OXFORD

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Interactive effects of reproductive assets and ambient predation risk on the threat-sensitive decisions of Trinidadian guppies

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Abstract

Threat-sensitive behavioral trade-offs allow prey animals to balance the conflicting demands of successful predator detection and avoidance and a suite of fitness-related activities such as foraging, mating, and territorial defense. Here, we test the hypothesis that background predation level and reproductive status interact to determine the form and intensity of threat-sensitive behavioral decisions of wild-caught female Trinidadian guppies *Poecilia reticulata*. Gravid and nongravid guppies collected from high- and low-predation pressure populations were exposed to serial dilutions of conspecific chemical alarm cues. Our results demonstrate that there was 'no effect of reproductive status on the response of females originating from a low-predation population, with both gravid and nongravid guppies exhibiting strong anti-predator responses to the lowest concentration of alarm cues tested. Increasing cue concentrations did not result in increases in response intensity. Conversely, we found a significant effect of reproductive status among guppies from a high-predation population. Nongravid females from the high-predation population exhibited a strong graded (proportional) response to increasing concentrations of alarm cue. Gravid females from the same high-predation population, however, shifted to a nongraded response. Together, these results demonstrate that accrued reproductive assets influence the threat-sensitive behavioral decisions of prey, but only under conditions of high-ambient predation risk.

Key words: asset protection, antipredator behavior, fitness trade-offs, ecological uncertainty, chemical alarm cues.

As a result of the nonconsumptive effects (NCEs) of predation (Preisser et al. 2005), strong selective pressures are exerted on the behavior, morphology, and life history traits of prey (Peacor and Werner 2004; Preisser et al. 2005; McCauley et al. 2011; Jefferson et al. 2014). These NCEs are potentially costly, as prey may be forced to trade-off between critical activities such as foraging or courtship and predator avoidance, or may be forced to utilize suboptimal habitats to offset predation risk (Preisser et al. 2005). Moreover, given that predation risk is rarely predictable in space and time (Sih et al. 2000; Dall et al. 2005), prey may be faced with marked variation and unpredictability in local threat levels (i.e., uncertain risks) requiring increased allocation to predator detection

and avoidance and associated higher costs of critical trade-offs (Brown et al. 2006; Ferrari et al. 2010; Brown et al. 2013).

Key to balancing these conflicts is the ability to reliably assess risks based on publically available information regarding the nature of local predation threats (Brown 2003; Dall et al. 2005). The threat-sensitive predator avoidance hypothesis (Helfman 1989; Helfman and Winkleman 1997) predicts that prey should benefit by matching the intensity of their predator avoidance response to the level of perceived acute predation risk. This model has received extensive support across a range of taxa, including aquatic and terrestrial invertebrates (Rochette et al. 1997; Persons and Rypstra 2001), amphibians (Laurila et al. 1997), reptiles (Amo et al. 2004), birds (e.g., Edelaar and Wright

2006), mammals (e.g., Swaisgood et al. 1999), and fishes (e.g., Bishop and Brown 1992; Chivers et al. 2001; Brown et al. 2006, 2012).

Recently, Brown et al. (2009) demonstrated that ambient or background predation risk alters the relative benefits of predator avoidance versus other behavioral activities such as foraging. In populations exposed to low-predation risk, female Trinidadian guppies *Poecilia reticulata* exhibited a nongraded response to varying concentrations of conspecific chemical alarm cues. When exposed to concentrations above a detectable threshold, these guppies exhibited a strong predator avoidance response and increasing the concentration of alarm cues did not increase the intensity of the behavioral response. Conversely, guppies from a high-predation population exhibited an increase in predator avoidance responses proportional to the concentration of alarm cues detected. Brown et al. (2009) suggest that under conditions of low-background predation risk, the lost opportunity costs associated with predator avoidance are relatively small compared to the costs of exhibiting stronger avoidance responses toward rare predation events. However, under conditions of high-background predation risk, prey may benefit from showing graded threat-sensitive responses. This should allow them to maintain some residual foraging benefits and to reduce or limit the NCEs of predation (Elvidge et al. 2014).

In addition to threat-sensitive responses to acute risks, Clark's (1994) Asset Protection model suggests that an individual's past successes at accruing fitness will shape its response to current predation threats. For example, juvenile coho salmon *Oncorhynchus kisutch* with higher growth rates are more risk averse than conspecifics with lower growth rates (Reinhardt and Healey 1999). This difference in risk-averse versus risk-prone tactics may be dependent upon biotic conditions (Reinhardt and Healy 1999; Reinhardt 2002). During the peak growth season when food abundance is high, smaller and/or slower growing coho salmon are more likely to engage in risky foraging behavior than are larger and/or faster growing conspecifics. However, as water temperatures drop near the end of the growth season, both phenotypes become more risk averse as food availability declines (Reinhardt and Healy 1999). Thus, prey may alter their behavioral decisions based on both the perceived intensity of acute risk and/or previously accrued fitness. However, how accrued assets impact threat-sensitive behavioral trade-offs in response to differing levels of risk is largely unknown.

Here, we test the potential interacting effects of background predation risk and accrued assets on the threat-sensitive response patterns of wild-caught Trinidadian guppies. Guppies are livebearers and reproductive status (gravid vs. nongravid) is readily assessed visually. As such, they are an excellent model species to address this question. Using a series of laboratory trials, we exposed gravid (high accrued fitness) versus nongravid (low accrued fitness) wild-caught Trinidadian guppies from high-versus low-predation populations (ambient risk) to varying concentrations of conspecific alarm cues (acute risk). We predicted that: 1) gravid females would exhibit stronger predator avoidance responses compared to nongravid females in accordance with the Asset Protection model, and 2) the effects of accrued fitness on these responses will be dependent upon background predation risk, with a more pronounced effect of reproductive status among females from the high-predation risk population.

Materials and Methods

Test fish

Gravid and nongravid female guppies were collected from the Upper and Lower Aripo Rivers, located in the Northern Range Mountains, Trinidad. The Lower Aripo River is characterized as a high-predation site (Croft et al. 2006; Botham et al. 2008) as it contains several predators that prey on both juvenile and adult guppies including the pike cichlid *Crenichichla spp.*, blue acara cichlid *Aequidens pulcher*, and brown coscorub *Cichlasoma taenia*. In addition, there are several predators that prey on smaller, juvenile guppies including Hart's rivulus *Anablepsoides hartii* and a predatory characin *Astyanax bimaculatus*. The Upper Aripo River is characterized as a low-predation site (Croft et al. 2006; Botham et al. 2008) as the predominant predators present are Hart's rivulus and a predatory freshwater prawn *Macrobrachium crenulatum*.

We collected female guppies using hand seines (3 mm mesh size) and transported them directly to the University of the West Indies, St. Augustine campus. Guppies were held in aerated 100-L aquaria (∼27 °C; 12:12 L:D cycle), sorted by population and reproductive status. Holding tanks contained a gravel and cobble substrate and were continuously filtered. We visually assessed gravidity based on the presence/absence of a gravid spot and distended abdomens (Wong and McCarthy 2010; Knapp et al. 2011). All fish were fed dry commercial flaked food twice daily.

Stimulus preparation

We generated chemical alarm cues from 91 Lower Aripo female (mean ± SD standard length = 1.85 ± 0.27 cm) and 73 Upper Aripo female (2.04 ± 0.22 cm). Alarm cue donors were euthanized via cervical dislocation (in accordance with Concordia Animal Care Committee Protocol AC-2011-BROW). We immediately removed the head and tail (at the caudal peduncle) and manually extruded any remaining visceral tissue. The remaining tissue (skin and skeletal muscle) was placed into 200 mL of aged tap water. We then mechanically homogenized the tissue samples, filtered them through polyester floss, and diluted the samples to the desired final stock concentration (0.1 cm^2 skin per mL) with the addition of aged tap water. This concentration of alarm cue is known to elicit predator avoidance responses in guppies (Brown et al. 2009, 2010). We collected a total of 210.40 cm^2, with equal total amounts from each population, of skin tissue (in a final volume of 2104 mL). A blend of Upper and Lower Aripo guppies were used for alarm cues to control for any population-specific response (Brown et al. 2010). This stock alarm cue was frozen in 10 mL aliquots at −20 °C and thawed immediately prior to use.

Experimental protocol

We placed shoals of 3 gravid or nongravid females into test tanks and allowed them to acclimate overnight prior to testing. Test tanks consisted of a series of 20 L glass aquaria filled with 18 L of dechlorinated water at ∼26 °C. These observation tanks were aerated but not filtered and contained a single airstone mounted along the back wall of the tank. We attached an additional 1.5 m length of tubing, terminating immediately below the airstone, to allow for the introduction of stimuli. Tanks were lined with white plastic on 3 sides for visual isolation between tanks. In addition, we visually divided the tank into 3 horizontal sections by drawing lines on the front and back walls to facilitate recording area use (see below).

Trials consisted of a 5-min pre- and post-stimulus injection observation periods. Prior to an observation, we withdrew and discarded 60 mL of tank water to ensure any stagnant water was not introduced into the tank. An additional 60 mL of tank water was removed and retained. Immediately following the pre-stimulus observation, we introduced 10 mL of stimulus and slowly flushed it into the test tank using the retained tank water. Stimuli consisted of alarm cue at the stock concentration (100%) or diluted with aged tap water to 50% or 25% concentrations. Aged tap water was used as a control (0%).

During both the pre- and post-stimulus observation period, we quantified 3 measures of antipredator behavior: area use, shoaling

index, and foraging attempts. Area use and shoaling index were recorded every 15 s. Area use was measured as the position of each guppy within the tank, whose scores range from 1 (bottom third of the tank) to 3 (top third of the tank); resulting area use scores ranged from 3 (all fish near the substrate) to 9 (all fish near the surface). Shoaling index score ranged from 1 to 3 (1 = no guppy within 1 body length of each other, 3 = all guppies within 1 body length of each other (Brown and Godin 1999; Brown et al. 2009b). Foraging attempts were measured as a directed lunge toward an object in the water column, at the surface or on glass, involving opercular expansion and opening of the mouth. A reduction in area use and foraging and an increase in shoaling index are indicative of a predator avoidance response in Trinidadian guppies (Brown and Godin 1999; Brown et al. 2009b). A total of 15–18 replicates were conducted per stimulus concentration for gravid and nongravid guppies from both the Lower and Upper Aripo populations ($n = 252$). Individual guppies were tested only once. Upon completion of the study, guppies were released back to the site of collection. Size at testing (mean ± SD) was 1.82 ± 0.31 cm; 0.14 ± 0.07 g (nongravid) and 2.08 ± 0.39 cm; 0.23 ± 0.12 g (gravid) for Upper Aripo females, and 1.66 ± 0.27 cm; 0.11 ± 0.05 g (nongravid) and 1.83 ± 0.25 cm; 0.14 ± 0.06 g (gravid) for Lower Aripo females.

Statistical analysis

We calculated the changes (post–pre) in area use, shoaling index, and foraging attempts, and used these difference scores as dependent variables. Initially, we tested for the effects of stimulus concentration, population (Upper vs. Lower Aripo River; "stream"), and reproductive status (gravid vs. nongravid) using a MANCOVA. Given that changes in each of the behavioral measures recorded are highly correlated, the use of a multivariate approach is justified. To account for the differences in size among females from high- and low-predation populations, we included standard length as a covariate in this initial analysis. To further explore population differences (see below), we conducted separate 2-way MANOVAs, for both the Upper and Lower Aripo populations. All data met the assumptions of parametric testing (Levene's test, $P > 0.05$ for all).

Results

Standard length was found to have no effect on the response pattern ($F_{3,233} = 1.63$, $P = 0.18$) and was omitted from subsequent analyses. Our overall MANOVA revealed a significant effect of stream population, reproductive status, and stimulus concentration on behavioral responses of guppies (MANOVA; $P < 0.05$; Table 1). We also found a significant interaction between population and stimulus concentration ($P < 0.05$; Table 1). When testing the Lower Aripo guppies alone, we found a significant effect of both stimulus concentration (MANOVA; $F_{3,119} = 24.99$; $P < 0.001$) and reproductive status (MANOVA; $F_{3,117} = 5.07$; $P = 0.002$; Figure 1, left column) on anti-predator response, with no significant threat level–asset interaction (MANOVA; $F_{3,117} = 1.88$; $P = 0.14$; Figure 1, left column). However, when testing the Upper Aripo guppies alone, we found only a significant effect of stimulus concentration (MANOVA; $F_{3,117} = 15.54$; $P < 0.001$; Figure 1, right column). There was no significant effect of reproductive status ($F_{3,115} = 0.68$; $P = 0.57$) nor an interaction between these factors ($F_{3,117} = 0.31$; $P = 0.82$; Figure 1, right column).

We conducted planned (polynomial) contrasts to further explore the effect of reproductive status among Lower Aripo females. Nongravid females, from high-predation risk populations, exhibited significant linear responses for all 3 behavioral measures, suggesting graded or proportional responses to increasing stimulus concentrations

Table 1. Results of the overall MANOVA for the effects of population, reproductive status, and concentration of alarm cues on the change in anti-predator behavior of guppies

	F	df	P
Population	3.27	3234	= 0.022
Reproductive status	5.14	3234	= 0.002
Stimulus	35.03	3236	< 0.001
Population × Reproductive status	1.41	3234	= 0.24
Population × Stimulus	2.83	3236	= 0.039
Reproductive status × Stimulus	1.24	3236	= 0.30
3-Way interaction	1.16	3235	= 0.34

Population = Upper Aripo (low predation) versus Lower Aripo (high predation) River; Stimulus = guppy alarm cue at 100% (stock concentration), 50% or 25% dilutions or a water control; Reproductive status = gravid versus nongravid.

(Table 2). However, gravid Lower Aripo females exhibited significant quadratic responses for the frequency of foraging attempts and shoaling index (Table 2). The area use response among gravid Lower Aripo females is best described by the linear coefficient (Table 2). This suggests that for 2 (of 3) behavioral measures, the response appears nongraded. Above a minimum threshold, increasing stimulus concentration does not increase the strength of the response.

Discussion

Initially, our results provide confirmation of previous reports (Botham et al. 2008; Brown et al. 2009) demonstrating that guppies from a high-predation population exhibit stronger overall predator avoidance responses and are more sensitive to the level of acute threat than are guppies from a low-predation population. More interesting, however, is the observation that under conditions of low-background predation risk (Upper Aripo River), there appears to be little influence of reproductive status on the response pattern of female guppies as both gravid and nongravid females exhibited similar responses to increasing concentrations of conspecific alarm cues, consistent with the response patterns reported by Brown et al. (2009). Female guppies collected from a high-predation site (Lower Aripo River), by contrast, exhibited a very different responses depending upon reproductive status. While nongravid females demonstrated proportional responses to increasing alarm cue concentrations, gravid females exhibited an overall stronger and nongraded response to the same range of cues. Combined, these data suggest that background risk and reproductive status (accrued assets) interact to shape the threat-sensitive response patterns of Trinidadian guppies.

Recent studies have demonstrated that reproductive status influence risk-taking tactics in other species. Gravid female ninespine sticklebacks (*Pungitius pungitius*), for example, are more likely to rely on social foraging cues in order to reduce predation risks compared to nongravid females (Webster and Laland 2011). Gravid three-spined sticklebacks (*Gasterosteus aculeatus*) exhibit an increase in predator inspection visits toward a realistic predator relative to nongravid females (Frommen et al. 2009). Given that predator inspection behaviors confer increased predator avoidance benefits to inspecting prey (Godin and Davis 1995; Brown 2003), the observations of Frommen et al. (2009) are consistent with increased risk-averse tactics. Similarly, Spanish terrapins (*Mauremys leprosa*) took longer to emerge from their shells following simulated predator attacks if they were gravid, and females with larger clutches took longer to emerge than those with smaller clutches (Ibánez et al. 2015). The responses of our Lower Aripo guppies
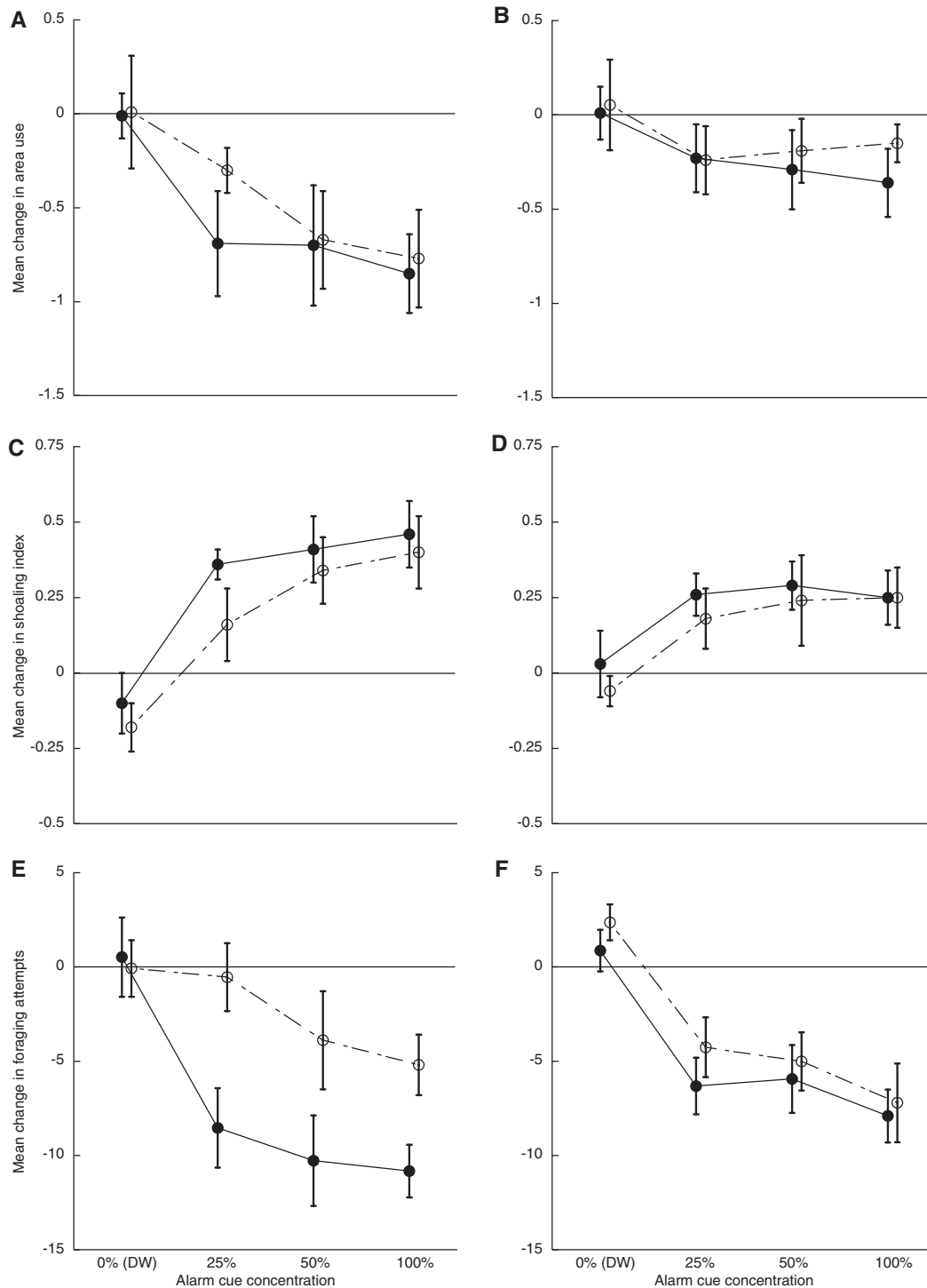
Figure 1. Mean (± *SE*) change in area use (A, B), shoaling index (C, D) and foraging attempts (E, F) for guppies originating from the Lower Aripo River (high predation; left column) and Upper Aripo River (low predation; right column) exposed to each of the alarm cue concentrations or a water control (DW). Solid circles and solid lines denote gravid females; open circles and dashed lines denote nongravid females.

show a similar shift toward increased reliance on risk-averse tactics among gravid females. Moreover, recent studies demonstrate that prey fishes (including poeciliids) are generally more risk prone under conditions of high-background predation compared to conspecifics under low-background predation (Brown et al. 2005; Harris et al. 2010; Elvidge et al. 2014). The shift toward risk-averse tactics by gravid, but not by nongravid females supports the supposition that accrued assets influence the risk-taking tactics of prey animals. As

the accrued assets (i.e., size, energy stores, reproductive investments) of prey increase, they are less likely to take risks when exposed to acute predation threats, consistent with the Asset Protection model (Clark 1994).

Threat-sensitive behavioral decisions are based on the ability of prey to reliably assess local predation risk (Brown 2003). The perception of acute predation threats, however, is known to be context-dependent. For example, juvenile convict cichlids *Amatitlania*

Table 2. Planned contrast values for linear and quadratic estimates for nongravid and gravid Lower Aripo (high predation) females exposed to varying concentrations of conspecific alarm cue

	Contrast	Coefficient	95% CI		P
Nongravid			Lower	Upper	
Foraging attempts	Linear	−4.19	−8.19	−0.18	= 0.041
	Quadratic	−0.42	−4.35	3.51	= 0.83
Shoaling index	Linear	0.43	0.21	0.66	< 0.001
	Quadratic	−0.14	−0.36	0.82	= 0.21
Area use	Linear	−0.66	−1.16	−0.16	= 0.01
	Quadratic	0.16	−0.34	−0.65	= 053
Gravid					
Foraging attempts	Linear	−8.00	−12.06	−3.90	< 0.001
	Quadratic	4.25	0.15	8.36	= 0.043
Shoaling index	Linear	0.39	0.21	0.51	< 0.001
	Quadratic	−0.26	−0.44	−0.08	= 0.006
Area use	Linear	−0.56	−1.05	−0.08	= 0.023
	Quadratic	−0.26	−0.23	0.75	= 0.29

nigrofasciata exhibit anti-predator responses to lower concentrations of conspecific alarm cues when tested as singletons or in small shoals than when tested in larger shoals (Brown et al. 2006). Similarly, hunger level (Smith 1981), distance to shelter (Turney and Godin 2014), and familiarity and relatedness among group membership (Ward and Hart 2003; Hoare et al. 2004) have all been shown to influence the perception of acute risk. Moreover, this context-dependent perception of risk should influence the shape of the response pattern. For example, juvenile cichlids tested as singletons exhibit a nongraded ("all-or-nothing") response, while they show a graded (proportional) response to increasing concentrations of alarm cues when tested as large shoals (Brown et al. 2006). Likewise, guppies from high-ambient predation populations exhibit graded responses while conspecifics from low-predation sites are decidedly more nongraded in their responses (Brown et al. 2009). Together, these studies suggest that factors that alter the relative value of predator avoidance versus other behavioral activities such as foraging influence the form and intensity of the response patterns to an acute predation threat.

Why should we expect to see a shift in threat-sensitive response patterns among guppies from the high-predation (Lower Aripo) site only? There are likely 2 complementary mechanisms that may account for our observed shifts in threat-sensitive response patterns among guppies from the high-predation (Lower Aripo) population. First, despite potentially higher rates of competitive interactions with conspecific and heterospecific foragers, prey are likely able to obtain sufficient foraging opportunities to offset short-term reductions due to infrequent predation risks under low-predation risk conditions (Elvidge et al. 2014). In this case, both gravid and nongravid females would benefit from exhibiting strong anti-predator responses as the relative costs associated with responding to rare acute threats are likely low. However, under high-background predation risk conditions, guppies may be under increased foraging stress resulting from reduced opportunities to forage (Fraser et al. 2004; Botham et al. 2008) or increased physiological costs (Cooke et al. 2003) associated with elevated predation risks. As a result, nongravid females should benefit from the residual foraging opportunities associated with graded response patterns (Brown et al. 2009; Elvidge et al. 2014). Gravid females would be expected to have similar benefits associated with residual foraging due to the increased energy but would also have potentially much higher costs associated with failing to respond to an acute threat (*sensu* Webster

and Laland 2011). Thus, selection should benefit increased behavioral plasticity among female guppies under conditions of high-ambient predation risk.

Second, the ability of gravid females to increase the overall intensity of predator avoidance responses relative to nongravid females may be constrained by foraging competition. Low-predation sites, such as the Upper Aripo River, generally have lower productivity due to greater canopy cover (Grether et al. 2001) and higher consumer density relative to high-predation sites (Magurran 2005). While the acute costs of lost foraging opportunities associated with responding to rare acute threats may be relatively low (see above), the behavioral plasticity of females may be constrained by competitive costs. As such, gravid females may be limited in the scope of their behavioral response intensities. Thus, selection under low-predation and high-competition populations may limit behavioral flexibility. Though we cannot eliminate this second mechanism, it is unlikely to be operating in our current experiment as all fish were fed *ad libitum* under low-competition conditions and we observed no difference in baseline foraging rates (Population $F_{1,247}=0.051$; reproductive status $F_{1,247}=0.003$; interaction $F_{1,247}=3.19$, $P > 0.05$ for all; data not shown). If gravid and nongravid Upper Aripo River females were under different levels of competitive stress, we would expect to see some difference in foraging rates in the absence of an acute threat.

Our results suggest a greater level of behavioral flexibility among the Lower (vs. Upper) Aripo guppies. Under conditions of ecological uncertainty (broadly defined as the absence of complete information regarding local conditions), the cost of balancing behavioral trade-offs likely increases. These costs, in turn, would favor plastic behavioral phenotypes (Dall et al. 2005; Ferrari et al. 2010; Brown et al. 2013) as it would allow prey to respond to short-term variability in risks. A significant contributor to ecological uncertainty is the diversity and degree of predation pressure within specific habitats (Brown et al. 2013). The Lower Aripo River contains a diversity of predators (see above) with differing foraging tactics, leading to increased uncertainty in acute predation threats compared to the Upper Aripo River. Our current results suggest a greater degree of behavioral flexibility among the Lower Aripo guppies, consistent with this hypothesis.

In summary, our results strongly suggest that reproductive status (accrued fitness) has a significant influence on the form and intensity of threat-sensitive behavioral decisions of female guppies, but only under conditions of high-background predation risks. However, caution in extrapolation is warranted, as our study only compares one high- versus one low-predation risk population. Future studies should compare multiple populations to establish the generality of our findings.

Acknowledgments

Funding

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