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Does donor group size matter? The response of Trinidadian guppies and Convict cichlids to disturbance cues from conspecific and heterospecific donors.

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Abstract

Prey are under immense pressure to make context specific, behavioural decisions. Prey use public information to reduce the costs associated with making inappropriate decisions. Chemical cues are commonly used by aquatic vertebrates to assess local threats and facilitate behavioural decision making. Previous studies on chemosensory assessment of risk have largely focused on damage-released alarm cues, with the cues released by disturbed or stressed prey (i.e. disturbance cues) receiving less attention. Disturbance cues are ‘early warning signals’ common among aquatic vertebrates, that may warn conspecific and heterospecific prey guild members of potential risk. Initially, we conducted a series of laboratory studies to determine: 1) if guppies (*Poecilia reticulata* Peters, 1859) produce and respond to disturbance cues and 2) if relative concentration (donor group size) determines response intensity. Secondly, we examined if guppies and Convict cichlids (*Amatitlania nigrofasciata* Günther, 1867) show similar response patterns to their own vs. heterospecific disturbance cues. Our results suggest that guppies exhibit increased predator avoidance behaviour to conspecific disturbance cues (relative to water from undisturbed conspecifics) and increased donor group size lead to stronger antipredator responses. However, while guppies and cichlids respond to each other’s disturbance cues, we found no effect of donor group size towards heterospecific disturbance cues. Our results suggest that disturbance cues are not generalized cues, and present a degree of species-specificity.

Key Words

Disturbance cues, Public information, Predator avoidance, Trinidadian guppy, *Poecilia reticulata* Peters, 1859, Convict cichlid, *Amatitlania nigrofasciata* (Günther, 1867), Heterospecific donors, Conspecific donors.

48 **Introduction**

49 Predation risk is a pervasive selection pressure, shaping the behavioural phenotypes of
50 prey animals. In fact, in response to the nonconsumptive effects of predation (Preisser et al.
51 2005), prey are often forced to trade-off time and energy devoted to activities such as foraging,
52 courtship and/or territorial defence in favour of increased vigilance and predator avoidance
53 (Lima and Dill 1990). Publically available information (risk assessment cues) regarding local
54 predators should allow prey to better balance these trade-offs (McNamara and Dall 2010) and
55 potentially increase survival during encounters with predators (Wisenden et al. 1999; Mirza and
56 Chivers 2001; Crane et al. 2018). However, risk assessment cues may vary in reliability
57 (Magrath et al. 2009), potentially restricting the ability to make correct choices (Dall et al. 2005),
58 and leading to inappropriate or costly behavioural decisions.

59 Within aquatic ecosystems, prey are known to rely on at least two main forms of
60 chemosensory information to assess local predation threats, damage-released chemical alarm
61 cues and disturbance cues to assess local risks (Ferrari et al. 2010; Chivers et al. 2012). The well
62 studied damage-released alarm cues are released from the skin (or visceral tissue) of a wide
63 range of taxonomically diverse aquatic prey following mechanical damage, as would occur
64 during a predator attack (Chivers and Smith 1998; Chivers et al. 2012). Alarm cues are reliable
65 and honest indicators of risk (Chivers et al. 2012) and can elicit dramatic short-term increases in
66 predator avoidance behaviour (Ferrari et al. 2010; Brown et al. 2011; Chivers et al. 2012).
67 Disturbance cues are argued to be metabolic by-products, released in the urine or across the gill
68 epithelium (Kiesecker et al. 1999; Vavrek et al. 2008; Brown et al. 2012) by aquatic vertebrates
69 when stressed or disturbed (Wisenden et al. 1995; Kiesecker et al. 1999; Abreu et al. 2016).
70 When detected by nearby conspecific and heterospecific prey, disturbance cues may function as

an ‘early warning’ cue of nearby predation threats (Wisenden et al. 1995; Kiesecker et al. 1999). Evidence suggests that disturbance cues play a vital role in the predation sequence, providing preliminary information used in the preparation of antipredator responses (Wisenden 2000, 2014). Like the damage-released alarm cues, disturbance cues have been reported in a wide variety of taxa (Hazlett et al. 1985; Bryer et al. 2001; Jordão 2004; Nishizaki and Ackerman 2005; Gonzalo et al. 2010; Bett et al. 2016; Bairos-Novak et al. 2017).

Unlike damage-released alarm cues, it has been suggested that disturbance cues are a generalized, non-species-specific source of information (Chivers et al. 2012). In phylogenetically related species, damage-released alarm cues appear to be evolutionarily conserved (Mirza and Chivers 2001; Kelly et al. 2006; Ferrari et al. 2010), as the intensity of responses to alarm cues decreases the more phylogenetically distant the heterospecifics are to the focal fish (Mirza and Chivers 2001; Mitchell et al. 2012). However, disturbance cues are thought to be metabolic by-products (Hazlett 1990; Bryer et al. 2001; Chivers et al. 2012), and lack species-specificity (Hazlett 1989, 1990; Vavrek et al. 2008). For example, Vavrek et al. (2008) demonstrated that juvenile convict cichlids (*Amatitlania nigrofasciata* (Günther 1867)) and rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) exhibited similar intensities of antipredator response to water from disturbed conspecifics and heterospecifics (disturbance cues). In addition, several authors have demonstrated that the relative concentration of chemosensory cues detected can provide sufficient information to allow for threat-sensitive behavioural decisions (Vavrek and Brown 2009). For example, wild caught Trinidadian guppies (*Poecilia reticulata* Peters, 1859) from high- predation environments exhibit a graded response to concentrations of alarm cues, responding with the strongest intensity to the highest concentration of alarm cue and decreasing commensurately with the concentration (Brown et al. 2009). Likewise, changes in the relative

concentration of disturbance cues elicit a marked response in prey fish (Vavrek and Brown 2009). Therefore, if disturbance cues are indeed a generalized cue, we might expect similar concentration-dependent responses to both conspecific and heterospecific disturbance cues.

We had three main goals to this study. Initially, we sought to confirm the presence of disturbance cues in Trinidadian guppies. Secondly, we tested if donor group size determines the relative concentration of disturbance cue and, as a result, the strength of the behavioural response in Trinidadian guppies. Thirdly, we tested if the cross-species response to disturbance cues is specific enough to observe an effect of relative concentration (donor group size). We exposed shoals of guppies to water from both stressed and unstressed conspecifics and tested for both a baseline response and an effect of concentration on the intensity of the response. Given the likelihood that disturbance cues are not species-specific (Vavrek et al. 2008) and that the concentration of conspecific disturbance cues influences antipredator responses (Vavrek and Brown 2009), there could be a cross-species effect of relative concentration on antipredator responses. To test this, we exposed independent shoals of Trinidadian guppies and convict cichlids to both conspecific and heterospecific disturbance cues at different relative concentrations.

Methods

Test Fish

In this experiment we used female Trinidadian guppies and Convict cichlids (male and female) from our laboratory stock populations. Convict cichlids and Trinidadian guppies are phylogenetically distance, allowing us to avoid potential confounds of using closely related

species. While Convict cichlids and guppies do not naturally co-exist in Trinidad, guppies are often sympatric with a number of con-familial cichlids (i.e. blue acara, *Aequidens pulcher* (Gill, 1858), brown coscarob *Cichlasoma taenia* (Bennett, 1831)). Prior to testing, the guppies were held in 110 L glass aquaria, and cichlids were held in 37 L glass aquaria. Donor aquaria had a gravel substrate and artificial vegetation and were filled with dechlorinated water that was continuously filtered through the tanks. For guppies, the water in the tanks was held at roughly 26-27° C, whereas cichlids were held at 22-23° C. Fish were exposed to a 12-12-hour light-dark cycle. The guppies were fed with commercial tropical fish flake food (Nutrafin) twice a day and cichlids were fed a diet of brine shrimp twice daily.

Stimulus Preparation

In order to collect disturbance cues and the water of undisturbed shoals, we placed either 10 donor fish in a 9.5 L tank (filled with 4.7 L of dechlorinated tap water) or 20 donor fish in a 35 L tank (filled with 9.5 L of dechlorinated tap water). This was done to ensure that the relative concentration of disturbance cues was not being affected (i.e. similar donor to water volume ratio). We placed the donor fish in tanks 24 hours prior to stimulus collection to allow an adequate time to acclimate because the tanks were wrapped in black garbage bags to ensure that they would not be disturbed prior to stimulus collection. We placed air stones in the back-right corner of each tank, which we then removed one hour prior to stimulus collection. Before collecting the disturbance cue, we removed 125 mL of tank water to use as the water from undisturbed conspecifics (control). To collect disturbance cues (experimental) we passed a realistic esocid predator model (15 cm) connected to a glass rod through the tank for 60 seconds. We were careful not to contact the fish to avoid the introduction of damage-released alarm cues. After passing the model through the tank, we waited 60 seconds and softly mixed the water

around the tank and removed 125 ml of tank water to be used as a disturbance cue. This procedure has previously been used to successfully collect disturbance cues (Vavrek and Brown 2009; Brown et al. 2012). We collected all cues immediately before the experiment and used them within an hour of collection.

Test Tanks

For both experiments, we used 37 L glass aquaria filled with 20 L of dechlorinated water as test tanks. We placed a gravel substrate to cover the bottom of our test tanks. Instead of continuously filtering through the tanks, we placed an air stone against the back wall of the tank. Directly above the air stone, we attached airline tubing to the back of the tank. The airline tubing was 1.5 m in length and we used it to inject the stimulus into the tank as to not disrupt the fish. We wrapped each tank in blue plastic to eliminate visual communication between fish in neighbouring tanks. To quantify area use, we drew three black horizontal lines on the exterior of the tanks, which divided the tank into three vertical sections. To allow an adequate amount of time to acclimate, we placed focal fish into test tanks 1 hour prior to testing. We tested guppies in shoals of three to accurately test shoaling index and area use. We tested cichlids in pairs, as prior experiments show that cichlids tested alone do not respond (Brown et al. 2006). For both guppies and cichlids, we selected donors and test fish randomly from the same stock population. After testing, we removed fish and placed them back into the stock population. We used donors and focal fish only once. We fed the focal fish *ad libitum* up to 1 hour before testing began. Therefore, food remained on the substrate during observations, allowing the quantification of foraging attempts without dispensing additional food (Brown et al. 2006).

Experimental Protocol

Behavioural testing consisted of two observation periods; 5-minute pre- and 5-minute post-stimulus periods. Immediately following the pre-stimulus observation period, we withdrew 60 mL of tank water through the stimulus injection tube and discarded it to ensure no residual cues remained in the injection tube. We removed and retained an additional 60 mL to be used to slowly flush the stimulus into the tanks. We then introduced 10 ml of either disturbance cue or undisturbed water. The stimulus was then slowly flushed into the test tank using the 60 mL of retained tank water. Post-stimulus injection observations began once the stimulus was fully introduced to the observation (test) tank.

Experiment 1: Response of guppies to disturbance cues and the effect of donor group size

The goal of this study was to confirm that Trinidadian guppies produce and respond to conspecific disturbance and to determine whether donor group size influenced the form or intensity of the observed response pattern. We collected disturbance cues and the water from undisturbed conspecifics from groups of 10 or 20 donors (described above). Mean ($\pm$ SD) total mass of donor group size was $4.61 \text{ g} \pm 0.15$ (10 donor treatment) and $10.08 \text{ g} \pm 0.13$ (20 donor treatment).

Behavioural observations consisted of paired 5 minute pre- and 5 minute post-stimulus injection observations and were conducted live by an individual. During both the pre- and post-stimulus observation periods, we scored shoaling index and area use as well documented measures of predator avoidance in guppies (Brown et al. 2009; 2010). We scored shoaling index and area use every 15 seconds throughout the 5 minute pre- and 5 minute post-stimulus

183 observation period. Shoaling index was recorded as the position of each test fish in relation to
184 one another. Scores ranged from 1 to 3 based on the proximity of the individuals being within 1
185 body length of each other (1 = no fish within 1 body length of each other; 3 = all fish within 1
186 body length of each other). Area use scores ranged from 3 (all fish in the bottom third of the
187 tank) to 9 (all fish in the top third of the tank). Both area use and shoaling index scores were
188 totaled and divided by the number of observations to obtain an average. Mean ($\pm$ SD) size of
189 test fish was 2.29 cm $\pm$ 0.17 standard length (SL) at the time of testing. Using the average
190 scores, we calculated the change in both shoaling index and area use (post-pre) as difference
191 scores. We used these changes as dependent variables. We conducted a total of 15 replicates per
192 treatment combination. These scores were rank transformed and due to the likelihood of the
193 highly autocorrelated behavioural measures, we performed a non-parametric *MANOVA* to test
194 the effect of stimulus treatment and group size on the response. We conducted analyses in R v. 3.
195 4. 3 using the package *stats* (R Core Team, 2017).

196

197 *Experiment 2: Effect of donor group size on response to conspecific and heterospecific*
198 *disturbance cues*

199 The goal was to determine whether the response to heterospecific disturbance cues is
200 specific enough to elicit a greater response as a result of exposure to different relative
201 concentrations. We collected disturbance cues from heterospecifics and conspecifics, as well as
202 the water from undisturbed heterospecifics and conspecifics from groups of 10 or 20 donors.
203 Mean ($\pm$ SD) mass of group size 10 donors were 5.20 g $\pm$ 0.36 (guppies) and 5.69 g $\pm$ 0.95
204 (cichlids), and for group size of 20 donors, 10.47 g $\pm$ 0.89 (guppies) and 10.44 g $\pm$ 0.87

(cichlids). For guppies, we scored shoaling index and area use during pre-and post-stimulus observation periods (*see above*). For cichlids, we recorded time spent moving, aggression rate and foraging rate. We measured time spent moving on one individual. For foraging, we measured the attempts per observation period, which we defined as pecking at the substrate at an angle greater than 45° relative to the gravel substrate. We quantified aggression as the amount of times an individual bit another. Both foraging rate and aggression rate were quantified on all individuals. Mean ($\pm$ SD) length of guppies was $2.31 \text{ cm} \pm 0.19$, and cichlid $2.18 \text{ cm} \pm 0.21$ at the time of testing. Similar to experiment 1, we calculated the change in behavioural measures by subtracting the post-stimulus observation from the pre-stimulus and we used the difference scores as dependent variables in statistical analyses. We rank transformed the data, and the overall effect of disturbance cue and whether it was from a conspecific or heterospecific, as well as donor number was tested for using a non-parametric *MANOVA* using the package *stats* in R v. 3.4.3. (R Core Team, 2017).

Results

Experiment 1

We found a significant effect of stimulus type on the response of Trinidadian guppies ($F_{1,55} = 52.14$, $P < 0.001$, Fig. 1). Guppies exhibited antipredator behaviours by increasing shoaling index and decreasing area use when exposed to disturbance cues, compared to water from undisturbed donors. Donor group size had a significant effect on antipredator responses, as we observed an increase in shoaling index and a decrease in area use with cues from 20 donors

compared to 10 ($F_{1,55} = 6.60$, $P = 0.002$, Fig. 1). There was a significant interaction between the presence of disturbance cues and the number of donors ($F_{1,55} = 4.49$, $P = 0.038$, Fig. 1).

Experiment 2

We found a significant main effect of disturbance cues from Trinidadian guppy donors on the response of both guppy ($F_{1,55} = 79.82$, $P < 0.001$, Fig. 2) and cichlid receivers ($F_{1,54} = 5.45$, $P = 0.002$, Fig. 3). Guppy receivers increased shoaling and decreased area use when exposed to conspecific disturbance cues, and cichlid receivers decreased time spent moving and the number of aggressive behaviours. However, there was no observed change in foraging rate. Both results are indicative of an antipredator response. From cichlid donors, there was a significant main effect of disturbance cues on the response of both guppies ($F_{1,55} = 80.20$, $P < 0.001$, Fig. 2) and cichlid receivers ($F_{1,54} = 27.68$, $P < 0.001$, Fig. 3). Guppies decreased area use and increased shoaling index when exposed to heterospecific disturbance cues. Cichlids decreased time spent moving, aggression rate and foraging rate when exposed to disturbance cues from conspecifics. However, donor group size did not have an effect on receiver responses to heterospecific disturbance cues for, guppies ($F_{1,55} = 0.15$, $P = 0.86$, Fig. 2) and cichlids ($F_{1,54} = 5.361$, $P = 0.659$, Fig. 3) and, also no significant interaction between disturbance cue and donor group size in guppies ($F_{1,55} = 3.14$, $P = 0.05$, Fig. 2) and cichlids ($F_{1,54} = 1.86$, $P = 0.147$, Fig. 3). Both guppies and cichlids displayed antipredator behaviours in response to heterospecific disturbance cues from both 10 and 20 donors, however the observed responses were similar. Donor group size did influence the response to conspecific disturbance cues in both guppies ($F_{1,55} = 3.81$, $P = 0.02$, Fig. 2) and cichlids ($F_{1,54} = 5.15$, $P = 0.003$, Fig. 3), as guppies and cichlids increased antipredator behaviours commensurately towards the relative concentration of disturbance cues.

In guppies there was a significant interaction between exposure to conspecific disturbance cues and donor group size ($F_{1,55} = 13.63$, $P < 0.001$, Fig. 2), there was no significant interaction in cichlids ($F_{1,54} = 2.52$, $P = 0.06$, Fig. 3).

Discussion

Our results confirm that Trinidadian guppies produce and respond to conspecific disturbance cues and represent the first time that the production and detection of disturbance cues has been recorded in the genus *Poeciliidae*. Guppies increased their predator avoidance behaviour in the presence of disturbance cues, relative to the water from undisturbed conspecifics. This adds to a growing list of aquatic prey that display a behavioural response towards chemosensory cues generated by disturbed conspecifics. For example, Sockeye salmon (*Oncorhynchus nerka* (Walbaum, 1792)) frequent areas marked with conspecific disturbance significantly less than those marked with water from undisturbed conspecifics (Bett et al. 2016). Iowa darters (*Ethostoma exile* (Girard, 1859)) reduce exploratory behaviour and alter movement patterns in response to disturbance cues (Wisenden et al. 1995). Likewise, Orange clownfish (*Amphiprion percula* (Lacepède, 1802)) show a significant antipredator response to disturbance cues (Manassa et al. 2013).

Under natural conditions, shoal sizes of guppies vary greatly (Magurran and Seghers 1994). Thus, when disturbed, the density of donors may differ. Our results provide evidence that donor group size (and resulting relative concentration) of early-warning disturbance cues influences the overall antipredator response of individuals. Similar results have been shown for juvenile convict cichlids and rainbow trout; Vavrek and Brown (2009) reported stronger

271 responses to the disturbance cues collected from 10 vs. 5 donors for both species tested. Our
272 results show that guppies exhibited a marked increase in antipredator responses when exposed to
273 cues from 20 vs. 10 donors. Previous studies have shown analogous concentration-dependent
274 response patterns for damage-released chemical alarm cues and learned predator odours (Jachner
275 and Rydz 2002; Dupuch et al. 2004; Zhao et al. 2006). For example, the intensity of antipredator
276 responses of Trinidadian guppies is dependent on the concentrations of alarm cues, as response
277 intensity increased commensurately with concentration (Brown et al. 2009). Likewise, the
278 adjustment of antipredator behaviours in northern redbelly dace (*Phoxinus eos* Cope, 1861) is
279 correlated to the concentration of chemical alarm cues (Dupuch et al. 2004).

280 Our results also suggest that the concentration-dependent response pattern is limited to
281 the disturbance cues of conspecifics. Both guppies and cichlids exhibited concentration (donor
282 group size) dependent responses to conspecific disturbance cues. However, when exposed to the
283 cues of heterospecific donors, we found no effect of donor group size in either guppies or
284 cichlids. These results extend those of Vavrek and Brown (2009) who demonstrated significant
285 cross-species responses to disturbance cues among juvenile convict cichlids and rainbow trout.
286 The lack of donor group size effects in our current study suggests some degree of species
287 specificity in disturbance cues. Similar to the well-studied damage-released chemical alarm cue,
288 which exhibit species-specificity to a certain degree (Brown et al. 2010; Chivers et al. 2012). It is
289 plausible that there is a chemical difference between disturbance cues from heterospecifics and
290 conspecifics, as found in damage-released alarm cues (Kelly et al. 2006). It is argued that
291 differences in metabolic mechanisms cause individuals to produce distinct chemical compounds
292 (Chivers et al. 2012). Since disturbance cues are thought to be a by-product of metabolised
293 protein (Vavrek and Brown 2009), variation in metabolic mechanisms between species can lead

to the development of chemical signatures that are species-specific. Studies suggest the composition of chemical cues could be a result of diet (Wisenden and Smith 1997), however in our study, diet was controlled (*see Methods*). Kelly et al. (2006) argued that purine compounds present in damage-released alarm cues are homologous in related species. Therefore, certain chemical compounds present in disturbance cues may be correlated to conspecifics. In urine, fish are known to have unique mixtures of chemical compounds to trigger behavioural responses in conspecifics (Sorensen and Stacey 1999). Fish excrete a variety of nitrogenous wastes, including methylamines, taurine and imidazole (Van Waarde 1988), which if released simultaneously with urea, may influence species-specific responses. Thus, a likely scenario for the cross-species response to disturbance cues could be that heterospecifics detect and respond to disturbance cues, however, the level of uniqueness in the mixture of chemical compounds leads to a limited response intensity. Alternatively, differences in species-specific olfactory sensitivity may likewise render heterospecific disturbance cues less effective. Regardless of the mechanism, our results clearly demonstrate a differential effect of concentration between conspecific and heterospecific disturbance cues.

The observed response could also be a result of the reliability of public information from heterospecific prey guild members. Since the relevance of public information plays a significant role in mediating antipredator behaviours (Dall et al. 2005), it could be suggested that information from heterospecifics is less relevant. Individuals respond to the information, but these responses are limited or capped. Therefore, the quality of information outweighs the quantity, and might explain why we did not observe a difference of donor group size. Our results provide evidence that conspecifics provide more relevant information compared to heterospecifics in terms of chemosensory cues. These findings support the notion that public

317 information use is widespread among species (Valone and Templeton 2002). However, it
318 demonstrates that there is a relative scale of importance of public information in its use by fish,
319 as conspecific information is more important than heterospecific information.

320 Our results can be attributed to the importance of information quantity in making
321 appropriate behavioural decisions (Dall et al. 2005). Increasing donor group size could be
322 defined as proliferating the amount of public information, enriching the value of the disturbance
323 cue, leading to an intensification of antipredator behaviour. It is well documented that the
324 amount of reliable public information influences predator avoidance (Dupuch et al. 2004;
325 Schoeppner and Relyea 2005; Ferrari and Chivers 2009) thus potentially providing an
326 explanation for function of disturbance cues. This is because disturbance cues are early-warning
327 signals to inform prey guild members of a potential threat (Wisenden et al. 1995). Thus, it could
328 be argued that an increase in quantity and underlying information tells nearby prey the degree to
329 which a threat is imminent, and that with more information comes a more viable or imminent
330 threat. Likewise, guppies exposed to varying concentrations of damage-released alarm cues
331 exhibit graded responses (Brown et al. 2009), thus they respond accordingly based on the
332 quantity of information. From this, we suggest that an increase in donor group size mimics a
333 difference in concentration, and that the greater the concentration, the more reliable the
334 information.

335 Notably, in response to undisturbed donors, guppies were shown to decrease shoaling and
336 increase area use and exhibited a graded response to relative concentration. This unexpected
337 result is contrary to our predictions as we would expect responses to undisturbed donor cues to
338 be similar in both the pre-stimulus and post-stimulus treatments, as reported in other species
339 (Vavrek and Brown 2009). However, our results suggest the possibility of competitive effects

from the undisturbed donor (control) on the behaviour of female guppies. This could be explained by an increase in perceived competition, leading prey to higher competition for resources. Guppies under low-risk conditions exhibit an increase in rates of competition (Chuard et al. 2016), thus we might postulate that water from undisturbed conspecific donors influence competitive behaviours.

Recent studies have highlighted the importance of making appropriate decisions under uncertain conditions (Johnson et al. 2013). Disturbance cues could be another source of information allowing for the reliable assessment of risk. Individuals who respond to a reliable threat are going to reduce mortality risk and maintain benefits (i.e. foraging). Public information provides individuals with the knowledge to reliably assess the quality of a resource (Coolen et al. 2003), or in this case a threat. When conditions become uncertain or unreliable, individuals who respond to something that is not a threat could incur short-term opportunity costs. For example, prey have been shown to overestimate risk under uncertain conditions driven by incomplete information (Bouskila and Blumstein 1992). Overestimation of risk could cause prey to respond to information that is ecologically redundant (Brown et al. 2014), forcing prey to misuse energy and incur fitness costs. We have shown that disturbance cues are not generalized cues and are to a certain degree species-specific. Therefore, under uncertain conditions, (i.e. when ambient predation risk is high) the reliability of public information released by disturbance cues may change. A study conducted on wood frog tadpoles (*Lithobates sylvaticus* (LeConte, 1825)) provides evidence that prior experience with background risk matters (Bairos-Novak et al. 2017). Future studies should test the effects of recent experience on the production and response to both conspecific and heterospecific disturbance cues.

363

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369

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Figure captions

Figure 1: Mean ($\pm$ SE) change in shoaling index (A) and area use (B) for Trinidadian guppies (*Poecilia reticulata*) exposed to disturbance cues (solid bars) and water from undisturbed conspecifics (open bars) collected from groups of ten or twenty conspecific donors. $N = 15$ for each treatment combination.

Figure 2: Mean ($\pm$ SE) change in shoaling index (A) and area use (B) for Trinidadian guppies (*Poecilia reticulata*) exposed to disturbance cues (solid bars) and water from undisturbed donors (open bars) collected from groups of ten or twenty heterospecific (convict cichlid; *Amatitlania nigrofasciata*) and conspecific (guppy) donors. $N = 15$ for each treatment combination.

Figure 3: Mean ($\pm$ SE) change in time moving (seconds; A), aggressive behaviours (B) and foraging attempts (C) for convict cichlids (*Amatitlania nigrofasciata*) exposed to disturbance cues (solid bars) and water from undisturbed donors (open bars) collected from groups of ten or twenty heterospecific (guppy; *Poecilia reticulata*) and conspecific (cichlid) donors. $N = 15$ for each treatment combination.