

## Article

# Sender and receiver experience alters the response of fish to disturbance cues

Jack A. GOLDMAN<sup>a,\*</sup>, Laurence E. A. FEYTEN<sup>a</sup>, Indar W. RAMNARINE<sup>b</sup>, and Grant E. BROWN<sup>a</sup>

<sup>a</sup>Department of Biology, Concordia University, 7141 Sherbrooke St. West, Montreal, QC, H4B 1R6, Canada and

<sup>b</sup>Department of Life Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago

\*Address correspondence to Jack A. Goldman. E-mail: jgoldma5@uwo.ca

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## Abstract

Predation is a pervasive selection pressure, shaping morphological, physiological, and behavioral phenotypes of prey species. Recent studies have begun to examine how the effects of individual experience with predation risk shapes the use of publicly available risk assessment cues. Here, we investigated the effects of prior predation risk experience on disturbance cue production and use by Trinidadian guppies *Poecilia reticulata* under laboratory conditions. In our first experiment, we demonstrate that the response of guppies from a high predation population (Lopinot River) was dependent upon the source of disturbance cue senders (high vs. low predation populations). However, guppies collected from a low predation site (Upper Aripo River) exhibited similar responses to disturbance cues, regardless of the sender population. In our second experiment, we used laboratory strain guppies exposed to high versus low background risk conditions. Our results show an analogous response patterns as shown for our first experiment. Guppies exposed to high background risk conditions exhibited stronger responses to the disturbance cues collected from senders exposed to high (vs. low) risk conditions and guppies exposed to low risk conditions were not influenced by sender experience. Combined, our results suggest that experience with background predation risk significantly impacts both the production of and response to disturbance cues in guppies.

**Key words:** disturbance cues, ecology of information, perceived risk, predator–prey interactions, Trinidadian guppy

The ability to reliably assess local predation threats allows prey to balance the often conflicting demands of reducing predation risks while still maintaining sufficient time and energy to engage in other fitness related activities such as foraging (Lima and Dill 1990; Ferrari et al. 2010). Chemosensory cues provide valuable sources of public information regarding the identity and intensity of local predation threats (Kats and Dill 1998), allowing for context appropriate behavioral trade-offs. For example, wood mice *Apodemus sylvaticus* reduce foraging behavior when in the presence of predatory chemical cues (Sunyer et al. 2013). Likewise, desert isopods *Hemilepistus reaumuri* use the presence vs. absence of the odor of scorpion *Scorpio palmatus* to make fine-scale risk assessments (Zaguri et al. 2018).

Within aquatic systems, prey are widely known to assess predation risk directly from chemosensory information originating from the predator (Kats and Dill 1998) or through cues released by conspecific and/or heterospecific prey-guild members (Vavrek et al. 2008; Ferrari et al. 2010). Perhaps most studied among aquatic prey are the damage-released chemical alarm cues, which are released via mechanical damage, as would occur during a predator attack (Chivers and Smith 1998; Brown 2003; Ferrari et al. 2010). Given the mechanism of release, these cues are reliable indicators of local predation risks (Chivers et al. 2007; 2012) and can elicit strong species-typical antipredator responses in nearby conspecifics and heterospecific prey-guild members (Ferrari et al. 2010, Chivers et al. 2012).

In addition to the damage-released alarm cues, many aquatic prey species rely on disturbance cues as sources of local threat assessment (Ferrari et al. 2010). Disturbance cues differ from the alarm cues, as they are non-injury released sources of risk assessment information, released in urine or across the gill epithelium of aquatic vertebrates when stressed or disturbed (Wisenden et al. 1995; Vavrek and Brown 2009; Brown et al. 2012; Abreu et al. 2016). Thus, disturbance cues would be released (and potentially detected) much earlier in the predation sequence (Wisenden et al. 1995) and elicit short-term increases in predator avoidance behavior (i.e., increased shoaling and decreased area use in guppies, Goldman et al. 2019). Given that they are released prior to an attack by a predator, disturbance cues are argued to function as early warning cues (Wisenden et al. 1995; Ferrari et al. 2010). Disturbance cues are widely distributed across phyla, as they have been identified in a diverse range of species, such as invertebrates (Hazlett 1985, 1990a, 1990b; Nishizaki and Ackerman 2005; Siepielski et al. 2016), amphibians (Kiesecker et al. 1999; Gonzalo et al. 2010; Crane and Mathis 2011) and fish (Wisenden et al. 1995; Manassa et al. 2013; Bett et al. 2016; Fulton et al. 2017; Goldman et al. 2019).

A growing body of research demonstrates that past experience with predation shapes how prey respond to damage-released alarm cues (Brown 2003; Ferrari et al. 2009; Brown et al. 2013). For example, guppies from high predation risk populations exhibit stronger antipredator and more threat-sensitive (Brown et al. 2009, 2014) responses to standardized predation threats compared to conspecifics from low predation risk populations. Indeed, recent studies demonstrate that exposure to conditions of elevated predation risk for even a few days is sufficient to induce “high risk behavioural phenotypes among prey” (Brown et al. 2013, 2015; Crane and Ferrari 2016; Mitchell et al. 2016). Given that alarm cues are “honest and reliable” indicators of acute predation risks (Brown 2003; Chivers et al. 2012), it is perhaps not surprising that prey may alter their response to these cues based on recent experience. However, Chivers et al. (2007) demonstrated that alarm cue senders do not show similar plasticity in the production and release of alarm cues; fathead minnows reared under high versus low predation conditions did not differ in the production of alarm cues.

Recent experience with predation risk has also been shown to influence how prey respond to disturbance cues. Wood-frog tadpoles *Lithobates sylvaticus* adjust behavioral responses according to background risk experience, exhibiting all-or-nothing responses among high-risk receivers, regardless of sender risk level (Bairos-Novak et al. 2017). However, tadpole receivers with low-risk experience exhibited stronger responses to the disturbance cues of high versus low-risk senders. In addition, Bairos-Novak et al. (2019a) have shown that the release of disturbance cue can be influenced by familiarity among group members in the fathead minnow. Thus, it appears that while the effects of ambient predation risk is limited to plasticity of the response in the case of damage-released alarm cues (Chivers et al. 2007; Brown et al. 2009), prey may adjust both the release of, and response to disturbance cues (Bairos-Novak et al. 2019a, 2019b).

Here, we test the effects of background predation risk on both the production of and response to disturbance cues in Trinidadian guppies in wild-caught guppies from populations with different background predation risk conditions (Experiment 1) and in a common laboratory population, in which we manipulated background risk experimentally (Experiment 2). Initially, we predict that guppies from a high predation risk population should respond to disturbance cues more intensely and produce disturbance cues that elicit a

stronger response compared to conspecifics from a low predation risk population. Second, if predicted responses of wild caught guppies are due to predation experience (and not population-specific differences), we predict the same response patterns in a laboratory strain of guppies experimentally exposed to high versus low background risk conditions.

## Materials and Methods

### Experiment 1: Effects of sender and receiver source population

Under laboratory conditions, we tested the effects of ambient predation risk on both the production and response to conspecific disturbance cues in wild-caught Trinidadian guppies. We collected female guppies from the Lopinot and Upper Aripo Rivers, Trinidad, using a 3-mm mesh seine net. The Lopinot River is a high predation stream, with a diverse predator guild including pike cichlids (*Crenicichla* sp.), blue acara *Andinocara pulcher*, brown coscarub *Cichlasoma taenia*, and wolf fish *Hoplias malabaricus* (Deacon et al. 2018). The Upper Aripo is characterized as low predation site with no aquatic predators of adult guppy present (Deacon et al. 2018). Guppies were transported to the University of the West Indies, St Augustine, Trinidad, and housed in 100-L glass aquaria. These holding tanks were kept at  $\sim 26^{\circ}\text{C}$ , under a 12: 12 h light: dark cycle and guppies were fed twice daily with commercial flake food and freeze-dried tubifex.

In order to generate disturbance cues (and the odor of undisturbed guppies), shoals of 10 Lopinot River or Upper Aripo River guppies were placed into 20 L tanks and allowed to acclimate for 24 h. Tanks were aerated, continuously filtered and wrapped in white plastic to prevent visual disturbance. We passed a realistic predator model (Feyten et al. 2019) connected to a glass rod through the tank for 60 s, being careful not to contact the fish. The 14 cm long model (3D printed in polycarbonate) was hand painted to emulate a pike cichlid, a common guppy predator. After passing the model through the tank, we waited 60 s and gently mixed the water and collected  $\sim 100$  mL to be used as a disturbance cue. We collected all cues immediately before use in laboratory trials. As a control, we collected water samples (odour of undisturbed conspecifics) from the sender tanks prior to presenting the model predator. This protocol has been shown to be a reliable way to collect disturbance cues (Vavrek et al. 2008; Goldman et al. 2019; Bairos-Novak et al. 2019a). We generated cues from a total of five ‘sender’ shoals from each population (i.e. each sender group was used to test 2 replicates for cue  $\times$  focal receiver population combination). Mean  $\pm$  SD size of cue senders was  $21.60 \pm 2.23$  mm SL for Lopinot River guppies and  $21.02 \pm 2.53$  mm SL for Upper Aripo guppies.

Behavioral observations were conducted in a series of 20 L glass aquaria, filled with 18 L of dechlorinated tap water ( $\sim 24^{\circ}\text{C}$ , 12: 12 L: D cycle). We affixed a single airstone to the back wall of the tank and attached an additional 1.5 m length of airline tubing to facilitate stimulus injection. We placed shoals of three female guppies into testing tanks and allowed  $\sim 2$  h to acclimate prior to testing (as in Brown and Godin 1999; Brown et al. 2009). We tested guppies in shoals, as singleton guppies typically exhibit high levels of baseline stress (i.e., pacing and dashing behavior, personal observations). Mean ( $\pm$  SD) size at testing was  $23.50 \pm 1.89$  mm SL for Lopinot River guppies and  $22.15 \pm 1.79$  mm SL for Upper Aripo guppies. Observations consisted of a 5 min pre-stimulus and a 5 min post-stimulus observation period. Immediately following the pre-stimulus observation period, we injected 10 mL of disturbance cue

**Table 1.** Summary of treatments and number of shoals tested per treatment combination

Receivers	Senders	Stimulus	N
Experiment 1: Effects of sender and receiver source population			
Lopinot River	Lopinot River	Disturbance cue	10
		Undisturbed conspecific cue	10
	Upper Aripo	Disturbance cue	10
		Undisturbed conspecific cue	10
Upper Aripo	Lopinot River	Disturbance cue	10
		Undisturbed conspecific cue	10
	Upper Aripo	Disturbance cue	10
		Undisturbed conspecific cue	10
Total number of shoals tested			N = 80
Experiment 2: Laboratory manipulation of risk			
High risk	High risk	Disturbance cue	12
		Undisturbed conspecific cue	11
	Low risk	Disturbance cue	11
		Undisturbed conspecific cue	9
Low risk	High risk	Disturbance cue	12
		Undisturbed conspecific cue	11
	Low risk	Disturbance cue	10
		Undisturbed conspecific cue	12
Total number of shoals tested			N = 88

Each test shoal consisted of 3 female guppies. See text for details for number of stimulus sender shoals.

(experimental) or the odor of undisturbed conspecifics (control) and began the post-stimulus observation. During both the pre- and post-stimulus observations, we recorded an index of shoaling and vertical area use every 15 s. Shoaling index scores ranged between 1 (no fish within one body length of another) to 3 (all fish within one body length of each other). Area use scores were recorded as the position of each guppy within the tank (1 = bottom third; 3 = top third), aided by horizontal lines drawn on the exterior of the tank. Area use scores ranged from 3 (all fish near the bottom) to 9 (all fish near the water surface). An increase in shoaling index and a reduction in area use (within shoals of three) is a reliable indicator of increased predator avoidance in guppies (Brown and Godin 1999; Brown et al. 2009, 2010, 2013, 2018; Goldman et al. 2019). The observations were made blind to treatment and the order of testing was randomized throughout the experiment.

We calculated the proportional change [(post-pre)/pre] in shoaling index and area use scores for each replicate as dependent variables in all subsequent analyses. We used ANOVAs (SPSS V24.0) to test the effects of disturbance cue (and undisturbed control) population, focal shoal population, and stimulus (disturbance cue vs. undisturbed cue) on the change in shoaling index and area use separately. All data met the assumptions of parametric tests. We conducted a total of  $N = 10$  observations per treatment combination (Table 1).

### Experiment 2: Laboratory manipulation of risk

Using a laboratory population of Trinidadian guppies, we manipulated background predation risk to further investigate the effects of ambient risk on the production and response to conspecific disturbance cues. Guppies were ~10th generation descendants of wild-caught Upper Aripo guppies. Prior to testing, guppies were held in 110 L glass aquaria, (~26°C, 12-12 L: D cycle). Guppies were fed with commercial tropical fish flake food (Nutrafin) twice a day. We generated conspecific damage-released chemical alarm cues

in order to manipulate background risk as in Brown et al. (2013). We used a total of 95 non-gravid (visually assessed) female guppies as alarm cue senders (mean  $\pm$  SD standard length =  $26.2 \pm 0.57$  mm and body depth =  $4.7 \pm 0.14$  mm). Senders were euthanized via cervical dislocation (in accordance with Concordia University Animal Research Ethics Protocol# 30000255). We immediately removed the head, tail (at the caudal peduncle), and internal visceral tissues. The remaining tissue was placed into 100 mL of chilled distilled water and then homogenized and filtered through polyester filter floss. We added distilled water to achieve our desired final volume. We collected a total of 181.6 cm<sup>2</sup> of skin (diluted to a final volume of 1210 mL). Alarm cues were frozen in 40 mL aliquots at  $-20^{\circ}\text{C}$  until required. Damage-released chemical alarm cues at this concentration are known to elicit reliable increases in predator avoidance behavior (increased shoal cohesion and reduced vertical area use) in guppies (Brown et al. 2009; 2013).

To create high versus low levels of background risk among receivers, we placed groups of size matched female guppies (16 shoals of 3 guppies each;  $N = 48$  per group) into 40 L flow-through bins. Each bin contained a gravel substrate and a single air stone affixed to the back right and was held at  $\sim 21^{\circ}\text{C}$ . Focal fish ‘bins’ were exposed to 10 mL of conspecific alarm cue (high risk) or 10 mL of distilled water (low risk) twice per day for 5 days (as in Brown et al. 2013; 2015). Approximately 30 min after exposure to a pre-conditioning cue, we conducted a partial water change ( $\sim 50\%$  volume). We fed each group of fish twice daily throughout the conditioning phase. We replicated this process three times, with each ‘block’ of high- versus low-risk focal guppies yielding four shoals of three guppies for each treatment combination (disturbance cue vs. odour of undisturbed conspecifics from high-risk and low-risk senders).

Using a similar procedure, we manipulated background risk for cue senders. Two shoals of 20 size-matched female guppies (one high-risk shoal and one low-risk shoal, replicated four times for each risk level) in 37 L aquaria containing a gravel substrate and an air stone. We increased the number of senders per tank (20 vs. 10) due to the larger tank volumes used in order to keep the relative concentration of disturbance cues similar. As above, sender tanks were exposed to either 10 mL of alarm cue or distilled water, twice daily for 5 days. We conducted  $\sim 50\%$  water changes 30 min following the introduction of a cue. As we replicated sender groups four times, each sender group yielded disturbance and undisturbed cues for three test replicates per treatment combination. Disturbance cues and the odour of undisturbed conspecifics were collected as described above.

Behavioral observations were conducted as described above, with the exception that shoals of guppies were tested in 37 L glass aquaria (filled with 20 L of dechlorinated tap water,  $\sim 24^{\circ}\text{C}$ , 12:12 L: D cycle). Mean ( $\pm$  SD) size at testing  $23.9 \pm 7.4$  mm. We calculated the proportional change in shoaling index and area use scores for each replicate (as per Mitchell et al. 2016). As focal guppies were pre-exposed to risk conditions as groups, we cannot consider them as truly independent. To account for this non-independence, pre-exposure round was included as a nested factor using ANOVAs (SPSS V24.0). Due to some guppies escaping test tanks during the acclimation period, final sample sizes ranged from 9 to 12 per treatment combination (Table 1). As above, observations were made blind to treatment and the order of testing was randomized throughout the experiment.

## Results

### Experiment 1

Our overall analysis revealed a significant main effect of focal population and stimulus, as well as a significant three-way interaction among the effects of sender and focal populations and stimulus type for the proportional change in shoaling index (Table 2; Figure 1A). Regardless of treatment combination, guppies appeared to increase shoal cohesion in response to disturbance cues (Figure 1A). However, both Lopinot (high risk) and Upper Aripo (low risk) River guppies exhibited stronger responses to the disturbance cues collected from Lopinot River senders, with the greatest response in the Lopinot receivers–Lopinot sender treatment combination (Figure 1A). For the proportional change in area use, we found only a significant effect of stimulus (disturbance cue vs. the odour of undisturbed conspecifics, Table 2; Figure 1B). We observed similar reductions in area use regardless of disturbance cue sender or focal population (Table 2; Figure 1B).

### Experiment 2

Our overall analysis suggests that the behavior of guppies pre-exposed to high versus low levels of background risk follows a similar pattern found among wild caught guppies from high versus low predation risk populations (Experiment 1). We found no significant effect of pre-conditioning block (nesting factor,  $P > 0.05$  for both shoaling index and area use; Figure 2, Table 3). We found a significant three-way interaction for the proportional change in shoaling index (Figure 2A, Table 3). As with wild caught guppies, laboratory guppies exhibited an increase in shoal cohesion in response to disturbance cues, regardless of background risk treatment. However, guppies pre-exposed to the high-risk background treatment exhibited greater increases in shoaling index in response to disturbance cues from high risk vs. low risk senders (Figure 2A; Table 3). Similar to the response of wild-caught guppies, we found a significant decrease in proportional area use in response to disturbance cues (Figure 2B; Table 3). However, the significant receiver risk level  $\times$  stimulus interaction term (Figure 2B; Table 3) suggests that high risk

guppy receivers exhibited stronger responses to disturbance cues than did low risk receivers. As above, sender experience had no effect on the observed change in area use (Figure 2B; Table 3).

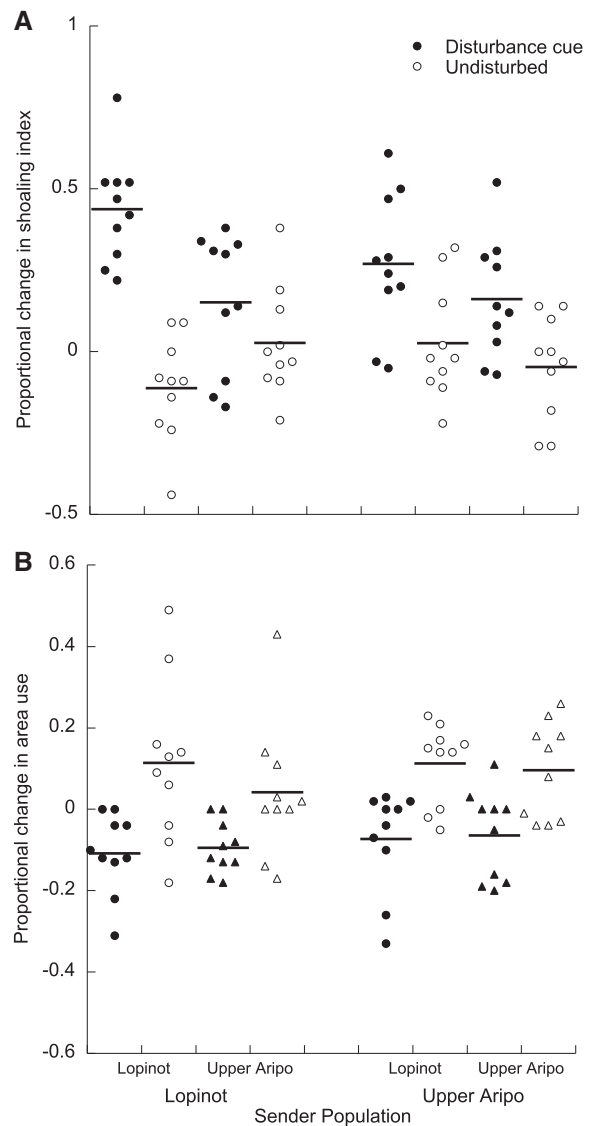
## Discussion

Our first experiment shows the combined effects of sender and receiver experience on the response to disturbance cues. Guppies from the Lopinot (high-risk) showed stronger overall responses compared to guppies from the Upper Aripo (low-risk). However, the response of Upper Aripo guppies did not differ based on the experience of the sender, whereas the response of Lopinot guppies differed based on sender experience. Our results suggest that under high-risk conditions, there is an interacting effect of receivers and senders, but under low-risk conditions there is no effect of senders. The results of

**Table 2.** Results of ANOVAs on the proportional change in shoaling index and area use for guppies tested in experiment 1

	<i>F</i>	<i>Df</i>	<i>P</i>
Shoaling index			
Sender	0.34	1, 72	0.56
Focal	4.09	1, 72	0.047
Stimulus	48.33	1, 72	<0.001
Sender $\times$ Stimulus	1.87	1, 72	0.18
Focal $\times$ Stimulus	8.04	1, 72	0.006
Sender $\times$ Focal	0.04	1, 72	0.84
Sender $\times$ Focal $\times$ Stimulus	5.78	1, 72	0.019
Area use			
Sender	1.05	1, 72	0.31
Focal	0.33	1, 72	0.57
Stimulus	37.21	1, 72	<0.001
Sender $\times$ Stimulus	0.11	1, 72	0.92
Focal $\times$ Stimulus	0.94	1, 72	0.34
Sender $\times$ Focal	0.19	1, 72	0.67
Sender $\times$ Focal $\times$ Stimulus	0.27	1, 72	0.61

Sender population (high vs. low predation risk), focal population (high vs. low predation risk) and stimulus (disturbance cue vs. odour of undisturbed conspecifics) were included as independent variables.  $N = 10$  per treatment combination.



**Figure 1.** Proportional change in shoaling index (A) and proportional change in area use (B) for guppies collected from high predation (Lopinot) and low predation (Upper Aripo) streams and exposed to the disturbance cue (solid circles) or odor of undisturbed guppies (open circles) from Lopinot or Upper Aripo senders.  $N = 10$  per treatment combination. Horizontal bars denote means.

**Table 3.** Results of nested ANOVAs for the proportional change in shoaling index and area use for guppies tested in experiment 2

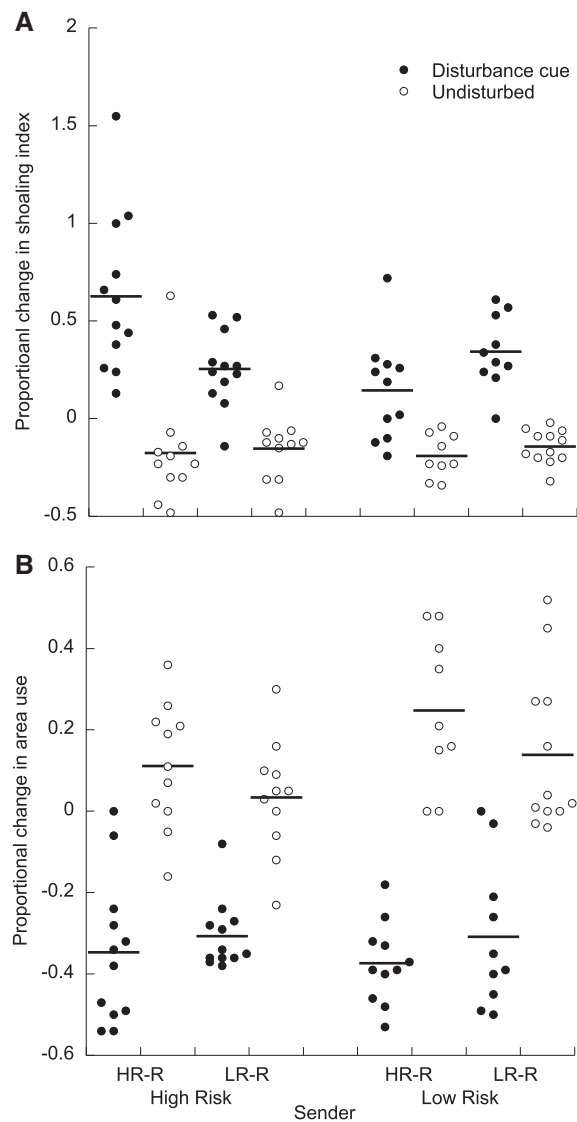
	<i>F</i>	<i>Df</i>	<i>P</i>
Shoaling index			
Sender	5.18	1, 76.34	0.017
Focal	0.34	1, 3.99	0.59
Stimulus	94.39	1, 76.54	<0.001
Sender × Stimulus	4.24	1, 76	0.043
Focal × Stimulus	1.82	1, 76	0.18
Sender × Focal	8.63	1, 76	0.004
Sender × Focal × Stimulus	7.25	1, 76	0.009
Nested factor	0.86	4, 76	0.49
Area use			
Sender	3.06	1, 76.52	0.08
Focal	0.18	1, 3.99	0.69
Stimulus	195.21	1, 76.81	<0.001
Sender × Stimulus	3.90	1, 76	0.052
Focal × Stimulus	5.17	1, 76	0.026
Sender × Focal	0.009	1, 76	0.93
Sender × Focal × Stimulus	0.201	1, 76	0.66
Nested factor	1.32	4, 76	0.27

Sender background risk (high vs. low), focal background risk (high vs. low) and stimulus (disturbance cue vs. odor of undisturbed conspecifics) were included as independent variables.  $N = 9\text{--}12$  per treatment combination.

our second experiment compliment those of Experiment 1, demonstrating that when background risk is induced, we also observed a combined effect of sender and receiver experience.

It is interesting to note that the observed effects of sender and receiver experience were only seen in one of two behavioral measures (shoaling index). Given that disturbance cues are released earlier in the predation sequence, they may be perceived as a lower risk form of information compared to sources of risk assessment such as alarm cues (Wisenden et al. 1995; Ferrari et al. 2010). Recent studies have shown that the response to disturbance cues is concentration dependant (Vavrek and Brown 2009; Goldman et al. 2019), consistent with previously documented threat-sensitive responses to damage-released alarm cues (Dupuch et al. 2004; Brown et al. 2006, 2009). It is possible that the low risk senders in the current experiment were simply producing lower quantities (concentration) of disturbance cues and that our observed response patterns are simply due to “detectable concentrations” of disturbance cues. However, Brown et al. (2009) demonstrate that while guppies from high-risk populations show stronger maximal responses to high concentrations of conspecific alarm cues, guppies from low-risk populations show significant response (increased shoaling and reduced area use) to much lower concentrations.

Our results represent the first demonstration that prey fish can modify the intensity of their antipredator responses proportionately to chemosensory cues based on information provided by the prior experience of both cue senders and receivers. We cannot rule out population-specific differences in Experiment 1. It is possible that population differences in, for example, competition or foraging opportunities may shape the response patterns observed. However, the results of experiment 2, in which we induce risk in a single population, show analogous response patterns and provide support for our initial hypothesis. Alongside our results, recent studies show that background risk affects receivers of chemosensory cues (Brown et al. 2009; Bairos-Novak et al. 2017). Our results extend this, showing that experience also shapes how senders produce information. Previous studies show that background risk does not alter



**Figure 2.** Proportional change in shoaling index (A) and proportional change in area use (B) for guppies pre-conditioned to high versus low predation risk and exposed to the disturbance cue (solid circles) or odor of undisturbed guppies (open circles) from high versus low risk senders. Receiver risk treatment denoted as HR-R (high risk receivers) versus LR-R (low risk receivers).  $N = 9\text{--}12$  per treatment combination. Horizontal bars denote means.

alarm cue production (Chivers et al. 2007). However, as disturbance cues are released earlier in the predation sequence than alarm cues (Wisenden et al. 1995), senders may have greater plasticity in disturbance cue production. Bairos-Novak et al. (2017) demonstrated interacting effects between background risk and response to disturbance cues in woodfrog tadpoles, although the observed responses were all-or-nothing. Our results suggest that there is a graded response based on interacting experience. However, guppies increase the intensity of their antipredator responses based on sender experience only when the receivers were high-risk. Therefore, the effects of a sender’s experience only matter for high-risk receivers. This suggests that high-risk receivers may be more sensitive to information that is conveyed by the sender.

Consistent with previous findings (reviewed in Ferrari et al. 2010), our current results suggest high risk receivers may benefit from enhanced predator avoidance responses to disturbance cues.



Perhaps more interesting, our results suggest that the release of disturbance cues by senders is also shaped by background predation risks. Recently, Bairos-Novak et al. (2017) have argued that disturbance cues may function as a source of social information among prey regarding local predation risks and that senders benefit by releasing disturbance cues. Consistent with this, we found that increases in shoaling behavior were greatest among high risk guppies in response to disturbance cues from high risk senders. If disturbance cues are, at least in part, released voluntarily by senders (Bairos-Novak et al. 2019a; Crane et al. manuscript in review), our current results suggest that prey exposed to high risk conditions may benefit from releasing more (or more potent) disturbance cues. Senders should benefit from enhanced antipredator responses among nearby prey guild members.

There is growing interest in understanding the ecology of information in predator–prey interactions (Schmidt et al. 2010; Luttbeg and Trussell 2013; Magrath et al. 2015; Evans et al. 2016). Aquatic environments tend to be extremely heterogeneous, which makes it important to quantify the degree to which variation within the environment shapes information. Moreover, given the high degree of fission-fusion nature of guppy shoals (Croft et al. 2003; Wilson et al. 2014), it is reasonable to expect considerable variation in recent predation experience among shoalmates. Our results demonstrate that predation risk alters the information conveyed by disturbance cues, and that prey benefit from responding to these cues and may also benefit from their release. Therefore, we highlight the importance of disturbance cues in predator–prey interactions and specifically, how they can play a crucial role in the predation sequence. Combined with recent studies (Bairos-Novak et al. 2017; 2019a, 2019b; Goldman et al. 2019), we provide evidence for the use of disturbance cues as a model to investigate the ecology of information in predator–prey interactions. Future studies should investigate whether these cues are in fact a signaling system to warn prey-guild members, whether they can also be utilized as an antipredator tactic by senders to increase survival and the degree to which other ecological factors shape their information content (i.e., competition or diet).

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## References

- Abreu MS, Giacomini ACV, Gusso D, Koakoski G, Oliviera TA et al. 2016. Behavioural responses of zebrafish depend on the type of threatening chemical cues. *J Comp Phys A* 202:895–901.
- Bairos-Novak KR, Mitchell MD, Crane AL, Chivers DP, Ferrari MCO, 2017. Trust thy neighbour in times of trouble: background risk alters how tadpoles release and respond to disturbance cues. *Proc R Soc Lond B* 284:20171465.
- Bairos-Novak KR, Ferrari MCO, Chivers DP, 2019a. A novel alarm signal in aquatic prey: familiar minnows coordinate group defences against predators through chemical disturbance cues. *J Anim Ecol.* doi: 10.1111/1365-2656-12986
- Bairos-Novak KR, Crane AL, Chivers DP, Ferrari MCO, 2019b. Better the devil you know? How familiarity and kinship affect prey responses to disturbance cues. *Behav Ecol* 30:446–454.
- Bett N, Hinch SG, Yun SS, 2016. Behavioural responses of Pacific salmon to chemical disturbance cues during the spawning migration. *Behav Processes* 132:76–84.
- Brown GE, 2003. Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish Fish* 4:227–234.
- Brown GE, Godin J-GJ, 1999. Chemical alarm signals in wild Trinidadian guppies *Poecilia reticulata*. *Can J Zool* 77:562–570.
- Brown GE, Bongiorno T, DiCapua DM, Ivan LI, Roh E, 2006. Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. *Can J Zool* 84:1–8.
- Brown GE, Macnaughton CJ, Elvidge CK, Ramnarine IW, Godin J-GJ, 2009. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. *Behav Eco Sociobiol* 63:699–706.
- Brown GE, Elvidge CK, Macnaughton CJ, Ramnarine I, Godin J-GJ, 2010. Cross-population responses to conspecific chemical alarm cues in wild Trinidadian guppies *Poecilia reticulata*: evidence for local conservation of cue production. *Can J Zool* 88:139–147.
- Brown GE, Jackson CD, Malka PH, Jacques E, Couturier M-A, 2012. Disturbance cues in freshwater prey fishes: does urea function as an ‘early warning cue’ in juvenile convict cichlids and rainbow trout?. *Curr Zool* 58: 250–259.
- Brown GE, Elvidge CK, Ramnarine I, Chivers DP, Ferrari MCO, 2014. Personality and the response to predation risk: effects of information quantity and quality. *Anim Cogn* 17:1063–1069.
- Brown GE, Elvidge CK, Ramnarine I, Ferrari MCO, Chivers DP, 2015. Background risk and recent experience influences retention of neophobic responses to predators. *Behav Ecol Sociobiol* 69:737–745.
- Brown GE, Ferrari MCO, Elvidge CK, Ramnarine I, Chivers DP, 2013. Phenotypically plastic neophobia: a response to variable predation risk. *Proc R Soc Lond B* 280:20122712.
- Brown GE, Chuard PJC, Demers EEM, Ramnarine IW, Chivers DP et al. 2018. Personality and the retention of neophobic predator avoidance in wild caught Trinidadian guppies. *Behaviour* 155:265–278.
- Chivers DP, Smith RJF, 1998. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Écoscience* 5:338–352.
- Chivers DP, Brown GE, Ferrari MCO, 2012. The evolution of alarm substances and disturbance cues in aquatic animals. In: Brönmark C, Hansson L-A, editors. *Chemical Ecology in Aquatic Systems*. Oxford: Oxford University Press. 127–139.
- Chivers DP, Wisenden BD, Hindman CJ, Michalak TA, Kusch RC et al. 2007. Epidermal ‘alarm substance’ cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. *Proc R Soc Lond B* 274:2611–2619.
- Crane AL, Mathis A, 2011. Predator-recognition training: a conservation strategy to increase postrelease survival of hellbenders in head-starting programs. *Zool Biol* 30:611–622.
- Crane AL, Ferrari MCO, 2016. Uncertainty in risky environments: a high-risk phenotype interferes with social learning about risk and safety. *Anim Behav* 119:49–57.
- Croft DP, Arrowsmith BJ, Bielby J, Skinner K, White E et al. 2003. Mechanisms underlying shoal composition in the Trinidadian guppy *Poecilia reticulata*. *Oikos* 100:429–438.
- Deacon AE, Jones FAM, Magurran AE, 2018. Gradients in predation risk in a tropical river system. *Curr Zool* 64:213–221.
- Dupuch A, Magnan P, Dill LM, 2004. Sensitivity of northern redbelly dace *Phoxinus eos* to chemical alarm cues. *Can J Zool* 82:407–415.
- Evans JC, Votier SC, Dall SRX, 2016. Information use in colonial living. *Biol Rev* 91:658–672.
- Ferrari MCO, Sih A, Chivers DP, 2009. The paradox of risk allocation: a review and prospectus. *Anim Behav* 78:579–585.
- Ferrari MCO, Wisenden BD, Chivers DP, 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool* 88:698–724.

- Feyten LEA, Demers EEM, Ramnarine IW, Brown GE, 2019. Predation risk assessment based on uncertain information: interacting effects of known and unknown cues. *Curr Zool* 65:75–76.
- Fulton J, LeMoine CMR, Bucking C, Brix KV, Walsh PJ et al. 2017. A waterborne chemical cue from Gulf toadfish, *Opsanus beta*, prompts pulsatile urea excretion in conspecifics. *Phys Behav* 171:92–99.
- Goldman JA, Singh A, Demers EEM, Feyten LEA, Brown GE, 2019. Does donor group size matter? The response of guppies *Poecilia reticulata* and convict cichlids *Amatitlania nigrofasciata* to disturbances cues from conspecific and heterospecific donors. *Can J Zool* 97:319–325.
- Gonzalo A, Lopez P, Martin J, 2010. Risk level of chemical cues determines retention of recognition of new predators in Iberian green frog tadpoles. *Behav Ecol Sociobiol* 64:1117–1123.
- Hazlett BA, 1985. Disturbance pheromones in the crayfish *Orconectes virilis*. *J Chem Ecol* 11:1695–1711.
- Hazlett BA, 1990a. Source and nature of disturbance-chemical system in crayfish. *J Chem Ecol* 16:2263–2275.
- Hazlett BA, 1990b. Disturbance pheromones in the hermit crab *Calcinus laevis manus*. *Crustaceana* 58:314–316.
- Kats LB, Dill LM, 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361–394.
- Kiesecker JM, Chivers DP, Marco A, Quilchano C, Anderson MT et al. 1999. Identification of a disturbance signal in larval red-legged frogs *Rana aurora*. *Anim Behav* 57:1295–1300.
- Lima SL, Dill LM, 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool* 68:619–640.
- Luttberg B, Trussell GC, 2013. How the informational environment shapes how prey estimate predation risk and the resulting indirect effects of predators. *Am Nat* 181:182–194.
- Magrath RD, Haff TM, Fallow PM, Radford AN, 2015. Eavesdropping on heterospecific alarm calls: from mechanisms to consequences. *Biol Rev* 90:560–586.
- Manassa RP, McCormick MI, Chivers DP, Ferrari MCO, 2013. Social learning of predators in the dark: understanding the role of visual, chemical and mechanical information. *Proc R Soc B* 280:20130720.
- Mitchell MD, Chivers DP, Brown GE, Ferrari MCO, 2016. Living on the edge: how does environmental risk affect the behavioural and cognitive ecology of prey?. *Anim Behav* 115:185–192.
- Nishizaki MT, Ackerman JD, 2005. A secondary chemical cue facilitates juvenile-adult postsettlement associations in red sea urchins. *Limnol Oceanogr* 50:354–362.
- Schmidt KA, Dall SRX, van Gils JA, 2010. The ecology of information: an overview on the ecological significance of making informed decisions. *Oikos* 199:304–316.
- Siepielski AM, Fallon E, Boersma K, 2016. Predator olfactory cues generate a foraging - predation trade-off through prey apprehension. *R Soc Open Sci* 3:150537.
- Sunyer P, Muñoz A, Bosnal R, Espelta JM, 2013. The ecology of seed dispersal by small rodents: a role of predator and conspecific scents. *Funct Ecol* 27:1313–1321.
- Vavrek MA, Brown GE, 2009. Threat-sensitive responses to disturbance cues in juvenile convict cichlids and rainbow trout. *Ann Zool Fennici* 46:171–180.
- Vavrek MA, Elvidge CK, DeCaire R, Belland B, Jackson CD et al. 2008. Disturbance cues in freshwater prey fishes: do juvenile convict cichlids and rainbow trout respond to ammonium as an ‘early warning’ signal?. *Chemoecology* 18:255–261.
- Wilson ADM, Krause S, James R, Croft DP, Ramnarine IW et al. 2014. Dynamic social networks in guppies *Poecilia reticulata*. *Behav Ecol Sociobiol* 68:915–925.
- Wisenden BD, Chivers DP, Smith RJ, 1995. Early warning in the predation sequence: a disturbance pheromone in Iowa darters *Etheostoma exile*. *J Chem Ecol* 21:1469–1480.
- Zaguri M, Zohar Y, Hawlena D, 2018. Considerations used by desert isopods to assess scorpion predation risk. *Am Nat* 192:630–643.

