). *F. grandis* is a top minnow, and although this species feeds throughout the water column on a variety of prey, *F. grandis* may be morphologically more adapted to feed at the water surface than centrarchid predators like *H. cyanoguttatus* and *L. cyanellus* (Rozas and Lasalle 1990). In fact, the feeding behavior and habitat use of *F. grandis* is somewhat similar to *G. geiseri*, which could increase the likelihood of *F. grandis* encountering and preying upon *G. geiseri*. Further investigation is needed to ascertain whether the effect of predator type in our results was due to the contrasting hunting behavior of the specific predator species used or a generalizable effect in the novelty of the predator.

It is also possible that differences between individual predators of the same species may play a role in the predator–prey interactions in our study. Emerging research suggests that individual predators of different behavioral types may interact in distinct ways with individual prey behavioral types (McGhee et al. 2013). Because our experimental design focused on between-species differences, we did not collect information on behavioral or morphological differences between individual predators. Complex interactions between individual predator and prey behavioral types could have contributed to the lack of an effect of prey behavioral score on survival in our study. Our results add support to the hypothesis that differences between predator individuals or predator species could contribute to the continued existence of multiple behavioral types in a population (Smith and Blumstein 2010; Wolf and Weissing 2010; Pruitt, Stachowicz, et al. 2012).

Proportion of time spent at the bottom of the predation tank was a significant predictor of survival, but the interaction between predator treatment and time at the bottom was not significant. This result suggests that the same behavior (water column position) was associated with survival regardless of predator species. We also noted that most fatal attacks occurred in the top zone of the water column, which reinforces our finding that remaining in the bottom zone is an effective strategy for *G. geiseri* to evade predation from the species we used in this experiment. Due to the constraints of filming trials from above, our behavioral assays in the first 2 experiments did not measure water column use, so it is unclear whether an individual's water column use is correlated to the other behaviors we measured. Due to the importance of water-column use in predicting survival of predators in our experiment, future work should explore whether this is a consistent behavioral trait for prey individuals.

To our knowledge, this is the first study to show that the likelihood an individual will escape a predator attack correlates with the behavioral type of these same individuals measured in another context. The existing literature on the fitness consequences of behavioral syndromes has focused largely on reproductive success, and although studies on fish have tended to find negative correlations between survival and boldness (Smith and Blumstein 2008), only a few studies have explored predation specifically as a mechanism for fitness consequences of behavioral types (Bell and Sih 2007; Smith and Blumstein 2010; Pruitt, Stachowicz, et al. 2012). The fact that the direction of the effect of behavioral score on escape from predator attacks depends on predator species suggests that multiple predators could select for the maintenance of several prey behavioral types. Further, our study shows the importance of integrating research on behavioral syndromes and invasion ecology. Our results show that novel predators may favor different behavioral types than native predators, which could have important implications for prey populations that experience invasion. Although behavioral type did not predict survival in one-on-one laboratory predation trials, in a natural environment surrounded by a shoal of conspecifics, ability to escape predators may have an effect on an individual's *relative* likelihood of survival. Further predation studies in more naturalistic settings are required to explore this possibility.

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REFERENCES

Archard GA, Braithwaite VA. 2011. Increased exposure to predators increases both exploration and activity level in *Brachyrhaphis episcopi*. J Fish Biol. 78:593–601.

ASB/ABS. 2012. Guidelines for the treatment of animals in behavioural research and teaching. Anim Behav. 83:301–309.

Bell AM. 2005. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J Evol Biol. 18:464–473.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. Anim Behav. 77:771–783.

Bell AM, Sih A. 2007. Exposure to predation generates personality in three-spined sticklebacks (*Gasterosteus aculeatus*). Ecol Lett. 10:828–834.

Biro PA, Abrahams MV, Post JR, Parkinson EA. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc Biol Sci. 271:2233–2237.

Brosnan SF, Earley RL, Dugatkin LA. 2003. Observational learning and predator inspection in guppies (*Poecilia reticulata*). Ethology. 109:823–833.

Brown C, Jones F, Braithwaite VA. 2005. In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. Anim Behav. 70:1003–1009.

Cheverud JM, Ruteledge JJ, Atchley WR. 1993. Quantitative genetics of development: genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 35:895–905.

Cote J, Fogarty S, Brodin T, Weinersmith K, Sih A. 2011. Personality-dependent dispersal in the invasive mosquitofish: group composition matters. Proc Biol Sci. 278:1670–1678.

Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A. 2010. Personality traits and dispersal tendency in the invasive mosquitofish (Gambusia affinis). Proc Biol Sci. 277:1571–1579.

Courtenay WR, Moyle PB. 1992. Crimes against biodiversity-the lasting legacy of fish introductions. Transactions of the Fifty-Seventh North American Wildlife and Natural Resources Conference. Charlotte (NC): Wildlife Management Institute. 365–372 pp.

Cox DR. 1972. Regression models and life-tables. J R Stat Soc B. 34:187–220.

Dingemanse NJ, Kazem AJ, Réale D, Wright J. 2010. Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol (Amst). 25:81–89.

Dingemanse NJ, Wright J, Kazem AJ, Thomas DK, Hickling R, Dawnay N. 2007. Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. J Anim Ecol. 76:1128–1138.

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

Ensminger AL, Westneat DF. 2012. Individual and sex differences in habituation and neophobia in house sparrows (*Passer domesticus*). Ethology. 118:1085–1095.

Hedrick AV, Riechert SE. 1989. Genetically-based variation between two spider populations in foraging behavior. Oecologia. 80:533–539.

Hillis LG, Milstead E, Campbell SL. 1980. Inland records of *Fundulus grandis* (Cyprinodontidae) in Texas. Southwest Nat. 25:271–272.

Hubbs C, Edwards RJ, Garrett GP. 1991. An annotated checklist of freshwater fishes of Texas, with key to identification of species. Tex J Sci (Suppl). 43:1–56.

Hubbs C, Lucier T, Garrett GP, Edwards RJ, Dean SM, Marsh E, Belk D. 1978. Survival and abundance of introduced fishes near San Antonio, Texas. Tex. J. Sci. 30:369–373.

Johnson JC, Sih A. 2005. Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioral syndromes. Behav Ecol Sociobiol. 58:390–396.

Kelley JL, Magurran AE. 2003. Learned predator recognition and antipredator responses in fishes. Fish Fish. 4:216–226.

McGhee KE, Pintor LM, Bell AM. 2013. Reciprocal behavioral plasticity and behavioral types during predator-prey interactions. Am Nat. 182:704–717.

Nakagawa S, Schielzeth H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev Camb Philos Soc. 85:935–956.

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

Pellegrini AF, Wisenden BD, Sorensen PW. 2012. Bold minnows consistently approach danger in the field and lab in response to either chemical or visual indicators of predation risk. Behav Ecol Sociobiol. 64:381–387.

Pruitt JN, Cote J, Ferrari MCO. 2012. Behavioural trait variants in a habitat-forming species dictate the nature of its interactions with and among heterospecifics. Funct Ecol. 26:29–36.

Pruitt JN, Ferrari MC. 2011. Intraspecific trait variants determine the nature of interspecific interactions in a habitat-forming species. Ecology. 92:1902–1908.

Pruitt JN, Stachowicz JJ, Sih A. 2012. Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. Am Nat. 179:217–227.

Reale D, Festa-Bianchet M. 2003. Predator-induced natural selection on temperament in bighorn ewes. Anim Behav. 65:463–470.

Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev Camb Philos Soc. 82:291–318.

Rehage JS, Barnett BK, Sih A. 2005. Behavioral responses to a novel predator and competitor of invasive mosquitofish and their non-invasive relatives (*Gambusia* sp.). Behav Ecol Sociobiol. 57:256–266.

Rehage JS, Dunlop KL, Loftus WF. 2009. Antipredator responses by native mosquitofish to non-native cichlids: an examination of the role of prey naivete. Ethology. 115:1046–1056.

Riechert SE, Hedrick AV. 1990. Levels of predation and genetically based antipredator behavior in the spider, *Agelenopsis aperta*. Anim Behav. 40:679–687.

Rozas LP, Lasalle MW. 1990. A comparison of the diets of Gulf killifish, *Fundulus grandis* Baird and Girard, entering and leaving a Mississippi brackish marsh. Estuaries. 13:332–336.

Sih A. 2013 Understanding variation in behavioral responses to human-induced rapid environmental change: a conceptual overview. Anim Behav. 85:1077–1088.

Sih A, Bell A, Johnson JC. 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol (Amst). 19:372–378.

Sih A, Bell AM, Johnson JC, Ziemba RE. 2004b. Behavioral syndromes: an integrative overview. Q Rev Biol. 79:241–277.

Sih A, Bolnick DI, Luttbeg B, Orrock JL, Peacor SD, Pintor LM, Preisser E, Rehage JS, Vonesh JR. 2010. Predator-prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos. 119:610–621.

Sih A, Cote J, Evans M, Fogarty S, Pruitt J. 2012. Ecological implications of behavioural syndromes. Ecol Lett. 15:278–289.

Sih A, Kats LB, Maurer EF. 2003. Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish–salamander system. Anim Behav. 65:29–44.

Smith BR, Blumstein DT. 2008. Fitness consequences of personality: a meta-analysis. Behav Ecol. 19:448–455.

Smith BR, Blumstein DT. 2010. Behavioral types as predictors of survival in Trinidadian guppies (*Poecilia reticulata*). Behav Ecol. 21:919–926.

Smith ME, Belk MC. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? Behav Ecol Sociobiol. 51:101–107.

Strauss SY, Lau JA, Carroll SP. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecol Lett. 9:357–374.

Swanson BO, Gibb AC, Marks JC, Hendrickson DA. 2003. Trophic polymorphism and behavioral differences decrease intraspecific competition in a cichlid, *Herichthys minckleyi*. Ecology. 84:1441–1446.

Thomas C, Bonner TH, Whitside BG. 2007. Freshwater Fishes of Texas, A Field Guide. College Station (TX): Texas A&M University Press.

Werner EE, Hall DJ. 1977. Competition and habitat shift in two sunfishes (Centrarchidae). Ecology. 58:869–876.

Wilson ADM, Godin JGJ. 2009. Boldness and behavioral syndromes in the bluegill sunfish, *Lepomis macrochirus*. Behav Ecol. 20:231–237.

Wolf M, Weissing FJ. 2010. An explanatory framework for adaptive personality differences. Philos Trans R Soc Lond B Biol Sci. 365:3959–3968.