

Effects of physical activity on the production of disturbance cues in Trinidadian guppies

*(Poecilia reticulata)*

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## ABSTRACT

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Disturbance cues are metabolic byproducts that are argued to function as ‘early warning signals’ where both the senders and receivers can benefit from their release. These disturbance cues are used by many aquatic prey to warn conspecifics of potential local threats and provide prey with valuable information that can help increase their probability of survival following a predatory encounter. Due to their identity as metabolic byproducts, it is shown that sender diet quality influences the information conveyed to a receiver. However, only one disturbance cue study has exposed fish to ecologically relevant, natural flowing water conditions. Thus, we assessed the influence of varying ambient current flow by conditioning sender guppies in current treatment buckets for 3 days with artificially increased current flow velocities and exposed receiver guppies to the collected disturbance cues and undisturbed odours. We found no effect of the cue received and the current experienced by senders. This suggests that disturbance cues are not affected by ambient current which suggests that the cues can continue to function when streams are altered. Since responses to disturbance cue are known to be influenced by background risk, we conducted a second experiment, where background risk was manipulated. We found that high background risk fish decreased their activity significantly when exposed to disturbance cues across all current flow ranges. Interestingly, the odours of undisturbed conspecifics may function as ‘social foraging cues’ for high background risk receivers who may be more willing to forage when they perceive safety. However, neither the current experienced by senders, nor the cue received influenced responses in low background risk fish. Overall, our results show that while

the current velocity the sender experiences and their resulting levels of physical activity do not influence the responses of receiver fish, fish exposed to higher background risk appeared to be more sensitive to chemical information from conspecifics.

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#### Contribution of authors

D Cao Van Truong contributed to the conception, design, collection of data, data analyses, and wrote the manuscript. GE Brown contributed to the conception, design, data analyses, and editing. A Brusseau, V Groves, J Domenicano, A Destarac, A Mansfield, C Nouvel, F Dumaresq-Synnott, A Preagola, A Vanier, and ME Felismino contributed to the collection of data for experiments 1 and 2.

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## Introduction

Predation is a pervasive selective pressure that strongly influences the morphological and behavioural phenotypes in many prey species (Lima and Dill 1990; Brown et al. 2011). Consequently, prey are forced to balance fitness-related activities such as foraging and courtship, with the successful detection and avoidance of predation threats (Kats and Dill 1990; Lima and Dill 1990). Additionally, prey are known exhibit behavioural plasticity in making their decisions. For example, decisions can be influenced by hunger levels which can change their overall probability of survival during a predatory encounter since fish are more willing to take foraging opportunities (Hansen et al. 2015). Likewise, past experience with high predation risk is also shown to influence decisions in prey (Goldman et al. 2020). Key to the ability of prey to make such behavioural trade-offs is the availability of sufficient information to reliably assess local predation risks which is critical to avoid inappropriate behavioural decisions (Brown 2003)

Within aquatic ecosystems, a wide variety of prey rely on chemosensory information to assess ecologically relevant threats (Ferrari et al 2010; Brown et al. 2011; Bairos-Novak et al. 2019). One type of widely used source of chemosensory information among aquatic prey is the damage-released chemical alarm cue system. Since the 1940s, it is well understood that small freshwater prey fishes exhibited a fright reaction when exposed to chemical cues from injured conspecifics, these substances are coined '*Schreckstoff*' (German for 'fear substance'; von Frisch 1942). Consequently, these 'fear substances' are the subject of numerous studies in attempts to identify such alarm cues among a wide variety of aquatic vertebrate and invertebrate taxa (Wisenden and Millard 2001; Chivers et al. 2012). Studies show that these alarm cues elicit anti-predator responses that when used appropriately can increase probability of survival during a predatory encounter (Ferrari et al. 2010). These alarm cues are contained within the skin and

undelying viscera, and are released upon mechanical damage or when captured by a predator (Chivers et al. 2012). Due to their mechanism of release, alarm cues serve as highly reliable indicators of acute predation risk since the detection of alarm cues imply a recent, nearby predatory attack. Additionally, there is a high degree of evolutionary conservation of alarm cues within a taxonomic group and response intensities decrease as phylogenetic distance between sender and receiver increases (Leduc et al. 2003; Wisenden & Barbour 2005).

In addition to the well-studied damage-released alarm cues, many aquatic prey species may utilize disturbance cues as a source of risk assessment information (Ferrari et al. 2010; Crane et al. 2022). While much is known regarding the form and function of damage released chemical alarm cues in a variety of aquatic prey species, much less is known regarding disturbance cues. Since 1985, there have been thousands of studies on alarm cues compared to ~ sixty studies on disturbance cues (Crane et al. 2022). Though alarm cues serve as honest and reliable cues where only the receiver gains a benefit since they are only present after injury, it is argued that disturbance cues may serve as signals where both the sender and receiver may benefit such as the initiation of group defences like shoaling behaviour (Bairos-Novak et al 2018; Bairos-Novak 2019; Crane et al. 2022). Conversely, prey fish release disturbance cues across their gill epithelia and/or via urine prior to an attack when they perceive a potential local predator (Vavrek et al. 2008; Crane et al. 2022). As such, disturbance cues typically elicit lower intensity predator avoidance responses, while increasing vigilance towards secondary sources of information such as visual cues (Ferrari et al. 2008; Crane et al. 2022). Among prey fishes, the relative concentration of disturbance cues provides sufficient information to allow for threat-sensitive behavioural trade-offs (Vavrek and Brown 2009). Also, responses to disturbance cues increase in intensity proportionally as its detected concentration increases; consistent with a

graded, threat-sensitive response (Vavrek and Brown 2009; Goldman et al. 2019). In guppies, it has been shown that disturbance cue responses are influenced by sender experience and sender-receiver familiarity in an additive manner (Goldman et al. 2019; Crane et al. 2020). These disturbance cues are shown to be crucial sources of risk assessment. Brook charr exposed to disturbance cues have increased probability of survival upon staged encounters with a predator by increasing vigilance, employing cryptic behaviours, or fleeing the area (Mirza and Chivers 2001; Mirza and Chivers 2002). Consequently, the largely unexplored disturbance cues are argued to provide an ‘early warning signal’ crucial for the detection of local predation risks (Wisenden 2000). Disturbance cues have been documented in a variety of invertebrate and vertebrate prey taxa in both terrestrial and aquatic systems (northern crayfish *Oronectes virilis*, Hazlett 1990; hermit crabs *Calcinus laevimanus*, Hazlett 1990; red-legged frogs *Rana aurora*, Kiesecker et al. 1999; Iowa darters *Etheostoma exile*, Wisenden et al. 1995; slimy sculpins *Cottus cognatus*, Bryer et al. 2001; red sea urchins *Strongylocentrotus franciscanus*, Nishizaki and Ackerman 2005; desert isopods *Hemilepistus reaumuri*, Zaguri et al. 2018).

While responses to other chemosensory cues (i.e. alarm cues) have been shown to be phylogenetically conserved within closely related species, disturbance cues do not show the same level of species-specificity (Vavrek et al. 2008). For example, phylogenetically distant species such as the rainbow trout (*Oncorhynchus mykiss*) and convict cichlids (*Amatitlania nigrofasciata*), respond to each other’s disturbance cues with similar intensities, while guppies (*Poecilia reticulata*) also respond to cichlid disturbance cues (Vavrek & Brown, 2008; Brown et al. 2012; Goldman et al. 2019). Due to the lack of species-specificity of disturbance cues, they are argued to elicit more generalized indicators of risk (Vavrek et al. 2008). The prevailing hypothesis posits that nitrogenous wastes released as metabolic byproducts function as

disturbance cues. Pulses of ammonia trigger predator avoidance responses in northern crayfish (Hazlett 1990) and red-legged frog tadpoles (Kiesecker et al. 1999) similar to responses depicted upon exposure to a conspecific disturbance cues (Hazlett 1990; Kiesecker et al. 1999). While it is suggested that ammonia likewise function as a disturbance cues in fish (Jordão and Volpato 2000; Bryer et al. 2001), more recent studies suggest that ammonia has no significant effect (Vavrek and Brown 2009). Pulses of urea higher in concentration than that of the surrounding environment (background noise hypothesis; Vavrek and Brown 2009) are shown to elicit antipredator responses in both convict cichlids and rainbow trout (Brown et al. 2012). Thus, disturbance cues are argued to be metabolic byproducts generalized across phyla (such as urea; Vavrek et al. 2008). Recent studies have shown the influence of sender diet quality and quantity on the chemistry and concentration of disturbance cues (Goldman et al. 2022). This change was observed through the response intensity to the disturbance cues produced, where high background risk fish were able to discriminate between high- and low-protein diet senders (Goldman et al. 2022). While, there is suggestion that diet-based cue production and discrimination can aid fish to correctly respond to disturbances, there is limited knowledge of the influence of sender metabolism on the production and response to disturbance cues.

While the relationship between sender diet quality and response intensity to the cue produced is previously established (Goldman et al. 2022), there is yet to be a link found between physical activity and the information conveyed in disturbance cues despite the influence activity has on metabolic rate (Wright et al. 2014). This may be an interesting next step due to the known effects of swimming speed and nitrogenous waste excretion in fish (Alsop et al. 1999). However, in the existing disturbance cue literature, only one study has been conducted under natural conditions with flowing water (Goldman et al. 2020). By looking at varying currents we will fill

a knowledge gap and test the ecological relevance of natural conditions where prey are exposed to a wide range of velocities. Since the vast work on disturbance cues are performed in static water conditions, we want to look at whether there is a difference by varying the flowing water component.

Recent studies focus on the presence of disturbance cues in guppies which suggest this species would serve as a good model system (Goldman et al. 2019; Crane et al. 2022). Previous work on intraspecific communication using disturbance cues revealed that response intensities vary with the sender and receiver experience with background risk, while the sender diet also has a role in shaping disturbance cue responses (Goldman et al. 2019; Goldman et al. 2020; Crane et al. 2020; Goldman et al. 2022). Using guppies, we explored the effects of elevated metabolic levels by increasing physical activity upon disturbance cue release. Since disturbance cues are argued to be a metabolic byproduct, by varying ambient current intensity and forcing the model organism to increase its activity and metabolism, we aimed to explore how the release of disturbance cues were affected. The increased physical activity may allow more disturbance cues to be released upon disturbance as more metabolic waste would be available with the higher levels of activity. This would mean that a higher concentration of metabolic waste is released as disturbance cues upon disturbance which may elicit higher intensity responses. Alternatively, due to the increased energetically costly levels of activity, we hypothesized that there would be more turnover of metabolic waste in guppies. As a result, less metabolic byproduct would be available to be released as disturbance cues upon disturbance by a predator. Thus, a lower concentration of disturbance cues may be detected by local conspecifics. By observing the intensity of response to the different collected disturbance cues from guppies exposed to varying ambient current treatments, the relationship between metabolic activity in the sender and the

response intensities in the receivers to disturbance cues can be evaluated. Additionally, we aim to assess how the responses to the different ambient stimuli vary with differing background risk regimes among the receivers (Goldman et al. 2019). Since disturbance cues responses are shown to be influenced by receiver background experience (Goldman et al. 2020), we will conduct an assessment to understand how physical activity influences the presumed metabolic cost of communication during periods of predation as well as how varying receiver background experience shapes responses to these cues. This can provide valuable insight into the intraspecific interactions that occur during periods of predation

## **Objectives**

Here, we tested the hypothesis where elevated ambient currents will influence both the production and response to disturbance cues in lab-raised Trinidadian guppies who are descendants from wild populations (Experiment 1). This is due to the effect swimming speed has on metabolic rate and nitrogenous waste production (Alsop et al. 1999; Wright et al. 2014). Therefore, upon a perceived encounter with a predator, we predict that prey fish would have more metabolic byproduct to release which can elicit more intense responses from local conspecifics. Alternatively, these disturbance cues may be continually excreted so there would be less metabolic byproduct to release during periods of perceived threat. Therefore, we may expect receiver guppies to respond less to stimuli from higher current treatments. Since the background risk of the receivers are known to influence responses to disturbance cues, we manipulated background risk in a second experiment where we tested the response of lab-raised receiver fish to the disturbance cues generated in experiment 1 (Experiment 2). In the second experiment, we predicted that receiver guppies conditioned to higher background risk should

respond to the disturbance cues from the varying ambient current treatments more intensely than those conditioned with the water treatment since responses to disturbance cues are previously shown to be influenced by sender experience (Goldman et al. 2020).

## **Methods**

*Test fish:* The adult guppies used in this study were a laboratory population who were descendants of a wild-caught population from Trinidad. Prior to testing, guppies were held in 110-L glass aquaria containing filtered, dechlorinated tap water (26°C, pH ~7.2), gravel substrate and artificial vegetation. Fish were exposed to a 12h light:12h dark cycle and fed twice daily with commercial flake food.

## **Experiment 1**

*Sender current velocity treatments:* In this experiment, the purpose was to test the influence of sender ambient current velocities on the responses to produced disturbance cues. Disturbance cues were acquired from female guppies exposed to a 3-day conditioning phase varying in current velocity. Three conditioning buckets (19L buckets) were set up, each containing a shoal of 20 guppies (mean  $\pm$  sd total length: 19.50  $\pm$  3.74 mm) in filtered tank water and were assigned one of three different treatments throughout the duration of the study: high current, low current, and no current. Artificial currents were established in the tanks using Sicce Voyager Nano water pumps set at different intensities (mean  $\pm$  SD current velocity: high current: 2 running pumps = 1.66  $\pm$  0.42 cm s<sup>-1</sup>; low current: 1 running pump = 1.13  $\pm$  0.26 cm s<sup>-1</sup>; no current = -0.116  $\pm$  0.34 cm s<sup>-1</sup>). Current velocity was measured using a Hach flow meter. The



sender fish were fed 3 times per day and a 50% water change was performed after the final feeding each day.

At the end of the 3 day conditioning period, water pumps were shut off. Sixty ml of bucket water was then extracted pre-disturbance and served as the odour of undisturbed conspecifics which we called our undisturbed odour. Disturbance cues were collected using a model predator (made with a fishing lure attached to a rod) waved 10 times around each bucket to disturb the sender fish, 60 mL of tank water containing released cues were collected immediately using a syringe (Goldman et al. 2019). The stimuli were stored in freezer bags and frozen at approximately -20 °C until use in the experimental phase.

The conditioning buckets were also filmed from a bird's-eye view on day 1 and day 3 of the conditioning period to collect biological activity data via 10 minute videos using a GoPro Hero 8. We replicated this 6 times, with each block containing 3 groups of 20 guppies yielding 3 30mL aliquots of disturbance cue or undisturbed odour for each current treatment each time (high current disturbance cue, high current undisturbed odour, low current disturbance cue, low current undisturbed odour, no current disturbance cue, no current undisturbed odour). We observed the number of visible fish every 30 seconds up until the 360-second mark. We also recorded the number of body undulations (how many times the body wagged while swimming) per minute of 5 fish in each conditioning tub at 3 different observation periods (90 seconds, 120 seconds, 150 seconds). The undulations per minute was then averaged across the 5 fish in each treatment bucket. After the stimuli are extracted from the buckets the sender fish are returned to their holding tanks.

*Behavioural testing of receivers:* Untested female receivers (focal guppies; mean total length:  $14.93 \pm 4.06$  mm) were moved into individual test tanks (37L-aquaria) an hour before the start of the trial. Immediately prior to each testing period, a pinch flake food was added into the tanks. Each trial consisted of a 5-minute pre-stimulus observation (blind to the treatments), followed by the injection of the stimulus (disturbance cue or undisturbed odour), and a 5-minute post-stimulus observation period. The test stimulus was 5mL of either undisturbed odour, or disturbance cue from each current-conditioning treatment (high current disturbance cue, high current undisturbed odour, low current disturbance cue, low current undisturbed odour, no current disturbance cue, no current undisturbed odour) randomly introduced into the centre of the tank using a syringe injected into an air tube that fed into the tank. During the observation periods, movement in a novel arena was observed by measuring the occurrence of calm swimming or foraging behaviour at 10 second intervals for the 5 minute pre- and 5 minute post-stimulus observation period. Calm swimming was recorded when fish were seen swimming continuously, often 1-5cm above the substrate when looking for food, this is a typical behaviour in the absence of risk for guppies (Smith 1992; Mathis et al. 1993). Foraging was recorded when food items were observed in the mouth of the fish or when engulfing food items at the surface (Crane et al. 2020). We tested  $n = 15$  individuals per current and stimulus treatment.

*Statistical analysis:* To assess the effect of current on the activity of senders, we compared the frequency of swimming undulations across the current treatments. We used a GLMM, to assess the effect of current on the number of undulations per minute made by senders. We included 'observation period' as a random factor to account for the repeated measures within a block.

In order to test the effect of sender treatment on the response to disturbance cues (vs. the undisturbed odour control), we initially calculated the number of 10 second observations with calm swimming + foraging behaviours and converted these to proportions ((calm + forage)/30; Crane et al. 2020). We then calculated the proportional change (post-stimulus – pre-stimulus), which we defined as our response index. Using this response index as our dependent variable, we conducted a GLMM with the cue received and the sender current treatment as our independent variables and our sender block as the random effect. Statistical analyses were conducted using SPSS V28.0.

## **Experiment 2**

*Manipulation of background risk:* Given the lack of strong responses in Experiment 1, we further explored the effects of ambient risk on the response to conspecific disturbance cues by manipulating background predation risk in the receiver guppies prior to testing. To alter background risk levels, we produced conspecific damage released chemical alarm cues (Brown et al. 2013). We euthanized a total of 96 male and female guppies as alarm cue senders (mean total length  $\pm$  sd mm :  $24.21 \pm 2.54$  mm) via cervical dislocation in accordance with Concordia University Animal Research Ethics Protocol #VPRGS-13). We promptly removed the head, tail, and internal visceral tissues. Then, the skin was placed in 100mL of distilled water and homogenized. The solution was filtered through polyester fiber floss. Distilled water was added until the desired final volume was achieved. A total of 0.96 cm<sup>2</sup> of skin was collected (diluted to a final volume of 1000 mL) as our alarm cues. Alarm cues were stored in 20mL aliquots at -20°C until needed for use. Damage-released alarm cues at this concentration are known to induce elevated predator avoidance behaviour in guppies (Brown et al. 2009; Brown et al. 2013).

*Receiver conditioning:* Female receiver guppies are exposed to a 3-day conditioning phase prior to the testing protocol. Shoals of 5 guppies were moved into 10L conditioning buckets. These buckets were split into two conditioning regimes where 6 buckets received 2.5ml of distilled water 3 times a day for 3 days, while another 6 buckets were given 2.5ml of alarm cue. Each bucket contained a single air stone and was held at 23°C. We fed fish in the conditioning phase once daily at least 30 minutes after the final stimulus injection and a partial water change (~50% volume) was conducted. We replicated this four times, with each block of alarm cue vs. water guppies yielding 12 shoals of 4 guppies for each disturbance cue and current regime treatment combination from experiment 1 (high current disturbance cue, high current undisturbed odour, low current disturbance cue, low current undisturbed odour, no current disturbance cue, no current undisturbed odour).

*Receiver testing and statistical analysis:* Behavioural observations on the conditioned receiver guppies (mean total length:  $14.93 \pm 4.06$ mm) were carried out as stated above. We tested  $n=13 - 15$  individuals per current and stimulus treatment. Using the same metric of the response index from experiment 1 as our dependent variable, we conducted a GLMM (SPSS V28.0) with the cue received, receiver background risk treatment, and sender current treatment as our independent variables. Our background risk conditioning block was then used as the random effect. We then conducted 2 further GLMMs with high background risk fish and low background risk fish separately with the response index as our dependent variable, and the cue received and sender current treatment as our independent variables. The conditioning block was also used as the random effect.

## Results

*Sender behaviour:* When exposed to the varying current velocities, we found that donors exposed to increasing current velocities showed significantly more undulations per minute proportional to current intensities (Table 1; Figure 2). However, there was no influence found of the observation period on the number of undulations per minute, nor were there interacting effects found between the current velocity and the observation period on the intensity of their swimming measured by the number of undulations per minute (Table 1).

*Experiment 1: response of receivers:* There was no significant influence found of neither the cue received, nor the sender current treatment on the response intensity (Table 2). There were also no significant 2 way interactions between cue received and the sender current treatment.

*Experiment 2: response of receivers::* The overall analyses revealed significant main effects of cue ( $P = 0.002$ ; disturbance cue vs. undisturbed odour) and a significant cue x background risk interaction (Table 3). All other effects were non-significant. As with Experiment 1, there was no effect of cue, current, or an interaction when we examined low background risk receiver guppies (Table 3; Figure 4) alone. Since we had a significant cue x background risk interaction, we were able to analyze of the responses of high background risk and low background risk fish separately. However, when examining the high background risk receivers, we found a significant effect of cue ( $P < 0.001$ ) but no effect of donor current treatment nor an interaction (Table 4; Figure 4). Overall, our results suggest that high background risk receivers exhibit similar intensity responses to disturbance cues, regardless of the donor current treatment.

## Discussion

Our results provide strong evidence that despite observed differences in the overall activity senders under increased current conditions, there was no overall effect of this increased activity in the production of disturbance cues (i.e. receiver response). Senders exposed to varying current treatments appeared to be proportionally more active, consistent with increased physical activity. The results of first experiment indicate that there were only weak (nonsignificant) responses among receiver guppies to disturbances cues collected from senders exposed to varying ambient current treatments. The predicted interacting effects between the cue received and the currents experienced by the sender were not observed. The results of the second experiment show that when background risk is induced, low background risk vs. high background risk guppies responded to chemosensory cues differently. High background risk guppies were observed to decrease their calm-foraging activity when exposed to disturbance cues and increased their activity when exposed to undisturbed odours of conspecifics regardless of ambient current treatment. However, low background risk fish did not respond in this manner. The differences in responses when background risk is induced is consistent with previous results (Ferrari et al. 2008; Vavrek et al. 2008; Goldman et al. 2019).

Experiment 2 demonstrated the difference in responses to chemosensory cues when background risk is induced. When receivers had experience with low background risk conditions, we did not see a strong response to conspecific disturbance cues. This is consistent with recent work showing that prey fish from low predation backgrounds may only perceive disturbance cues as a low risk early warning signal, insufficient to elicit an observable behavioural response (Wisenden et al. 1995; Ferrari et al. 2010; Goldman et al. 2022). However, in the presence of complimentary information (i.e. additional visual or social information), the same cues may elicit

a response (Ferrari et al. 2008). The detection of disturbance cues is shown to increase vigilance towards and responses to other cues, such as alarm cues (Ferrari et al. 2008). In a low predator density environment, responding to a conspecific disturbance cue when there might not be an acute predatory threat may result in lost opportunity costs to perform other fitness-related activities (such as foraging and courting; Kats and Dill 1990, Preisser et al, 2005). Alternatively, our use of a single fish assay may not have the full ecologically relevant information that a shoaling fish would have under fully natural conditions. Perhaps, a multiple-fish assay may be better able to capture the subtle differences in behavioural response to these conspecific cues in future studies (Dyer et al. 2009; Piyapong et al. 2011; Davis et al. 2017)

It was interesting to see that the effects of the cue received were only observed in the high background risk fish. Recent studies also show that background risk affects receivers of chemosensory cues (Brown et al. 2009; Bairos-Novak et al. 2018). This may be because in high predator environments, it is more likely to encounter a predator so it may increase chances of survival to be less likely to stay exposed when there is social information conveying a potential local predation risk. Alternatively, when high background risk fish were exposed to the undisturbed odour of conspecifics there appeared to be an increase in calm-foraging activity. This is interesting as this may indicate potentially increased competition, leading to increased activity (Goldman et al. 2019). Fish who experience high background risk are shown to be more willing to take opportunities to get competitive advantages which would usually be more rare in such environments, the undisturbed odour may influence high background risk guppies to take foraging opportunities to avoid lost opportunity costs (Pettersson et al. 1993; Chuard 2016). These results are consistent with recent findings where undisturbed odour of conspecifics influence high background risk fish to increase their foraging activity (Vavrek et al. 2008;

Goldman et al. 2019). This suggests that high background risk receivers may be more sensitive to chemical information from conspecific senders.

Our results suggest that there was no observable effects of physical activity on the production of disturbance cues by cue senders, as initially predicted. Despite recent observations on the plasticity of disturbance cue responses shown to be influenced by the quality and quantity of the sender's diet (Goldman et al. 2022), sociality and sender-receiver familiarity (Bairos-Novak et al 2018; Crane et al. 2022), we found that the sender's physical activity did not have an effect on the information conveyed by the sender. While current velocity was not directly measured, we avoided very high current velocities due to the possibility of induced stress. Thus, we aimed to exposed senders to current velocities that were similar to those of natural guppy streams (G. E. Brown, personal observations). Thus our findings suggest that under natural conditions, the function of disturbance cues as an early-warning signal would not be impaired by current velocities within the normal range experienced by guppies.

## **Implications**

Freshwater ecosystems are subject to changing river flow regimes due to several anthropogenic stressors, such as large-scale damming, hydrological change, pollution, sediment mining, and climate change which challenge their integrity (Willems & Lloyd-Hughes, 2016; Best, 2019). Consequently, it is crucial to understand how freshwater prey fish will be affected by environmental change which can bring on ecological uncertainty and variations in predation risk. Prey fish rely on chemosensory cues (such as disturbance cues) as sources of information to assess their changing and unpredictable environment and make learned associations which will increase their ability to accurately detect predation risks (Ferrari et al. 2010). Since disturbance



cues are metabolic byproducts, altered river flow regimes can influence the physical activity levels of prey fish which will affect the release of these cues (Vavrek et al. 2008). When the release of these cues is impacted, there may be a shift the ability of prey to perceive predation risks. As a result, prey may be more susceptible to death, experience reduced fitness or the dynamics of social chemosensory communication may be altered (Bairos-Novak et al. 2019). Research into the physiological and behavioural consequences of human-induced changes in river flow regimes on prey fish can reveal how animal behaviour and biodiversity will be impacted. The results of our study show that while prey can be subject to varying current flow periods, the sender can still release usable disturbance cues that elicit appropriate responses in conspecifics. While the majority of disturbance cue studies have been conducted in laboratory or seminatural conditions (Vavrek et al. 2008; Crane et al. 2022), only one study shows disturbance cues responses under fully natural conditions (Goldman et al. 2019). The results of this experiment suggest that disturbance cues are still viable and convey information under periods of relatively high current. However, it may be interesting to evaluate the response of receivers exposed to ambient current treatments as well as estimating increases metabolic rates and how disturbance cue concentrations are influenced in future research.

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**Table 1:** Results of the GLMMs on the number of body undulations observed per minute in sender guppies exposed to increasing ambient current treatments (high current vs. low current vs. no current), which were the independent variables, during the conditioning phase prior to stimulus collection. Observation period was included as a random factor.

<b>Factor</b>	<b>F</b>	<b>df</b>	<b>P</b>
Current	513.34	2, 4	<0.001
Observation period	0.56	2, 5.69	0.600
Current * Observation period	0.018	4, 39	0.99

**Table 2:** Results of GLMMs on the difference in proportions of calm-foraging behaviours between stimulus injection periods for guppies in Experiment 1. Sender current treatment (high current vs. low current vs. no current) and cue received (disturbance cue vs. undisturbed odour) were used as the independent variables. The conditioning block was included as a random factor. N = 16 per treatment combination.

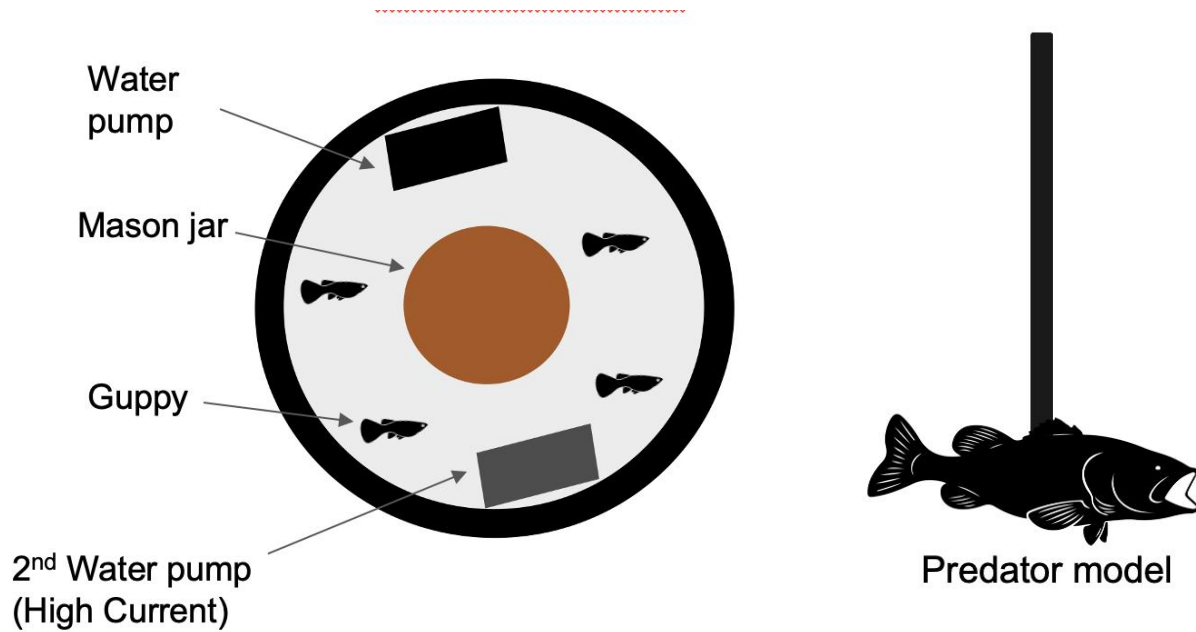
<b>Factor</b>	<b>F</b>	<b>df</b>	<b>P</b>
Cue	1.30	1, 172.05	0.26
Current	0.36	2, 5.69	0.71
Block	0.92	14, 171	0.53
Current * Cue	0.95	2, 171	0.39

**Table 3:** Results of GLMMs on the difference in proportions of calm-foraging behaviours between stimulus injection periods for risk induced receiver guppies in Experiment 2. Sender current treatment (high current vs. low current vs. no current), cue received (disturbance cue vs. undisturbed odour) were used as the independent variables. The conditioning block was included as a random factor. N = 13 - 15 per treatment combination.

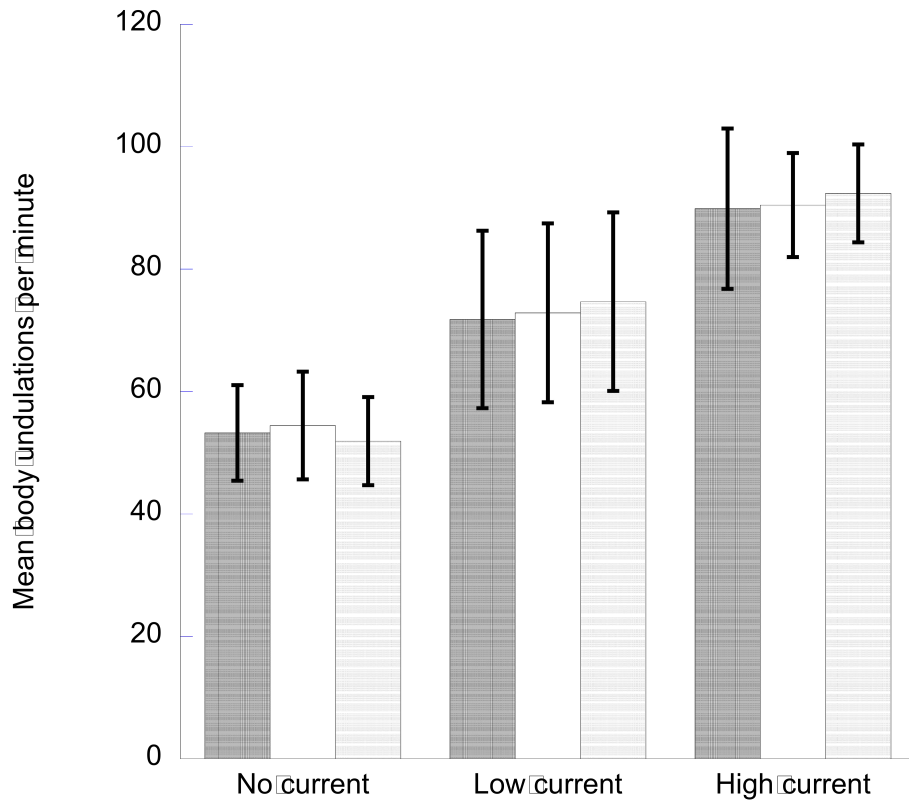
<b>Factor</b>	<b>F</b>	<b>df</b>	<b>P</b>
Cue	10.078	1, 154.27	0.002
Sender Current	0.100	2, 151.93	0.91
Background	0.83	1, 9.94	0.38
Block	1.27	10, 150	0.25
Background * Cue	4.83	1, 150	0.030
Background * Donor	0.039	2, 150	0.96
Cue * Donor	0.16	2, 150	0.86
Background * Cue * Donor	0.046	2, 150	0.96

**Table 4:** Results of GLMMs on the difference in proportions of calm-foraging behaviours between stimulus injection periods for low background risk (A) and high background risk (B) guppies in Experiment 2. Sender current treatment (high current vs. low current vs. no current) and cue received (disturbance cue vs. undisturbed odour) were used as the independent variables. The conditioning block was included as a random factor. N = 13 – 15 per treatment combination.

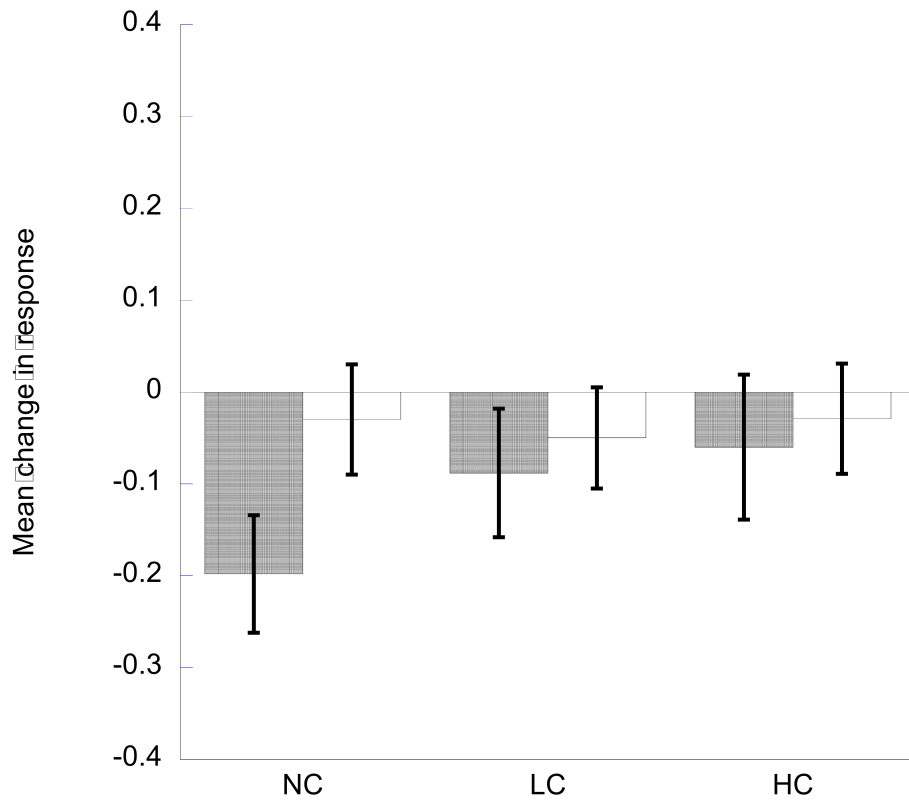
<b>Factor</b>	<b>F</b>	<b>df</b>	<b>P</b>
<b>Low background risk</b>	0.62	1, <b>74.85</b>	0.43
Cue			
Current	0.045	2, 75.42	0.96
Block	0.21	2, 75.41	0.81
Current * Cue	1.27	5, 74	0.28
<b>High background risk</b>			
Cue	13.36	1, 79.41	<0.001
Current	0.088	2, 76.45	0.92
Block	0.030	2, 77.34	0.97
Current * Cue	1.39	2, 76	0.24



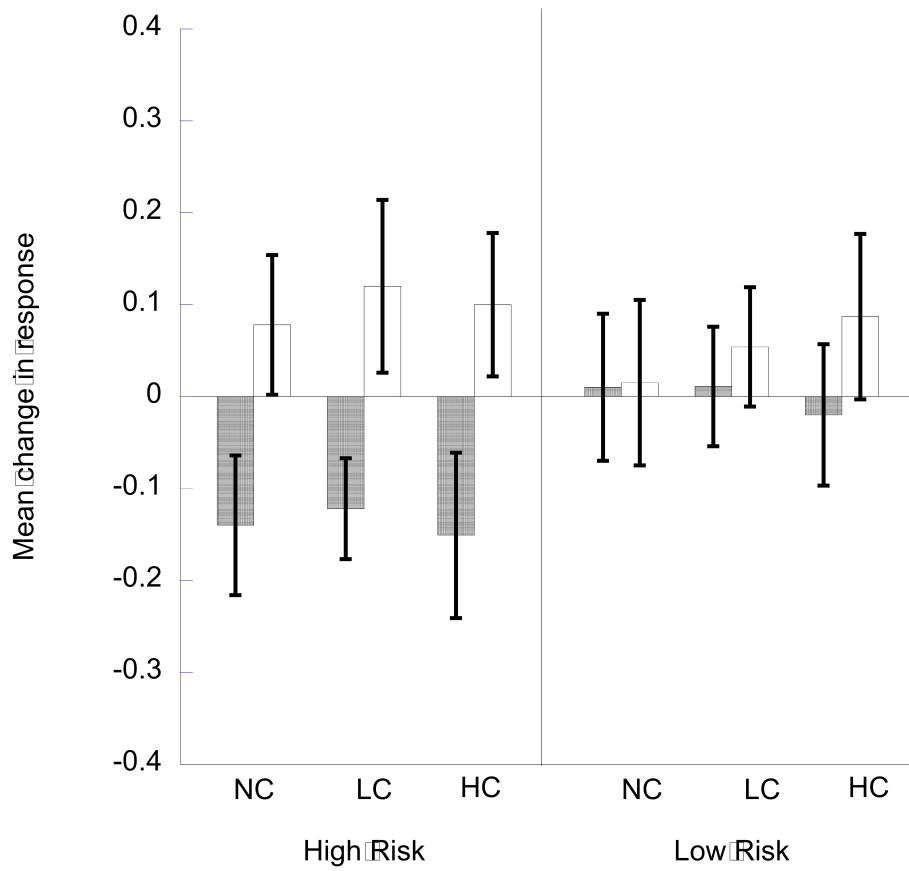
**Figure 1:** Three conditioning buckets (19L buckets) were set up, each containing a shoal of 20 guppies (mean  $\pm$  sd total length:  $19.50 \pm 3.74$  mm) in filtered tank water and were split into three different treatments: high current, low current, and no current. Mason jars were placed in the centre of each bucket, held down with gravel, to establish a circular circuit within the bucket. Artificial currents were established in the tanks using Sisce Voyager Nano water pumps set at different intensities (high current: 2 running pumps (mean  $\pm$  sd current velocity:  $1.66 \pm 0.42$  cm/s); low current: 1 running pump (mean  $\pm$  sd current velocity:  $1.13 \pm 0.26$  cm/s); no current: no running pumps (mean  $\pm$  sd current velocity:  $-0.116 \pm 0.34$  cm/s)) proportional to each treatment. Current velocity was measured using a Hach flow meter. A predator model was made using a fishing lure attached to a glass rod and waved ten times around the circuit to extract disturbance cues from the guppies.



**Figure 2:** Mean number of body undulations per minute observed in guppies exposed to the increasing current treatments. Observations were taken 3 times at the 60-second mark (dark grey bar), at the 180-second mark (white bar), and the 300-second mark (light grey bar). N = 5-6 per treatment



**Figure 3:** Mean proportional change ( $\pm$  SE) in calm-foraging behaviours between stimulus injection periods for guppies exposed to disturbance cue (shaded bars) or the odour of undisturbed guppies (open bars) from high- vs. low- vs. no-current senders. N = 16 per treatment combination.



**Figure 4:** Mean change in ( $\pm$  SE) in calm-foraging behaviours between disturbance cue (shaded bars) or undisturbed odour (open bars) injection periods for guppies conditioned to high- or low-background risk. N=13 - 15 per treatment combination.