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Disturbance cue communication is shaped by emitter diet and receiver background risk in Trinidadian guppies

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Abstract

In animal communication systems, individuals that detect a cue (i.e., "receivers") are often influenced by characteristics of the cue emitter. For instance, in many species, receivers avoid chemical cues that are released by emitters experiencing disturbance. These chemical "disturbance cues" appear to benefit receivers by warning them about nearby danger, such as a predator's approach. While the active ingredients in disturbance cues have been largely unexplored, by-products of metabolized protein are thought to play a role for some species. If so, the content (quality) and volume (quantity) of the emitter's diet should affect their disturbance cues, thus altering how receivers perceive the cues and respond. Guppies *Poecilia reticulata* are a species known to discriminate among disturbance cues from different types of donors, but dietary variation has yet to be explored. In this study, we found evidence that diet quality and quantity can affect disturbance cues released by guppy emitters (i.e., experimental "donors"). Receivers discriminated between donor cue treatments, responding more strongly to cues from donors fed a protein-rich bloodworm diet (Experiment 1), as well as an overall larger diet (Experiment 2). We also found that receivers exposed to higher background risk were more sensitive to disturbance cue variation, with the strongest avoidance responses displayed by high-risk receivers toward disturbance cues from donors fed the high-quality diet. Therefore, diet, and perhaps protein specifically, affects either the concentration or composition of disturbance cues released by guppies. Such variation may be important in information signaling in social species like the guppy.

Keywords: alarm cues, chemical cues, diet quality, diet quantity, protein

Communication plays a fundamental role in animal ecology, where sensory "cues" (e.g., the sights, sounds, and smells of others) can serve as critical sources of information (Bradbury and Vehrencamp 1998; Beecher 2017). Such cues are transmitted from "emitter" individuals to "receiver" individuals (or as intentional "signals" from "senders") that can then use the information in making ecological decisions (Dill 1987; Stevens 2013; Wisenden 2015b). In all communication systems, there are a variety of intrinsic (e.g., physiological constraints) and extrinsic (environmental variables) factors that can affect the production and emission of cues (i.e., the information availability), as well as their subsequent detection and perception by receivers (i.e., information retrieval) (Blumstein and Bouskila 1996; Ferrari et al. 2010a).

The transmission of chemical cues is a key modality for communication in many species (often detected via olfaction: Mathis and Crane 2017). For example, such cues can travel around visual obstructions and be detected at night (or under turbid aquatic conditions), making them critically important in detecting visually cryptic predators (Hickman et al. 2004). Indeed, many species recognize the chemical odors of predators via innate and learned mechanisms. Many aquatic species also possess chemicals in their skin that are released into the water upon physical damage from a predator (i.e., "alarm cues": Ferrari et al. 2010b). Receivers of the alarm cues are alerted to a nearby predator

attack, providing an opportunity for predator avoidance via antipredator behaviors.

In contrast to alarm cues, which have received substantial attention among chemical and behavioral ecologists, little is known about *disturbance cues*. A variety of aquatic species are known to possess disturbance cues (e.g., Kiesecker et al. 1999; Bryer et al. 2001; Jordao 2004; Watson et al. 2004), which are released from individuals upon being "disturbed but not injured" (Hazlett 1985; Wisenden 2015a). For example, being chased by a predator can cause the release of disturbance cues prior to any physical contact, thus providing receivers with an "early warning" (Mirza and Chivers 2002; Ferrari et al. 2008; Goldman et al. 2020a). Like alarm cue responses, receivers respond to disturbance cues in a "threat-sensitive" manner, where higher cue concentrations elicit stronger avoidance responses (Vavrek and Brown 2009).

In general, disturbance cue chemistry remains poorly understood (Bairos-Novak 2018). However, among some invertebrate and anuran species, a key component appears to be ammonium/ammonia (Hazlett 1985; Manteifel et al. 2005), whereas some fishes appear to use pulses of urea (Vavrek et al. 2008; Brown et al. 2012). Another possibility is that cortisol, or other endogenous correlates of stress, are active components of disturbance cues, although there is currently little support for such a role (Barcellos et al. 2014; Wisenden 2015a). Regardless of the specific ingredients, the

production of disturbance cues presumably depends on an animal's diet. For example, urea is a by-product of metabolized protein (Robin et al. 1987; Cai et al. 1996), so variation in dietary protein might drive variation in disturbance cues in some species. Diet is, of course, known to affect other chemical cues used in communication, such as in foraging (Larcher and Crane 2015), mating (Walls et al. 1989), and in agonistic (Heuring et al. 2017) contexts. Moreover, there is some evidence that food sources affect the production of chemical alarm cues (Brown et al. 2004). However, to our knowledge, there have been no previous reports of diet effects on disturbance cues.

Some fishes have been observed to discriminate between disturbance cues based on different characteristics of the emitters. For example, spotted sorubim *Pseudoplatystoma corruscans* responded more strongly to disturbance cues from donors that were exposed to a simulated predator chase compared with donors that were disturbed by physical confinement (Giaquinto and Hoffmann 2012). Bairos-Novak et al. (2019) found that fathead minnows *Pimephales promelas* responded more strongly to disturbance cues from groups of donors that were familiar with one another, compared to donors within recently formed groups (i.e., an audience composition effect). Guppies *Poecilia reticulata* have also shown disturbance cue discrimination based on the donors' intra-group familiarity (Crane et al. 2020b), as well as donor group size (Goldman et al. 2019), and the donor's background predation risk (Goldman et al. 2020b). In each case, the disturbance cues must have varied in either composition or concentration. Such discrimination might allow receivers to improve decisions and increase survival, although the adaptive mechanisms remain unclear. In contrast, wood frog tadpoles *Lithobates sylvaticus* were found not to discriminate between the disturbance cues from donor groups that differed in intra-group familiarity and kinship, suggesting that such discrimination may be restricted to more social species (Bairos-Novak et al. 2020).

The aforementioned guppy is a gregarious species that experiences frequent social interactions (Dugatkin and Godin 1992; Swaney et al. 2001; Croft et al. 2004; Chapman et al. 2008). When exposed to predation risk, for example, guppies coordinate avoidance behaviors by tightening their shoals and decreasing the shoal's spatial area use (Brown and Godin 1999). As mentioned above, guppies can discriminate between disturbance cues released by donors in various contexts. However, these effects were observed only among receivers that had experienced a high level of predation risk in their environment, whereas their low-risk counterparts showed little to no discrimination (Goldman et al. 2019, 2020b; Crane et al. 2020b). Such environmental risk has been found to strengthen guppy social networks by increasing shoal cohesion and promoting more differentiated, stable, and longer relationships between preferred individuals (Kelley et al. 2011; Hasenjager and Dugatkin 2017; Heathcote et al. 2017; Ioannou et al. 2017).

We hypothesized that diet affects disturbance cue production in guppies, with higher quality and higher quantity diets facilitating the release of more potent cues. Thus, we predicted that (1) receivers could detect this variation and would then show greater avoidance of cues from high-diet donors. We also predicted that (2) receivers exposed to high background risk would show higher overall vigilance toward disturbance cues and (3) greater discrimination

between cues released by donors with different diets.hat these receivers

Materials and Methods

Experimental overview

In this study, we simulated background risk for receivers using repeated exposures to conspecific alarm cues (Brown et al. 2013). Then in separate experiments (Figure 1), we tested the responses of receiver shoals (3 per group) to disturbance cues (or undisturbed cues) from donors fed diets differing in either quality (i.e., content—Experiment 1) or quantity (i.e., total amount—Experiment 2). Specifically, we measured changes in shoaling tightness and vertical area use, 2 variables that are commonly used to assess guppy antipredator behavior (Brown and Godin 1999; Crane et al. 2020b).

Test species and maintenance

This study involved guppies *P. reticulata* from our laboratory stock population that were descendants (~10 generations) of wild-caught individuals from a site on the Upper Aripo River, Trinidad. This site is considered a "low predation" site, lacking aquatic predators of adult guppies (Deacon et al. 2018; Crane et al. 2020b). Like previous studies on risk avoidance in guppies, we used only females in our experiments, as males are thought to be less responsive, being focused on mate competition instead (Magurran and Seghers 1990; Godin 1995; Nordell 1998; Brown and Godin 1999). Our stock population is housed in 110-L glass "holding" aquaria (~25°C, 12–12 L: D cycle, 100–150 individuals per tank). Each holding tank is filled with continuously filtered dechlorinated tap water (hereafter, "water"), gravel substrate, and artificial vegetation. Guppies are fed
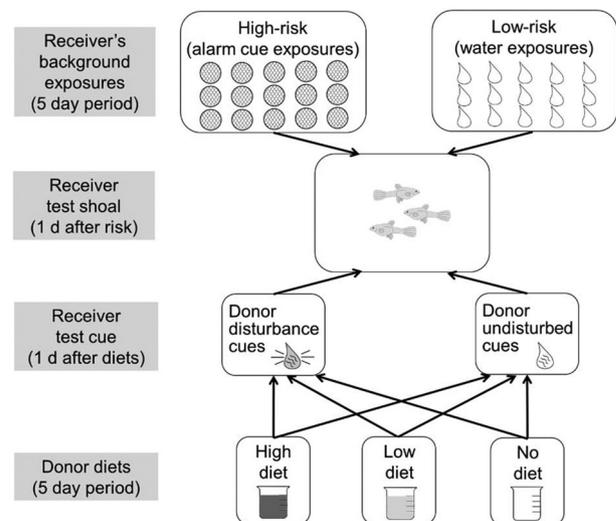

Figure 1. Experimental design where receivers exposed to high background risk (alarm cue exposures—depicted by circles with scales) or low background risk (water exposures—depicted by white droplets) were tested in response to disturbance cues or undisturbed cues from donors that were fed diets differing in quality (Experiment 1) or quantity (Experiment 2). Note that arrows indicate all possible treatment combinations, but each receiver shoal was tested in only 1 treatment group ($n = 2$ test shoals from 6–7 tank replicates per treatment group).

commercial flake food (Nutrafin) twice daily unless noted otherwise.

Alarm cue collection

To manipulate background risk for cue receivers, we collected alarm cues from 112 non-gravid females ($-x \pm s$ standard length = 28.35 ± 0.42 mm). These individuals provided all of the alarm cues used in these experiments, while also yielding ample cues that were frozen for use in future experiments. We followed standard procedures for obtaining alarm cues (as in Brown et al. 2013; Goldman et al. 2020b), euthanizing individuals via cervical dislocation immediately prior to removal of the head, tail (at the caudle peduncle), and internal visceral tissue (in accordance with Concordia University Animal Research Ethics Protocol #30000255). We then placed the remaining tissue into 150 mL of chilled distilled water and homogenized and filtered the solution through polyester filter floss. In total, we collected 201.84 cm² of skin, diluted with distilled water to reach a final volume of 1,900 mL and frozen (–20° C).

Background risk phase

For the background risk phase of the experiments, we moved groups of 36 female guppies from the holding tank into separate 40-L tanks (experiment 1: $N = 14$; experiment 2: $N = 12$). These "background tanks" also contained 20 L of water, a gravel substrate, and a charcoal filter (~24°C, 12:12 L:D cycle). To simulate high background risk, we followed an exposure regime known to induce lasting behavioral effects in guppies (e.g., Brown et al. 2015b; Goldman et al. 2020b). For 5 days and 3 times per day, we injected 10 mL of alarm cues into half of the tanks (Experiment 1: $n = 7$; Experiment 2: $n = 6$) to simulate high background risk, whereas the other half of tanks received exposures to distilled water (i.e., low background risk) (Figure 1). Each tank received a 50% water change 30 min after the third exposure on each day.

Experiment 1: diet quality
Diet manipulation

To manipulate the diet quality of guppies that would later serve as disturbance cue donors, we moved 60 female guppies from a holding tank into 3 15-L "donor tanks" (20 individuals per tank). Guppy body mass at the beginning of the treatment was similar across tanks ($P > 0.20$). Each tank contained 9.5 L of water with a gravel substrate and a charcoal filter placed in the rear left corner of each tank, being maintained at ~23°C under a 12:12 L:D cycle. The tanks were also wrapped with opaque plastic on 3 sides, blocking visual communication among tanks and thus maintaining tank independence, while also allowing us to monitor guppies from the front of the tanks. For 5 days, "high quality" donors were fed Omega One Freeze Dried Bloodworms, "low quality" donors were fed Omega One Super Veggie Red Seaweed, and a control group of donors was food deprived. The high- and low-quality diets differed markedly in protein content (55% versus 24% crude protein, respectively), as well as other ingredients to a lesser extent (e.g., a 2% difference for fiber; Supplementary Table S1). It should also be noted that we lack information on the specific molecules that vary between the 2 food types. Feedings occurred twice daily (10:00 and 17:00), with 2.5 cm³ of food. Partial water changes (~50%) were conducted 1 h after the final feeding each day.

Test cue collection

We collected disturbance cues and undisturbed cues using standard procedures (Vavrek and Brown 2009; Goldman et al. 2019; Crane et al. 2020b). This occurred on the morning following the 5-day diet-manipulation period. First, we removed the filters from the donor tanks. Then, guppies were left "undisturbed" for 30 min before tank water was gently collected to serve as "undisturbed cues." Although the donors may have been slightly disturbed during this phase, this disturbance level would be far exceeded by our disturbance treatment (i.e., the 2 cues were relative). For this, we performed a sudden 60-s "chasing" period where we passed a realistic predator model (15 cm long and connected to a glass rod) through each donor tank. We then waited an additional 60 s before gently mixing and collecting the water. For both cues, we removed a 700 mL of tank water from each donor tank, freezing the mixtures in 20-mL aliquots at –20° C. Because donor guppies shared a tank for the 5 days prior to cue collection, and shared a holding tank before that, they were familiar with each other at the time of cue collection (Crane et al. 2020b).

Receiver tanks

We tested receivers in shoals of 3 individuals. Each background risk tank of 36 individuals generated 12 test shoals, 2 of which were used for each of 6 cue treatment combinations (Figure 1). Each shoal consisted of 3 individuals that were removed from their shared background tank (i.e., they were familiar with one another before testing), measured for standard length ($-x \pm s = 20.6 \pm 1.6$ mm), and then moved into a testing tank (1 shoal per tank). These tanks (37-L) were filled with 20 L of water, lacked a filter, and were equipped with an air stone and 1.5-m "injection hose" (airline tubing) connected to the back wall. They also were wrapped on 3 sides with blue opaque plastic sheeting to prevent visual communication between tanks, keeping tanks statistically independent, while allowing us to observe guppy behavior. Horizontal lines on the tank walls facilitated scoring of vertical area use (Brown and Godin 1999; Brown et al. 2015b; Goldman et al. 2019). Shoals were given at least 1 h to acclimate and were swimming calmly before testing began.

Receiver testing

At the time of testing, receivers had not shared a tank environment with the cue donors for 6 days. Trials were conducted blind to the treatments, with the order being randomized throughout the experiment. Each trial consisted of a 5-min pre-stimulus observation period, followed by an injection of 10 mL of disturbance cue or the undisturbed cue, before a 5-min post-stimulus observation period. During both periods, we recorded an index of shoaling tightness and vertical area use at 15-s time intervals (20 per observation period). Shoaling index ranged between 1 (no fish within one body length of another) and 3 (all fish within one body length of each other). Area use was recorded as the vertical position of a guppy within the tank (1 = bottom third; 2 = middle third; 3 = top third; range of 3–9 for all 3 shoal members). The scores for each response variable were then averaged across the time intervals within each observation period, thus yielding a single value for each response variable for each shoal before and after the injection of the cue. Each receiver was tested in only

one shoal in one trial. We used 14 shoals per treatment group (2 from 7 background tanks per group).

Experiment 2: diet quantity

In Experiment 2, we used 60 donors that were not previously used in Experiment 1. Here, they were fed differing amounts of the same food source (flake food) twice daily for 5 days. The amount of each feeding was either 2.5 cm^3 ("high food"), 0.625 cm^3 ("low food"), or none (i.e., food deprived). Receivers were 19.97 ± 1.82 mm ($\bar{x} \pm s$) in standard length at the time of testing. We tested 12 shoals per treatment group (2 from 6 background tanks per group). All other experimental details matched Experiment 1.

Statistical analyses

The pre-stimulus baseline data were similar across treatment groups (all P-values > 0.05; Supplementary Table S2). For each response variable, we calculated the change in response due to the test cue by subtracting the pre-stimulus values from the post-stimulus values. These variables were highly correlated (Experiment 1: $r = -0.56$, $P < 0.001$; Experiment 2: $r = -0.51$, $P = 0.001$) and were combined using factor analysis. This process yielded a single response variable for each experiment, where higher values indicated stronger avoidance. This "avoidance score" accounted for 77.9% of the variance in Experiment 1 and 75.5% in Experiment 2.

We analyzed the avoidance score for each experiment using 4-way nested ANOVAs, testing the effects of receiver background risk (high or low), cue donor diet (high, low, or none), and test cue type (disturbance cue or undisturbed cue) as fixed factors. We also included the receiver background tank as a nested (random) factor to account for the non-independence of receivers exposed to background risk within the same tank. Hence, "tank," rather than "shoal," was the level of replication for each treatment group. The models also included all possible 2-way interaction terms and the 3-way interaction term. To interpret significant interactions, we split the data for post hoc testing (smaller nested ANOVAs) of the avoidance score, first to analyze each background risk treatment separately, and then for each cue type within each background treatment if necessary. In these post hoc models, we again included the tank as a nested (random) factor and tested for interactions between the fixed factors. When concluding on significant main effects, we used Tukey tests, as the effect of background tank was nonsignificant in all tests (see below). All analyses were conducted using SPSS V. 26 with $\alpha = 0.05$.

Results

Experiment 1: diet quality

In Experiment 1, avoidance responses were shaped by significant interactions involving receiver background risk, test cue type, and donor diet quality (background risk × cue type: $P = 0.002$; diet × cue type: $P = 0.009$; Table 1 and Figure 2A–D), while the background tank had no significant effect ($P = 0.09$; Table 1). Post hoc testing revealed that low-risk receivers responded strongly to disturbance cues overall (cue type: $P < 0.001$) and slightly more to cues from high-quality donors compared with food-deprived donors (diet: $P = 0.030$; Table 2 and Supplementary Table S3), with no interaction between the factors (cue type × diet: $P = 0.35$; Table 2 and Figure 2C and D). Compared with these low-risk receivers, high-risk receivers showed an even stronger response to the

Table 1. ANOVA output from Experiment 1, testing the fixed effects of receiver background risk (high or low), donor diet quality (high, low, or none), and test cue type (disturbance cue or undisturbed cue), and their interactions, on avoidance scores

	F	*df*	*P*
Cue type	112.25	1, 144	< 0.001
Diet	6.89	3, 53.38	0.01
Background risk	2.40	1, 12	0.15
Cue type 0× diet	4.84	2, 144	0.0090*
Cue type 0× background risk	10.12	1, 144	0.0020*
Diet 0× background risk	1.76	2, 144	0.18
Cue type 0× diet 0× background risk	1.06	2, 144	0.35
Background tank	1.61	12, 144	0.09

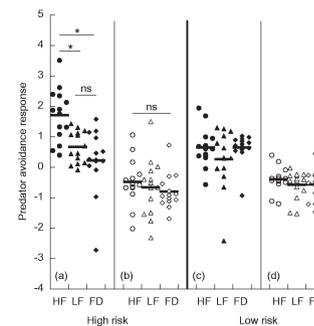Asterisks and bold type represent significant terms of interest.

Figure 2. Experiment 1 avoidance responses (bold lines = means). High-risk receivers (A, B) and low-risk receivers (C, D) were exposed to disturbance cues (black symbols) and undisturbed cues (white symbols) from donors that were fed a high-quality diet (HF: circles), a low-quality diet (LF: triangles), or were food deprived (FD: diamonds). Asterisks represent significant differences at $\alpha = 0.05$, ns = nonsignificant, and $n = 14$ shoals from 7 background tanks per treatment combination.

high-quality cues, and this response was significantly greater than their responses to the low-quality and food-deprived cues (diet × cue: $P = 0.023$; Table 2, Supplementary Tables S4 and S5, and Figure 2A and B).

Experiment 2: diet quantity

For Experiment 2, we found significant main effects of cue type ($P < 0.001$) and donor diet quantity ($P = 0.013$), again, revealing that responses were stronger toward disturbance cues overall, as well as toward the cues released by high-food donors compared with food-deprived donors (Table 3, Supplementary Table S6, and Figure 3A–D). Unlike Experiment 1, however, we found only marginal interactions (risk × diet: $P = 0.056$; cue × diet: $P = 0.059$; Table 3) that indicated tendencies for background risk to promote stronger responses toward disturbance cues from high-quantity donors and weaker responses toward disturbance cues from food-deprived donors (Figure 3A). Again, the background tank had no significant effect ($P = 0.88$; Table 3).

Discussion

In this study, disturbance cues from guppies fed high-quality diets (Experiment 1), and high-quantity diets (Experiment

Table 2. Post hoc ANOVA output from Experiment 1, testing the fixed effects of test cue type (disturbance cue or undisturbed cue), donor diet quality (high, low, or none), and their interactions, on avoidance responses separately for high- and low-risk receivers

	F	*df*	*P*
High-risk background			
Cue type	74.44	1, 72	< 0.001
Diet	9.27	2, 72	< 0.001
Cue type 0× diet	3.99	2, 72	**0.0230***
Background tank	1.47	6, 72	0.20
Low-risk background			
Cue type	37.89	1, 72	**< 0.0010***
Diet	3.12	2, 72	**0.030**
Cue type 0× diet	1.12	2, 72	0.33
Background tank	1.87	6, 72	0.10

Asterisks and bold type represent significant terms of interest.

Table 3. ANOVA output from Experiment 2, testing the fixed effects of receiver background risk (high or low risk), donor diet quantity (high, low, or none), test cue type (disturbance cue or undisturbed cue), and their interactions, on avoidance scores

	F	*df*	*P*
Cue type	12.78	1, 66.6	**< 0.0010***
Diet	4.51	2, 122	**0.0130***
Background risk	2.37	1, 10	0.16
Cue type 0× diet	2.90	2, 122	0.059
Cue type 0× background risk	0.77	1, 122	0.38
Diet 0× background risk	2.96	2, 122	0.056
Cue type 0× diet 0× background risk	1.48	2, 122	0.23
Background tank	0.51	10, 122	0.88

Asterisks and bold type represent significant terms of interest.

Figure 3. Experiment 2 avoidance responses (bold lines = means). High-risk receivers (A, B) and low-risk receivers (C, D) were exposed to disturbance cues (black symbols) and undisturbed cues (white symbols) from donors that were fed a high-food diet (HF: circles), a low-food diet (LF: triangles), or were food deprived (FD: diamonds). Asterisks represent significant differences at $\alpha = 0.05$, ns = nonsignificant, and $n = 12$ shoals from 6 background tanks per treatment combination.

2), elicited more intense avoidance responses in cue receivers (prediction 1). The diet treatments likely caused differences in the concentration of disturbance cues, although we cannot rule out the possibility of changes in chemical composition. The avoidance responses that we observed were most pronounced when donors had been fed the high-quality bloodworm diet, in comparison to the low-quality seaweed diet and the food-deprivation treatment. A major difference between these diets was the amount of protein, and thus, our data suggest that variation in protein may be an important factor in driving responses to disturbance cues in guppies. This is consistent with the hypothesis that disturbance cues are pulses of metabolic waste products, although investigations into their exact chemical composition are much needed. Higher-quality diets should also increase body condition, which may allow for the production of more potent cues, as has been observed for chemical alarm cues in cichlids *Amatitlania nigrofasciata* (Roh et al. 2004). In our study, we assessed body size prior to the diet treatment rather than afterward, and thus, changes in body condition may have also played a role in our results.
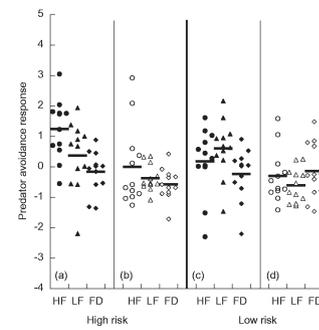
In the low-diet treatments, we observed a general pattern of intermediate responses between the high-diet and food-deprived treatments. This was expected, as these poorer diets should have had an intermediate level of the active ingredients in disturbance cues. Less concentrated cues would likely indicate to receivers that the disturbance is farther away or that the emitter is less disturbed. We should also note that our food deprivation treatment was likely a stressor, In some cases, food deprivation may induce the release of disturbance cues despite an absence of physical disturbance. Abreu et al. (2016) found that tank water from zebrafish *Danio rerio* that were acutely fasted (48 h) caused avoidance responses in receivers. However, water from donors that were chronically fasted (30 days) did not. This suggested that food restriction caused the release of disturbance cues until the dietary ingredients needed to produce the disturbance cues had become depleted. In our study, we used a donor food restriction of 5 days, resulting in receiver responses that generally matched those toward undisturbed cues. Hence, the food-deprived donors in our study did not appear to be producing disturbance cues at the time of cue collection. Whether guppies in our study released disturbance cues in response to food restriction initially was not tested, but future work on depletion of disturbance cues is an interesting area of future research.

Another important finding in our study was that receivers exposed to high background predation risk avoided disturbance cues more strongly than their low-risk counterparts (prediction 2). This is consistent with the hypothesis that background risk can promote a lasting sensitivity to cues that are potential threats, being widely reported in previous studies (Crane and Ferrari 2017). Thus, environmental riskiness appears to play an important role in disturbance cue communication. Although we did not employ a "medium" background risk treatment in this study, previous work indicates that such treatment induces intermediate effects (Brown et al. 2014, 2015a), so we would expect reduced risk effects in such a scenario.

We also observed that high-risk receivers showed increased discrimination between the disturbance cue treatments (prediction 3), consistent with previous studies on guppies (Goldman et al. 2019, 2020b; Crane et al. 2020b). Specifically, the most intense avoidance responses in our study were exhibited by high-risk receivers toward disturbance

cues from donors fed a high-quality diet. One explanation is that the high-risk receivers perceived the high- and low-quality cues as resulting from a nearer threat or a more dangerous type of disturbance such as a predator's approach. Such discrimination would be useful in high-risk environments, as it could correctly facilitate intensified responses to predators rather than other types of disturbances. Because low-risk individuals rarely experience predation attempts, such strong antipredator tactics in response to disturbance cues would likely be unnecessarily costly in terms of lost time devoted to other activities (Johnson et al. 2013; Crane et al. 2020a).

In some cases, exposure to risk "unlocks" contextual effects where the importance of other variables emerges only after experiencing high-risk conditions (Wirsing et al. 2020). Examples include age- and sex-specific morphology (Meuthen et al. 2019), habitat-specific antipredator behavior (Garcia and Sih 2003), and predator-specific learning rules (Chivers et al. 2014). Thus, background risk plays a major role in disturbance cue communication in guppies. Notably, this study would not have shown disturbance cue discrimination had we not tested high-risk individuals. Because many laboratory studies only involve study subjects that have experienced a low-risk captive environment, the potential for risk-induced interactive effects may be often overlooked.

As mentioned previously, the guppy is a group-living and highly social fish species. Males and females compete for mates (Kodric-Brown 1992), and females copy the choices of others (Dugatkin and Godin 1992). They have cohesive social networks (Hasenjager and Dugatkin 2016) and become familiar with individuals in their group (Swaney et al. 2001; Heathcote et al. 2017). These groups forage together in shoals (Swaney et al. 2001; Reader et al. 2003) and coordinate their predator defenses (Dugatkin and Godin 1992; Elvidge et al. 2016; Hasenjager and Dugatkin 2017; Heathcote et al. 2017). Guppies learn from others in their social group (Brown and Laland 2002; Chapman et al. 2008), and have been observed to rely on specific individuals as "leaders" (Lachlan et al. 1998; Brown and Irving 2014). We still know little about many aspects of disturbance cues, but their use in guppy communication, potentially as signals (Crane et al. 2020b), likely has important implications for guppy social dynamics and group decision-making.

Author Contributions

J.A.G. and G.E.B. conceived the study. J.A.G., L.E.A.F., and E.C. collected the data. G.E.B. conducted the analyses. A.L.C. drafted the manuscript. All authors contributed to the final version.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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