Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies

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Abstract

The antipredator behaviour of prey organisms is shaped by a series of threat-sensitive trade-offs between the benefits associated with successful predator avoidance and a suite of other fitness-related behaviours such as foraging, mating and territorial defence. Recent research has shown that the overall intensity of antipredator response and the pattern of threat-sensitive trade-offs are influenced by current conditions, including variability in predation risk over a period of days to weeks. Here, we tested the hypothesis that long-term predation pressure will likewise have shaped the nature of the threat-sensitive antipredator behaviour of wild-caught Trinidadian guppies (*Poecilia reticulata*). Female guppies were collected two populations that have evolved under high- and low-predation pressure, respectively, in the Aripo River, Northern Mountain Range, Trinidad. Under laboratory conditions, we exposed shoals of three guppies to varying concentrations of conspecific damage-released chemical alarm cues. Lower Aripo (high-predation) guppies exhibited the strongest antipredator response when exposed to the highest alarm cue concentration and a graded decline in response intensity with decreasing concentrations of alarm cue. Upper Aripo (low-predation) guppies, however, exhibited a nongraded (hypersensitive) response pattern. Our results suggest that long-term predation pressure shapes not only the overall intensity of antipredator responses of Trinidadian guppies, but also their threat-sensitive behavioural response patterns.

Introduction

The threat-sensitive predator avoidance hypothesis (Helfman 1989) predicts that prey individuals should be selected to adjust the form and/or intensity of their behavioural responses to a threat of predation in proportion to the level of the perceived threat. This ability to make such threat-sensitive behavioral decisions presumably allows prey to trade-off the often conflicting demands of successful detection and avoidance of potential predation threats against other fitness-related activities such as foraging, mating and territorial defence, so as to maximize fitness (Godin & Smith 1988; Lima & Dill 1990; Lima & Steury 2005; Brown *et al.* 2006a). To date, the threat-sensitive predator avoidance hypothesis has received extensive support across a range of taxa, including aquatic invertebrates (e.g., Rochette *et al.* 1997), terrestrial invertebrates (e.g., Persons & Rypstra 2001), amphibians (Laurila *et al.* 1997), reptiles (e.g., Amo *et al.* 2004), birds (e.g., Edelaar & Wright 2006) and mammals (e.g., Swaisgood *et al.* 1999). In addition, several authors have shown threat-sensitive responses to both visual (Bishop & Brown 1992; Chivers *et al.* 2001) and chemosensory cues (Lawrence & Smith 1989; Dupuch *et al.* 2004; Brown *et al.* 2006a; Vavrek & Brown in press) associated with a predation threat by a variety of prey fishes.

Threat-sensitive responses range from 'pure threat-sensitive' patterns to 'hypersensitive' ones (Helfman & Winkleman 1997; Brown *et al.* 2006a), depending on the relative benefits of successful predator avoidance versus those associated with other activities such as foraging. In the case of pure threat-sensitive (graded) responses, prey exhibit antipredator behaviour at an intensity directly proportional to the level of perceived risk. Conversely, hypersensitive (non-graded) responses are characterized by

prey responding at or near maximal intensity when a threat is detected above some minimum behavioral response threshold (Helfman & Winkleman 1997; Brown *et al*. 2001). For example, Helfman (1989) and Helfman & Winkleman 1997) examined the antipredator response patterns (i.e., visual reaction distance, duration of response) in two sympatric damselfish species exposed to model predator threats. Threespot damselfish (*Stegastes planifrons*), which are primarily benthic territorial herbivores, exhibit pure threat-sensitive antipredator responses to visual predator cues. However, the sympatric bicolour damselfish (*Stegastes partitus*) exhibits antipredator responses at or near maximal intensities when they detect a predation threat above some minimal level (i.e., hypersensitive response), likely due to their more risky planktivorous foraging tactics (Helfman & Winkleman 1997). More recently, Brown *et al*. (2006a) have shown that juvenile convict cichlids (*Amatitlania nigrofasciata*) shift from a non-graded to a graded response pattern as shoal size increased. The form of the threat-sensitive response pattern should be shaped by the relative benefits associated with successful predator avoidance versus those associated with other fitness-related behaviours. Thus, moment to moment changes in the local risk of predation can shape threat-sensitive trade-offs (Lima & Bednekoff 1999; Brown *et al*. 2006a).

Variability in the ambient level of predation risk over periods of a few days to weeks is also known to influence the antipredator responses of prey. Lima and Bednekoff (1999) and Sih *et al*. (2000) argued that the degree of variability in the level of risk should dictate the nature of the trade-offs between predator avoidance and other activities. In fact, a growing body of literature shows that, in an environment characterized by frequent predation threats, prey exhibit significantly lower intensities of antipredator behaviour when they perceive a predation threat and increased foraging activity in the absence of any perceived threat (e.g., Hamilton & Heithaus 2001; Sih & McCarthy 2002; Koivisto & Puesenius 2003; Foam *et al*. 2005a; Ferrari *et al*. 2008). Brown *et al*. (2006b) have recently shown that such variability in ambient predation risk influences the threat-sensitive decisions made by juvenile convict cichlids. Cichlids pre-exposed to a high-frequency risk treatment exhibited lower intensity antipredator responses than did cichlids under a low-frequency risk treatment. However, high-frequency treatment cichlids compensated for their reduced response intensity by responding at considerably lower levels of predation threat (Brown *et al*. 2006b). Thus, it is becoming increasingly clear that threat-sensitive behavioural responses are shaped by both immediate cost-benefit trade-offs and short-term variability in predation risk. Any immediate or short-term factor that increases potential foraging benefits (or some other fitness related activity) relative to antipredator benefits should favour a graded-response pattern. However, factors that decrease foraging benefits relative to antipredator benefits should favour a hypersensensitive response pattern (Brown *et al*. 2006a).

Populations may also experience different ambient predation pressures over generational time scales. It is well established that long-term ambient predation pressure can determine the overall intensity of antipredator behaviour in a number of prey fishes (Giles & Huntingford 1984; Kelley & Magurran 2006), including the Trinidadian guppy (Magurran 2005). Such effects may be due to multigenerational selection or individual experience within a generation (Magurran 2005), or both. However, it is unknown if

long-term predation pressure shapes the threat-sensitive response pattern (i.e., graded versus nongraded). In the current laboratory study, we addressed the question of how longer-term predation risk might have shaped threat-sensitive antipredator response patterns in the Trinidadian guppy. Under laboratory conditions, we exposed guppies collected from high- and low-predation sites within the Aripo River (Northern Range Mountains, Trinidad) to conspecific chemical alarm cues at varying concentrations. A variety of taxonomically diverse freshwater prey fishes (Chivers & Smith 1998; Wisenden & Chivers 2006), including the Trinidadian guppy (Nordell 1998; Brown & Godin 1999), rely on damage-released chemical alarm cues to assess local predation threats. These cues are typically found in the epidermis and are only released following mechanical damage, as would occur in a predation event (Chivers & Smith 1998). Given the nature of their release, alarm cues are a reliable indicator of local predation threats (Brown 2003; Wisenden & Chivers 2006). Previous work has shown that the relative concentration of alarm cue detected is a reliable indicator for the level of immediate predation threat in aquatic prey organisms (Lawrence & Smith 1989; Dupuch *et al.* 2004; Ferrari *et al.* 2005; Brown *et al.* 2006a). Here, we predicted that, given the comparatively high frequency and high risk of predation experienced by guppies from the Lower Aripo River (high-predation population), they should exhibit a more intense overall antipredator response and a more graded (proportional) response to chemical alarm cues compared to guppies from the Upper Aripo River (low-predation population).

Methods
Study populations and fish collection
We collected adult female guppies, using beach and hand seines, from the two locations within the Aripo River, Northern Mountain Range, Trinidad. The Lower Aripo population is characterized as a 'high-predation' site (Magurran 2005), containing several species that prey on juvenile and adult guppies, including pike cichlids (*Crenichichla alta*), blue acara cichlids (*Aequidens pulcher*), and black acara cichlids (*Cichlasoma bimaculatum*). In addition, there are several predators which prey on small, juvenile guppies, including Hart's rivulus (*Rivulus hartii*) and the twospot astyanax (*Astyanax bimaculatus*). The Upper Aripo population is located above a barrier waterfall and contains only Hart's rivulus and a predatory freshwater prawn (*Macrobrachium crenulatum*), both of which are restricted to preying on small, juvenile guppies (Endler & Houde 1995; Magurran 2005). As such, it can be characterized as a 'low-predation' site (Magurran 2005). Prior to being used either as stimulus donors or test fish, guppies were housed in 350 L glass aquaria at ~24°C in the laboratory and fed twice daily with commercial flake food (Tetramin™). The tanks were aerated and cleaned daily. Wild-caught guppies were allowed to adjust to laboratory conditions for at least 24 hours prior to testing and were used in behavioural triads within three days of collection in the field.

Alarm stimulus preparation
We collected alarm cue from non-gravid (assessed visually) female guppies from both the Upper and Lower Aripo River populations. Cue donors were sacrificed by cervical dislocation. We immediately removed the head and tail (at the caudal peduncle) and all internal visceral tissue. We placed the remaining tissue (skin and underlying skeletal muscle) into 100 mL of aged tap water. Tissue samples were then homogenized

and filtered through polyester filter floss and diluted to the desired final volume with the addition of aged tap water. To control for any confound originating from population bias in the behavioural response to alarm cues (Brown & Godin 1999, unpublished data), we blended equal quantities of alarm cue from Upper and Lower Aripo guppies and used the combined solution as our stimulus alarm cue.

We used 11 Upper Aripo females (mean ± SD standard length = 2.65 ± 0.37 cm) and 14 Lower Aripo females (2.34 ± 0.35 cm) as alarm cue donors. We collected a total of 31.66 and 31.84 cm^2 of tissue surface area (Upper and Lower Aripo donors, respectively) and adjusted the final volume to 612 mL. The final concentration (~0.1 cm^2 mL^{-1}) was similar to those used in previous studies with the Trinidadian guppy (Brown & Godin 1999, unpublished data). We froze the alarm cue in 25 mL aliquots at –20°C until needed.

Experimental protocol

Behavioural observations of test fish were conducted in a series of 23 L glass aquaria filled with 18.5 L of dechlorinated tap water (~ 25 °C). Each tank contained a single air stone and an additional 1.5 m length of airline tubing to allow for the injection of the chemical stimuli without disturbing the focal fish. To facilitate the quantification of area use (see below), we divided the tank into three equal horizontal sections by drawing lines along the front and back walls of the test tanks. At least 4 h prior to a trial, we placed shoals of three nongravid (assessed visually) female guppies into a test tank to acclimate. We chose to test shoals of three females as this particular shoal size falls within the natural range of guppy shoal sizes in nature (e.g., Magurran & Seghers 1991) and to minimize the total number of guppies required. We used females as focal guppies because they tend to be more responsive to predation threats than males (Magurran 2005). Test guppies from were matched for body length, both within and between focal populations.

Trials consisted of a 5 min pre-stimulus and a 5 min post-stimulus injection observation period. Immediately prior to the pre-stimulus observation period, we withdrew and discarded 60 mL of tank water through the stimulus injection tube. We then removed and retained an additional 60 mL of tank water. Following the pre-stimulus observation, we injected 5 mL of stimulus alarm cue at one of four concentrations or 5 mL of aged tap water as a control, and slowly flushed the stimulus into the test tank using the retained 60 mL of tank water. Alarm cue was used either at the stock concentration (100%) or diluted with dechlorinated tap water to 50%, 25% or 10% of stock concentration.

Once the stimulus was injected, we began the 5 min post-stimulus injection observation period. During both the pre- and post-stimulus observation periods, we recorded: 1) an index of area use, 2) a shoaling index, 3) the occurrence of dashing and freezing behaviour. Area use was recorded every 15 s as the position of each guppy within the tank (1 = bottom third of the tank, 3 = top third of the tank). Thus, possible area use scores ranged from 3 (all fish near the substrate) to 9 (all fish near the surface). We also recorded a shoaling index every 15 s, which ranged from 1 (no fish within one

body length of each other) to 3 (all fish within one body length of each other). Dashing was defined as a sudden burst of seemingly disoriented swimming, and freezing was defined as the cessation of all movement with the guppy settling to the substrate for at least 30 s. For dashing and freezing, we recorded either their presence or absence within each 5-min observation period. A reduction in area use and an increase in shoaling index, dashing and/or freezing are typical antipredator responses towards conspecific alarm cues in guppies (Brown & Godin 1999). We conducted 20 replicates per stimulus concentration for both Upper and Lower Aripo populations. Fish were used only once. Mean (± SD) length at testing was 2.49 ± 0.52 cm. All observations were made blind to the experimental treatments (stimulus concentrations or control).

We calculated the change in area use and shoaling index between the pre- and post-stimulus observation periods (post – pre) and used these difference scores as dependent variables in all subsequent analyses. As both area use and shoaling index data had unequal variances, we tested for the effects of focal population and alarm cue donor population using the Scheirer-Ray-Hare extension of the Kruskall-Wallis test, which is a nonparametric analysis of variance (Sokal & Rohlf 1995; Quinn & Keough 2002). To directly test for graded versus non-graded response patterns, we conducted polynomial planned contrasts for area use and shoaling behaviour for each focal population separately (Quinn & Keough 2002). Planned contrasts, as employed, test the total variance accounted for by linear versus quadratic models. The presence of significant quadratic terms suggests that the response pattern is best explained by a non-linear (non-graded) trend. For the occurrence of dashing and freezing behaviour, we tested for effects of focal population and alarm cue concentration using a two-factor G-test (Sokal & Rohlf 1995).

Results:
Conspecific alarm cue concentration significantly affected both area use and shoaling index (Figure 1, Table 1), with guppies exhibiting significantly stronger responses with increasing concentrations of alarm cues. There was no significant main effect of focal population (Figure 1, Table 1). However, we did find a significant concentration by focal population interaction for both area use and shoaling index (Figure 1, Table 1), suggesting that the response pattern differed for Upper Aripo (low-predation) and Lower Aripo (high-predation) guppies. Planned contrasts analysis confirmed this observation. For the Upper Aripo population, both area use and shoaling index exhibited significant quadratic terms, whereas the Lower Aripo population exhibited only significant linear terms (Table 2, Figure 1). Significant linear terms suggest graded response patterns, whereas significant quadratic terms, over and above the linear term, suggest nongraded or hypersensitive response patterns (Table 2). In addition, we found significant stimulus concentration by focal population interactions in the occurrence of both dashing (Likelihood ratio $\chi^2 = 18.19$, df = 4, P = 0.001) and freezing ($\chi^2 = 16.07$, df = 4, P = 0.003) behaviour (Figure 2).

Our results show that, at the 100% stimulus concentration, the overall antipredator response intensity was highest for Lower Aripo (high predation) guppies and that response intensity decreased in a graded (proportional) fashion with decreasing stimulus

concentration. Conversely, despite an overall lower intensity antipredator response to the highest stimulus concentration by Upper Aripo (low predation) guppies, decreasing stimulus concentration did not result in proportionally lower intensity responses. Thus, our results suggest differing response patterns to varying concentrations of conspecific alarm cues between these two study populations.

Discussion

Our results demonstrate that the overall intensity of antipredator behaviour and the form of threat-sensitive response patterns to conspecific chemical alarm cues in wild-caught Trinidadian guppies is dependant upon their provenance. When exposed to the highest test concentration of conspecific alarm cues, Lower Aripo (high-predation) guppies exhibited consistently higher responses intensity than did Upper Aripo (low-predation) guppies. This finding is consistent with previous work, which demonstrates that guppies from high-predation populations respond with a higher overall intensity to a standardized predation threat than do those from low-predation populations (Magurran 2005). More important, however, is our novel finding that the pattern of threat-sensitive response is likewise dependent upon provenance. Our results reveal that guppies from the Lower Aripo site exhibited a graded threat-sensitive response pattern in which the intensity of antipredator behaviour was proportional to the concentration of alarm cue presented. In comparison, guppies from the Upper Aripo site exhibited a non-graded (hypersensitive) response pattern to the same range of alarm cue concentrations. Collectively, these findings support our hypothesis that long-term predation pressure shapes threat-sensitive behavioural trade-offs in prey organisms.

Although it is clear that threat-sensitive response patterns differ between the Upper and Lower Aripo River populations, it is unclear if this plasticity is due to population-specific experiences or the product of differential selection, or both. A wealth of evidence has shown that individual experience with predation pressure results in context-appropriate antipredator behavioural responses (reviewed in Brown 2003; Kelley & Magurran 2006). For example, guppies from high-predation populations exhibit more risk aversive behaviour patterns towards model predators compared to conspecifics from low-predation populations (Kelley & Magurran 2003a,b). However, this difference was absent between their laboratory-reared offspring, demonstrating that experience and learning play an important role in shaping predator avoidance behaviour (Kelley & Magurran 2003a,b). However, there is also strong evidence demonstrating the role of selection in the evolution of population-specific antipredator response patterns. For example, Magurran *et al.* (1992) examined the shoaling and predator inspection behaviour of laboratory-reared offspring of guppies transplanted from a high-predation to a low-predation population some 26-36 generations previously. They then compared their antipredator behaviour to that of the laboratory-reared offspring of the original high-risk population. Their results demonstrate that when individual experience is controlled for, guppies transplanted from high- to low-predation sites exhibit typical 'low-predation site' antipredator responses. Thus, the results of Magurran *et al.* (1992) demonstrate that antipredator behaviour patterns can be modified by selection over multiple generations. Selection may act directly on the overall patterns of antipredator behaviour (i.e., direct genetic effects, O'Steen *et al.* 2002) or indirectly by favouring phenotypic plasticity

(Magurran 2005). Clearly, future research should examine the non-mutually exclusive roles of experience and selection on the threat-sensitive response patterns of Trinidadian guppies.

Successful predator avoidance is costly in terms of time and energy available for other fitness-related activities (Lima & Dill 1990; Welton *et al.* 2003; Lima & Steury 2005). By exhibiting an antipredator response intensity proportional to the level of a perceived threat (i.e., alarm cue concentration), Lower Aripo guppies are likely to maintain foraging and courtship activities, albeit at a reduced level, under the risk of predation (Croft *et al.* 2004; Foam *et al.* 2005b). Alternatively, responding to frequent predation threats with high intensity predator avoidance behaviour would likely result in a dramatic reduction in fitness due to lost foraging or courtship benefits (Lima & Bednekoff 1999; Sih et al. 2000). However, given that the frequency and/or intensity of predation threats are lower for Upper Aripo guppies, they may optimize threat-sensitive trade-offs by exhibiting 'maximal' antipredator responses whenever a threat is detected over some minimal behavioural threshold (Brown *et al.* 2001).

Our current results extend our understanding of factors that shape an individual's pattern of threat-sensitive behavioural decision making in animals. Brown *et al.* (2006a) have shown that juvenile convict cichlids switch from hypersensitive (nongraded) response as solitary individuals to a graded response pattern when tested in shoals of six conspecifics. Individuals tested alone or in small groups may be at a higher level of predation risk than are individuals in larger groups (Hoare *et al.* 2004). Thus, the elevated risk associated with small shoal size appears to shift the form of the threat-sensitive response from a graded to a hypersensitive one. By doing so, individuals presumably gain antipredator benefits at the cost of lost foraging opportunities. Conversely, the threat reduction associated with larger shoal sizes might favour a more graded response pattern, which would allow prey to continue to forage whilst concurrently benefitting from a group-mediated reduction in individual risk of predation.

Likewise, Brown *et al.* (2006b) have shown that frequency of predation risk (temporal variability) over the period of a few days also influences threat-sensitive decision making. In this study, Brown *et al.* (2006b) pre-exposed cichlids to either an infrequent or a frequent predation threat regime and tested their response to varying concentrations of conspecific alarm cue. They found that cichlids pre-exposed to a high frequency of risk exhibited consistently lower intensity responses compared to those pre-exposed to a low frequency of risk, consistent with the predictions of the predation risk allocation hypothesis (Lima & Bednekoff 1999; Ferrari *et al.* 2008). Moreover, cichlids appear to compensate for the reduced response intensity by lowering the threshold concentration of conspecific alarm cue needed to elicit an overt behavioural response (Brown *et al.* 2006b). Interestingly, Brown *et al.* (2006b) found that short-term variability in perceived predation threats did not induce a change in the pattern of response. They reported that cichlids exposed to high versus low frequencies of predation risk still exhibited a nongraded response pattern, suggesting that threat-sensitive decisions are shaped by immediate factors (Vainikka et al. 2005; Brown et al. 2006b; Ferrari & Chivers 2006). Thus, variability in local predation threats over multiple

temporal scales interact to shape both the overall intensity and pattern of antipredator behaviour (Lima & Bednekoff 1999; Brown *et al.* 2006 a,b; current study).

How prey perceive predation risk and how they integrate perce ived risk over multiple time scales are amongst the most important unanswered questions in the field of predator-prey dynamics (Lima & Steury 2005). Previous studies have shown that both immediate and short temporal scale events can shape individual behavioural decisions. Our current results suggest that long-term predation pressure also influences threat-sensitive decisions. Taken together, these studies highlight a high degree of intraspecific plasticity in behavioural decision making and suggest that the form of the threat-sensitive antipredator trade-off in prey is dynamic. Rather than simply adopting 'risk aversive' versus 'risk taking' strategies (Welton *et al.* 2003), the dynamic threat-sensitivity paradigm proposes that prey continually adjust their behavioural response according to immediate, intermediate or long-term patterns of predation risk (Brown et al. 2006a,b).

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Table 1: Results of two-way nonparametric ANOVA, testing for the effects of alarm cue concentration, focal population (Upper vs. Lower Aripo guppies) and the two-way interaction for the observed change in area use and shoaling index. N = 20 per treatment combination.

		H*	df	P
Area use	Concentration	19.85	4, 190	**< 0.001**
	Focal population	0.07	1, 190	= 0.79
	Concentration x Focal population	2.93	4, 190	**= 0.022**
Shoaling index	Concentration	10.21	4, 190	**< 0.001**
	Focal population	0.01	1, 190	= 0.99
	Concentration x Focal population	2.75	4, 190	**= 0.029**

* Scheirer-Ray-Hare two-way extension of the Kruskall-Wallis test.

Table 2: Planned contrast values and 95% confidence intervals for linear and quadratic estimates for Upper Aripo and Lower Aripo guppies exposed to varying concentrations of conspecific alarm cues. N = 20 per treatment combination.

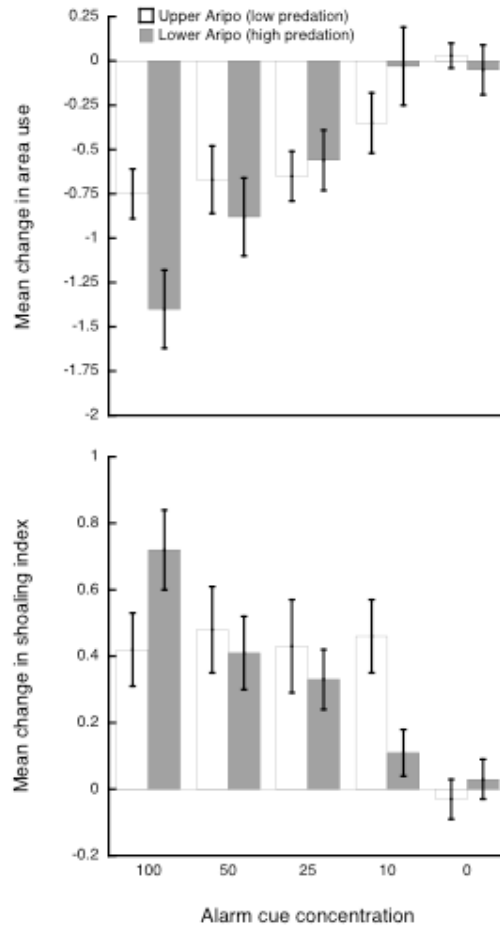|		Contrast difference	95% CI		P
			Lower	Upper	
Upper Aripo					
Area use	Linear	52.50	60.69	74.32	**< 0.001**
	Quadratic	23.33	1.51	45.14	**= 0.036**
Shoaling	Linear	-34.11	-59.02	-9.20	**= 0.008**
	Quadratic	-30.92	-55.83	-6.00	**= 0.016**
Lower Aripo					
Area use	Linear	82.83	61.28	104.38	**< 0.001**
	Quadratic	-11.57	-33.12	9.98	= 0.29
Shoaling	Linear	-65.09	-86.63	-43.56	**< 0.001**
	Quadratic	4.68	-16.86	26.21	= 0.68

Figure 1: Mean (± SE) change in area use and shoaling index for Upper Aripo (open bars) and Lower Aripo (shaded bars) guppies exposed to conspecific chemical alarm cues at the stock concentration (100%), diluted to 50%, 25%, or 10%, or a dechlorinated tap water control (0%). A reduction in area use and an increase in shoaling index indicate an antipredator response. N = 20 per treatment combination.
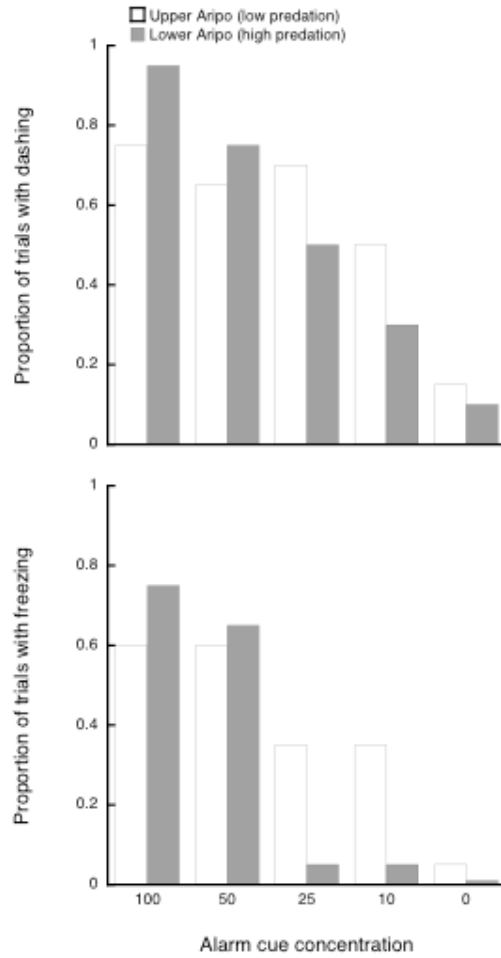
Figure 2: Proportion of trials in which dashing and freezing behaviour were observed for Upper Aripo (open bars) and Lower Aripo (shaded bars) guppies exposed to conspecific chemical alarm cues at the stock concentration (100%), diluted to 50%, 25%, or 10%, or a dechlorinated tap water control (0%). N = 20 per treatment combination.