Depletion of Disturbance Cues in Convict Cichlids (*Amatitlania nigrofasciata*)

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This is to certify that the thesis prepared

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ABSTRACT

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Within aquatic ecosystems, disturbance cues provide a critical source of chemosensory predation risk information. Unlike the well-documented damage-released chemical alarm cues, disturbance cues do not require direct physical interactions with predators to facilitate their release. Rather, disturbance cues are hypothesized to be nitrogenous wastes produced as the result of protein metabolism. Due to their release mechanism and chemical makeup, disturbance cues can be considered a generalized cue, lacking species specificity. Experiment 1 tested the generalized metabolic byproduct hypothesis, comparing the response of convict cichlids (*Amatitlania nigrofasciata*) conditioned to high or low background risk to the disturbance cues from similarly sized or large conspecific donors, controlling for donor biomass. We predicted that as disturbance cue is a metabolic byproduct, we may see a weaker response to larger individuals as they tend to excrete less nitrogen per unit mass. Our results, however, found no effect of donor size on receiver response. In experiments 2 and 3, we tested whether stores of disturbance cue, as a metabolic byproduct, could be depleted through repeated predator exposure at 30- (experiment 2) or 5- (experiment 3) minute intervals. We predicted that if depletion was occurring, disturbance cues collected later would elicit a weaker response from receivers, and that if replenishment was occurring, we would see a more dramatic decrease in response in the shorter time interval. Consistent with our hypothesis, our results showed no evidence of depletion at 30-minute increments, but at 5-minute increments, depletion was reached at the fifth collected disturbance cues. Ultimately, the body size experiment showed a generalized response to disturbance cues from different sized donors, and the depletion experiment supported the metabolic byproduct hypothesis.

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Table of Contents

List of Figures

List of Tables

Introduction

Predation poses one of the strongest selective pressures acting on prey populations, shaping prey behaviour, morphology, and life history through both plastic and genetically fixed effects (Lima and Dill 1990, Brönmark and Miner 1992, Sih 2005). As predator encounters are often unpredictable, reliable information is essential for prey animals to balance predator avoidance with other activities such as foraging and mate-seeking behaviours (Crane et al. 2022, 2024). Expressing anti-predator behaviours unnecessarily can result in missed foraging and mating opportunities, known as lost opportunity costs (Lima and Dill 1990, Dugatkin and Godin 1992b). However, the cost of ignoring information in favour of continuing foraging or mating activities may be death. Within aquatic ecosystems, chemicals released by both predators and prey can serve as critical sources of risk information (Wisenden and Chivers 2006).

The predation sequence describes the different stages of a predator-prey encounter and their possible outcomes. Broadly, these stages can be divided into the categories of detection, attack, capture, and ingestion. Different categories of chemical cues are available at different stages of the predation sequence and thus carry different information (Wisenden and Chivers 2006, Wisenden 2015, Fig. 1). For example, predator odours (kairomones) are available throughout the predation sequence but are most commonly attributed to the "detection" stage (Wisenden and Chivers 2006). They are a well-studied category of chemical cues that receivers (the individual detecting a cue) can recognize as an indicator of risk through learning or genetically fixed effects (Magurran 1990, Chivers and Smith 1994, Kats and Dill 1998). Damage-released chemical alarm cues, stored in prey skin and visceral tissues, are available only during the capture stage. Also well-studied, they are considered an honest indicator of risk to receivers because they indicate the nearby injury or death of the donor (the individual emitting the cue) (Chivers and Smith 1998, Wisenden 2015)(Fig. 1). Prey confer strong fitness benefits from the ability to detect and respond appropriately to alarm cues and kairomones, as it allows them to accurately assess predation risk (Chivers and Smith 1998, Kats and Dill 1998). Donors of these cues, however, are not thought to benefit from the receiver response to the cue (Kats and Dill 1998, Chivers et al. 2012).

Disturbance cues are a lesser-studied chemical cue observed mostly from aquatic vertebrates and invertebrates, but occasionally in terrestrial species (Crane et al. 2022). They are released in response to acute disturbance and may not necessarily indicate predation is occurring. They are available earlier in the predation sequence than alarm cues, and are therefore considered an early warning cue (Wisenden et al. 1995, Crane et al. 2022). Evidence suggests that they can be voluntarily emitted, and that senders may be able to control their release (Bairos-Novak et al. 2019, Crane et al. 2020). Release of both disturbance and alarm cues have been shown to elicit anti-predator responses in receivers, but alarm cue has been shown to provoke a stronger response (Vavrek and Brown 2009, Achtymichuk et al. 2025). For both types, cue concentration in the system correlates positively with receiver response intensity (Brown et al. 2006a, 2009, Vavrek and Brown 2009). This phenomenon of prey response plasticity according to the level of perceived risk is referred to as threat sensitivity (Helfman 1989). Within the hypothesis of threat sensitivity, when the receiver response strength is closely correlated with the level of perceived risk, the response style is referred to as being “graded” (Helfman and Winkelman 1997). In the case of chemical disturbance and alarm cues, cue concentration can be used as a proxy for perceived predation risk to the receiver as it can indicate the proximity to or the time elapsed since a disturbance/predation event (Ferrari et al. 2010). While alarm cue must be collected from whole-body or skin homogenates of donors, disturbance cue can be collected as the tank water of disturbed individuals (Wisenden et al. 1995, Vavrek and Brown 2009, Brown et al. 2012, Goldman et al. 2020b, 2022, Achtymichuk et al. 2025).

The chemistry of disturbance cues remains poorly understood, but the functional component is thought to be nitrogenous waste released through the urine or across the gill epithelia (Hazlett 1990, Wisenden et al. 1995, Vavrek and Brown 2009, Brown et al. 2012). Some larval amphibians and aquatic invertebrates show anti-predator responses to acute exposures of ammonium (Hazlett 1990, Kiesecker et al. 1999). When this was tested in convict cichlids (*Amatitlania nigrofasciata*) and rainbow trout (*Oncorhynchus mykiss*), they did not respond to ammonium (Vavrek et al. 2008), but they did respond to urea (Brown et al. 2012). Teleost fishes excrete 80-90% of nitrogenous waste as ammonia, and the rest as urea, which is generally excreted in pulses across the gills (Wilkie 2002). This makes urea an ideal candidate for the functional ingredient of teleost disturbance cues.

The proposed chemistry of disturbance cues was further supported by a dietary manipulation experiment in Trinidadian guppies (*Poecilia reticulata*). It was shown that disturbance cue collected from donors fed a high protein diet elicited a stronger anti-predator response than that of individuals fed a low protein diet, and that of fasted individuals elicited an even weaker response (Goldman et al. 2022). This points strongly to nitrogenous wastes, which are byproducts of metabolized protein (Wilkie 2002). As a result, disturbance cues are thought of as a generalized cue (i.e. not species specific). Within teleost fish, disturbance cues appear to have a shared chemistry with no evidence of phylogenetic conservation. When cross-species responses were tested on two distantly related teleosts, rainbow trout and convict cichlids, they did not respond differently to conspecific vs. heterospecific disturbance cues (Vavrek and Brown 2009). The same was also found between convict cichlids and guppies (Goldman et al. 2019).

This is in contrast to alarm cues, which are made of more specialized chemical components (Chivers et al. 2012) and show a high degree of conservation, even to the population level (Brown et al. 2010, Ferrari et al. 2010).

The response of prey to disturbance cues can also be influenced by the background level of predation risk experienced by the receiver. Receivers from high predation environments have been shown to respond more strongly to disturbance cues, and are better at discriminating between different cue qualities/concentrations (Bairos-Novak et al. 2017, Goldman et al. 2020a, 2020b, 2022). This trend is also observed in alarm cue detection (Brown et al. 2009, 2010). The effects of background predation experience can be induced in as few as three days under laboratory conditions by giving receivers regular exposures to conspecific alarm cue (Foam et al. 2005, Brown et al. 2006b, 2013, Bairos-Novak et al. 2017, Goldman et al. 2020b, 2022).

As mentioned above, receivers can show a graded response to disturbance cue where higher concentrations yield stronger responses. This has been shown by testing different dilutions of disturbance cue (Vavrek and Brown 2009), or by manipulating the ratio of donor group size (also interpretable as biomass) to water volume, as a correlate of concentration (Goldman et al. 2019). If disturbance cue is a nitrogenous byproduct of metabolized protein (henceforth metabolic byproduct), then factors that mediate mass-specific excretion rate, such as body size, may influence disturbance cue release. It has been demonstrated that in many taxa (including fishes), nitrogen excretion rate increases allometrically to body size, where larger individuals tend to have a lower mass-specific excretion rate than smaller individuals (Allgeier et al. 2015, Oliveira-Cunha et al. 2022).

In experiment 1, we tested high and low-risk juvenile (0.5-2g) convict cichlids to disturbance cues collected from shoals of either similarly sized or large (9-15g) convict cichlids while controlling for the ratio of donor biomass to water. We predicted that if the scaling of excretion rate to body size is extrapolatable to disturbance cue release, we might expect high-risk receivers to demonstrate a stronger response to disturbance cue from smaller donors. As low-risk receivers are not as sensitive to disturbance cues, they may not show the same pattern of response.

The proposed chemistry of disturbance cues would also mean that as a metabolic byproduct, disturbance cue is limited and cannot be released in perpetuity. This would mean that if donors were stimulated to repeatedly release disturbance cue, this should eventually result in depletion. Achtymichuk et al., (2025) tested wood frog (*Lithobates sylvaticus*) tadpoles by exposing them to two predator chases across a short (5 min) or long (2 h) time interval. No difference in conspecific receivers' responses was found between 1st and 2nd disturbance cues across the short time interval, but across the long time interval, the 2nd disturbance cues yielded a reduced response. This showed some (yet not complete) depletion, supporting the hypothesis that for amphibians, disturbance cues are released in pulses and not via bladder emptying. As mentioned above, fishes are also hypothesized to release disturbance cues in pulses.

In experiment 2, we tested the hypothesis that a repeated predator stimulus will result in depletion of disturbance cue stores. We exposed donor shoals of juvenile convict cichlids (*Amatitlania nigrofasciata*) to five repeated simulated predator chases at 30-minute increments. The resulting five disturbance cue treatments were tested on juvenile conspecific receivers conditioned to high risk and compared to a dechlorinated water control treatment. To limit the number of treatments, no low-risk receivers were used, as it has been repeatedly demonstrated in the primary literature that elevated background risk increases receiver sensitivity to disturbance cues (Bairos-Novak et al. 2017, Goldman et al. 2020a, 2020b, 2022). We then conducted a companion experiment changing the increment between predator stimuli to 5 minutes (experiment 3).

We predicted that if depletion was occurring, we would see a continuous decline in receiver response from 1st to 5th disturbance cues across the “time series.” If complete depletion had been reached, this would be shown if the response to the disturbance cue treatment was not significantly different to the control. Alternatively, if no depletion occurred, we would see no significant differences between any of the disturbance cue treatments, and all would be significantly different to the control (Fig. 2). If we saw a weaker effect of depletion in experiment 2 than experiment 3, this would suggest that disturbance cue replenishment was occurring.

Methods

Study Species and Captive Husbandry

We used captive-bred and raised convict cichlids (*Amatitlania nigrofasciata*), housed and managed by the Concordia University Animal Care Facilities (ACF). Convict cichlids are an ideal study species because they have been repeatedly shown to demonstrate threat-sensitive responses to conspecific disturbance cues (Vavrek et al. 2008, Vavrek and Brown 2009, Brown et al. 2012). They are also widely available, and uncomplicated to breed and maintain in captivity long-term. Fish were housed at a constant temperature of ~25.5°C, pH ~8.2 in dechlorinated water with a 12:12 photoperiod. They were fed commercial flake food (Nutrafin Basix Staple Food) daily. All tanks were filtered with mechanical and biological filtration.

Disturbance Cue Collection

To collect disturbance cues, donors were housed in aquaria for a 24-hour acclimation period before cue collection. Donor tanks were covered on all sides with opaque plastic to prevent fish from responding to seeing the researcher. Unless mentioned otherwise, all donor tanks were 40L aquaria filled to 9.5L with an airstone placed near the surface for oxygenation. Upon moving into their temporary tanks, donors were allowed to acclimate for 24 hours without food.

The disturbance cue collection protocol began by removing the airstone to prevent donors from crowding around it during the simulated predator chase. After 10 minutes, ~200 mL of tank water was scooped out with a beaker. This was then divided into smaller aliquots and frozen and functioned as an "undisturbed cue" control in experiment 1. Experiments 2 and 3 used a dechlorinated water control, but we kept the same collection methods for the sake of consistency.

After undisturbed cues were collected, donors were subjected to a 60 second simulated predator chase using a realistic predator model attached to a glass rod and manipulated by the researcher. Following the predator chase, 60 seconds were allowed for cue dispersion throughout the system, and then ~200 mL of tank water was collected via a beaker. This was separated into smaller aliquots and frozen.

Alarm Cue Collection

To manipulate background risk, alarm cue must be collected from conspecifics. In consideration of the reduction principle, adults were used to minimize the number of donors. Using adults also permitted the use of skin fillets as opposed to whole-body homogenates, which better isolates alarm cue. Males and females were used in equal proportions. In accordance with standard protocols (Chivers and Smith 1994, Mirza and Chivers 2001, Foam et al. 2005, Brown et al.

2006a, Brusseau et al. 2023, Dumaresq Synnott et al. 2025), alarm cue donors were euthanized via blunt impact to the head followed by cervical dislocation. Skin fillets were then removed from the flanks and fillet length and width were measured. All skin fillets were placed into 400mL of dechlorinated water, homogenized, and strained. The solution was then diluted to achieve a $1cm^2$ ml^{-1} concentration of skin/water. The diluted alarm cue solution was frozen in 30 mL aliquots for use in conditioning.

Background Risk Conditioning

Prior to testing, receivers were housed in 7.5L opaque pails filled with 6 L of dechlorinated water with an airstone and an additional length of airline tubing to inject stimuli with minimal disturbance. High-risk receivers were exposed to 5mL of conspecific alarm cue followed by a 50% water change 20 minutes later. Low-risk receivers underwent the same conditioning protocol but were given dechlorinated water instead of alarm cue. Three exposures were done a day at 3-hour increments for three consecutive days before testing on the fourth. During conditioning, receivers were fed a pinch of their regular food once every day.

Behavioural Observations

Juvenile convict cichlids were used as receivers. Both males and females were used, as they are difficult to sex at the size used (>1g) and no behavioural differences are expected. While there is evidence that in sexually dimorphic species there can be sexual differences in risk response (Brusseau et al. 2023), there is also support for these differences only appearing after sexual maturity (Dumaresq Synnott et al. 2025).

Receivers were placed individually in 7.5L opaque pails filled with 2L of water with lines on the bottom forming a 3x3 grid. Movement was measured by counting the number of lines crossed during the observation periods. Observations were recorded via USB webcams mounted above the pails. Receivers were given 5-8 minutes to acclimate to their surroundings followed by a 4-minute pre-stimulus observation period. Then, 5 mL of stimulus (disturbance cue, undisturbed cues, or water) was injected down the wall of the pail, followed by a 4-minute post-stimulus observation period. Response strength was calculated as the difference between lines crossed post-stimulus and lines crossed pre-stimulus. A more drastic reduction in movement is interpreted as a stronger risk-averse response.

Experiment 1: Donor Body Size

In this experiment, disturbance cue was collected from donor shoals of small (0.5-2g) and large (7-17g) donors and tested on small (< 1g) high- and low-risk receivers. Large donors were in pairs of one female and one male, to remove any confounding sex effect. They were weighed before their introduction in the donor tank. Combined biomass ranged from 23.5-27.8 g. A plastic divider with mesh windows (to allow surface agitation from the airstone across the entire tank) was placed in the donor tank to prevent any aggression during the acclimation period and was removed 30 minutes before cue collection.

Small donors were juveniles individually weighing < 1g. Shoals of 20 were collected and weighed (shoal biomass was 5.2-6.6 g), and water volume was calculated to equal the ratio of large donor biomass to water volume for that replicant. For example, if large donors weighed 24g together in 9.5L of water (approximately 2.53g/L) and small donor shoal biomass was 6g, small donors would be housed in 6g/2.53g/L = 2.37L of water. Because of the small volume to compensate for large donors, small donors were tested in 7.5L opaque pails rather than aquaria. There was still ample room for the predator model to move around.

Undisturbed cues for each donor size were used as controls for this experiment. This resulted in a 2 x 2 x 2 design, with receiver background risk (high vs. low), cue (disturbed vs. undisturbed), and donor body size (large vs. small) as independent variables. The final dataset consisted of *n* = 172 receivers.

Experiment 2: 30-minute Depletion

Donor shoals were groups of 9-12 juvenile convict cichlids with a group biomass of 20-21g.This chapter required five simulated predator chases to be performed on the same donor group for each replicant. To avoid the confound of disturbance cue building up in the system, a continuous water change was performed after every collection. A length of siphon tubing was left in the donor tanks throughout the protocols, as well as a length of tubing supplying new dechlorinated water. The water in and outflow speed were calibrated so the total volume of water in the tank would not change. Setups were tested using red food dye to determine if 100% of the tank water would be replaced in the allotted time.

In experiment 2, the simulated predator chase was immediately followed by a 10-minute continuous water change. The next predator chase occurred exactly 30 minutes after the start of the previous one. The five disturbance cue treatments (from initial to final predator chase) are labeled as T0, T30, T60, T90, and T120. Each receiver was tested once to only a single treatment.

In this experiment and experiment 3, dechlorinated water was used as the control to avoid the confound of repeated collection on the undisturbed cues. The final dataset consisted of *n* = 134 receivers.

Experiment 3: 5-minute Depletion

In experiment 3, the continuous water change was shortened to 5 minutes, but with a higher flow rate accomplished by wider tubing. The concern was that an aggressive flow may disturb donors between predator chases, however they did not appear to respond, even displaying foraging responses towards the surface disturbed by the incoming water. The following predator chase began as soon as the water change was complete. The predator chases were closer to 6-7 minutes apart than 5, but they were still much closer together than in the 30-minute experiment. The disturbance cue treatments for this experiment are labeled as T0, T5, T10, T15, and T20. The final dataset for consisted of *n* = 129 receivers.

Data Analysis

In all experiments, we used a Generalized Linear Model to analyze the data. The difference between lines crossed post- and pre-stimulus was used as the dependent variable. In experiment 1, the cue treatment was the only independent variable. However, in experiments 2 and 3, receiver background risk, donor body size, cue (disturbed or undisturbed), and their interactions were all tested as predictors of variation in changes in receiver line crosses. Analyses were conducted using SPSS V 29.0.

Results

Experiment 1: Donor Body Size

Our overall GLM found no significant effect of body size ($F_{(1, 164)} = 1.20$, P = 0.28; Table 1), and no significant effect of its interactions with other predictors (Risk × Body Size, $F_{(1, 164)} = 0.17$, P = 0.68; Body Size × Cue, $F_{(1, 164)} = 0.97$, P = 0.33; Risk × Body Size × Cue, $F_{(1, 164)} = 0.33$, P = 0.57; Table 1). Receiver background risk also did not have a significant effect on receiver response ($F_{(1, 164)} = 0.70$, $P = 0.40$). Cue was found to be the greatest predictor of difference in receiver movement ($F_{(1, 164)} = 15.32$, $P < 0.001$).

High-risk receivers demonstrated a significantly greater reduction in movement to disturbed cues than undisturbed, with no significant difference in cues from large or small donors (large donors, $F = 6.93$, $P = 0.012$, small donors, $F = 5.23$, $P = 0.28$, Fig. 3). Low-risk receivers reduced their movement significantly more to large donor disturbed cue than to large donor undisturbed cue ($F = 8.47$, $P = 0.006$) but showed no significant difference in response to small donor disturbed and undisturbed cues ($F = 0.44$, $P = 0.51$, Fig. 4).

Experiment 2: 30-minute Depletion

Our overall GLM found some variance was explained by cue treatment ($F_{(5, 128)} = 3.83$, $P = 0.003$). In post hoc testing, it was shown that there were no significant differences in line crosses between the five disturbance cues treatments collected from the same donor pool, and all showed a more significant reduction in line crosses than the water control (Fig. 5).

Experiment 3: 5-minute Depletion

Our overall GLM found cue treatment to be a better predictor of data variance than in experiment 1 ($F_{(5, 125)} = 4.97$, $P < 0.001$). T0, T5, and T10 disturbance cues elicited a significantly greater reduction in line crosses than the control and T20 treatments (Fig. 6). Additionally, the T15 treatment showed significantly greater movement reduction than the control treatment, but no significant difference to any other treatments. The T20 treatment, the fifth and final disturbance cue collected from the same donor pool at 5-minute increments, had no significant difference to the water control (Fig. 6).

Discussion

Disturbance cues are a critical source of risk information for a variety of aquatic taxa. Unlike alarm cues, which are only available in the "consumption" stage of the predation sequence, disturbance cues provide an early warning of predator presence. The chemistry of disturbance cues is not very well understood, but they are hypothesized to be a pulse release of metabolized protein byproduct (Crane et al. 2022). It is likely that the functional ingredient of disturbances cues in amphibians and certain freshwater crustaceans is ammonia/ammonium (Hazlett 1990, Kiesecker et al. 1999), while that of teleost fishes is suggested to be urea released in pulses in the urine or across the gill epithelia (Brown et al. 2012).

In experiment 1, we tested whether small convict cichlids would respond differently to disturbance cues from large or similar sized conspecific donors. As the ratio of donor shoal biomass to water was likely to present a confound (Goldman et al. 2019), we controlled for donor biomass to test the effect of donor body size. We predicted that we may see a weaker response to larger-sized donors, as it has been shown in fishes that larger individuals tend to excrete less nitrogen relative to their mass (Allgeier et al. 2015, Oliveira-Cunha et al. 2022).

Our results suggest no effect of donor body size on receiver response to disturbance cue. This provides support for the hypothesis of disturbance cue as a generalized metabolic byproduct. This hypothesis has received previous support from the lack of species-specificity in disturbance cues (Wisenden and Chivers 2006, Ferrari et al. 2010, Chivers et al. 2012, Wisenden 2015, Crane et al. 2022). Conversely, alarm cues show a high degree of phylogenetic conservation (Ferrari et al. 2010). Mirza and Chivers (2001) tested three salmonid species from different genera to conspecific alarm cues, heterospecific alarm cues from the other two salmonid species, and swordtail (*Xiphophorus helleri*) alarm cue. In all three salmonid species, it was shown that they responded the strongest to conspecific alarm cues, showed a weaker response to heterospecific salmonid alarm cue, and did not respond to swordtail alarm cue. Generally, the receiver response is directly correlated with the degree of taxonomic relatedness to the donor. Brown et al. (2010) even found evidence of alarm cue conservation in different populations of guppies (*Poecilia reticulata*) in Trinidad.

The same is not seen in disturbance cues. Not only have teleosts shown no differential response between conspecific and distantly related heterospecific cues (at least no difference that can be conclusively attributed to donor species) (Vavrek and Brown 2009, Goldman et al. 2019), but the same has been seen in amphibians. Brown et al. (unpublished data) found that larval giant gladiator tree frogs (*Boana boans*, family Hylidae) and cane toads (*Rhinella marina*, family Bufonidae) in Trinidad showed no significant difference to each other's disturbance cues. However, juvenile guppies and larval cane toads did not respond to each other's disturbance cues (Brown et al., unpublished data). This is consistent with the metabolic byproduct hypothesis, as teleosts had been hypothesized to use urea as the primary functional component, while amphibians likely use ammonia/ammonium (Crane et al. 2022). The lack of species-specificity observed within teleosts or amphibians suggests that within larger clades, the chemistry of disturbance cues between species is highly similar, ergo generalized.

It was important for experiment 1 to be conducted on laboratory bred and raised stock whom where fed the same diet ad libitum, to prevent any confounding effects of diet (Goldman et al. 2022) Conversely, donor size effects have been demonstrated in other chemical cues. For example, Mirza and Chivers (2002) found that small receiver brook char (*Salvelinus fontinalis*) reduced their movement more in response to alarm cues from similar sized donors than from larger donors, and large receivers also demonstrated a stronger response to similarly sized donors than to smaller donors. There is also evidence that teleost fishes can make similar distinctions with kairomones (Kusch et al. 2004) and conspecific odours (Giaquinto and Volpato 2005).

Despite no overall effect of body size or any interaction of body size and background risk (Table 1), we found that low-risk receivers did not respond to the disturbance cues of small donors (Fig. 4). This is likely because the quantity of disturbance cue they were exposed to fell below the minimum threshold for an overt behavioural response. It has been shown that receivers conditioned to lower background risk will have a higher minimum threshold for an overt behavioural response than higher risk receivers (Brown et al. 2006b), which would explain the difference in high- and low-risk receivers' responses to the disturbance cue of small donors. This also suggests a difference in disturbance cue concentration collected from large and small donors. It is not yet clear, however, why we found this potential concentration difference. Perhaps being housed as pairs with an opaque barrier caused higher stress during the acclimation period of the large donors, which could have affected their disturbance cue release. Small donors, in shoals of 9-12, were unlikely to be particularly stressed as their acclimation conditions were more like their normal housing conditions. High-risk receivers did not respond differentially between these potential different concentrations of disturbance cue. This is not unexpected, as they tend to be more sensitive to disturbance cue overall (Bairos-Novak et al. 2017, Goldman et al. 2020b, 2022)..

While our small donors were in familiar groups as they were normally housed in the same tank outside of experiments, large donors were not. Adults and subadults are housed in large sex-specific tanks to mitigate aggression, meaning that the large donor pairs were an unfamiliar male and female. The expected effect based on Bairos-Novak et al.'s (2019) findings would therefore be for the small donors to elicit a stronger response in receivers, but in low-risk receivers, the opposite was found. Perhaps familiarity did not interfere with our results because donors were kept at low background risk (Crane et al. 2020). We would still recommend controlling for donor familiarity in future experiments of this kind.

In controlling for diet, a primary driver of disturbance cue potency (Goldman et al. 2022), and cue concentration (donor biomass to water volume) (Goldman et al. 2019), we designed this experiment to assess whether there was a qualitative or quantitative difference in disturbance cue from different-sized donors. The lack of discrimination between the disturbance cues of small and large donors by high-risk receivers distinguishes it from alarm cues, supporting the hypothesis that disturbance cue is generalized. This also suggests that in future experiments, mismatch between size of conspecific donors and receivers of disturbance cues will not have a significant effect on the results. Based on precedent in previous literature, we do still recommend controlling for donor diet and biomass to water volume for standardization when collecting disturbance cues (Goldman et al. 2019, 2022).

In experiments 2 and 3, we tested whether a repeated predator stimulus on the same donor shoal at 30 (experiment 2) or 5 (experiment 3) minute increments would result in depletion of disturbance cue stores. Based on the hypothesis of disturbance cue as a metabolic byproduct, we expected disturbance cue collected from repeated predator stimuli to elicit weaker responses in receivers as we moved from first to last collected. In experiment 2, we found no evidence of depletion (Fig. 5). It could be inferred that 30 minutes between predator stimuli is enough time for replenishment to occur, even if donors had gone over 24 hours without food. Then, in experiment 3, we tested whether a repeated predator stimulus on the same donor shoal at 5-minute increments would result in depletion. We found that depletion was reached by the fifth predator stimulus, signified by no significant difference between the receiver response to the 5th disturbance cue collection (T20) and the water control (Fig. 6). The results of experiment 3 suggest that 5 minutes is not enough time for replenishment of disturbance cue to occur.

Combined, these experiments strongly support the hypothesis that fishes release disturbance cues in pulses, which had been suggested from the way they excrete urea (Wilkie 2002, Brown et al. 2012). Achtymichuk et al. (2025) also found support for this hypothesis in larval amphibians. However, they only found evidence of partial depletion from two predator stimuli 2 hours apart, and no difference at the 5-minute increment. This suggests that amphibian disturbance cues may be released slowly, in contrast to our findings. Our results indicate that teleost disturbance cues are released in acute pulses that are quickly replenished unless disturbances are intense and frequent.

In a companion experiment, guppies were found to deplete by the fifth disturbance at 30-minute increments, and by the third disturbance at 5-minute increments (Haney et al., unpublished data). This suggests that 30 minutes is not enough time for them to fully replenish, and that they deplete quicker than convict cichlids at 5-minute increments. It is possible that guppies are releasing more disturbance cue in every pulse relative to their "stores." Guppies are highly gregarious fish (Dugatkin and Godin 1992a, Swaney et al. 2001), likely more so than convict cichlids. They could rely more on disturbance cue as a source of social information, and this may cause them to release larger pulses in response to the disturbance cues of familiar conspecifics. This could also be a difference on the receivers' side. It is possible they have a higher minimum threshold for an overt behavioural response. However, this has not been suggested in previous comparative literature (Goldman et al. 2019), and guppy receivers were also conditioned to high background risk like the cichlids in our experiments. A cross-species experiment could address these questions.

The hypothesis that disturbance cues may be depleted via repeated exposure to acute risks should be further examined by elucidating whether disturbance cue is completely depleted at a certain point or if it still elicits "covert" responses. Our only measure of response was movement, which is appropriate for overt responses, but it is shown that exposure to risk cues under the minimum concentration for an overt behavioural response can still inform future behavioural decisions. Brown et al. (2004) showed this by exposing glowlight tetras (*Hemigrammus erythrozonus*) to subthreshold concentrations of hypoxanthine-3-*N*-oxide (the presumed functional ingredient of Ostariophysan alarm cues) paired with the sight of a conspecific performing an overt behavioural response. When the individuals were exposed to the subthreshold chemical and visual stimulus a second time, they displayed an overt behavioural response. If this phenomenon is applicable to disturbance cues, this means that even "depleted" cues may be a useful indicator of risk.

It has also been found that disturbance cues collected from donors kept at higher temperature elicited a stronger response in receivers (Laatsch & Brown, unpublished data). This strongly supports the metabolic byproduct hypothesis, as body temperature directly correlates to metabolism in ectotherms (Gillooly et al. 2001). Future experiments are required to investigate the influence of donor temperature on disturbance cue depletion. We expect donors held at higher temperatures to reach depletion faster, as the previous data suggest that at higher temperatures, disturbance cue may be released in either larger pulses or may be more potent.

It is also well-documented that donor background experience with predation affects disturbance cue release (Bairos-Novak et al. 2017, Goldman et al. 2020b). In guppies, senders conditioned to high risk tended to elicit stronger behavioural responses in high-risk receivers than any other combinations of sender and receiver background risk (Goldman et al. 2020b). It is not clear if this is because high-risk senders release larger pulses, but this may be elucidated by comparing their depletion to low-risk senders (which we used in these two experiments). If high-risk senders are truly releasing larger pulses of disturbance cue, then we would expect depletion to occur sooner than in low-risk senders.

Currently, disturbance cues are referred to as chemical cues, but in previous literature they have also been referred to as pheromones or signals (Crane et al. 2022). The difference between these terms pertains to how the communication type evolves in senders and/or receivers. Cues do not evolve in senders; the sender has no net fitness gain or loss from the receiver response to the cue, but receivers receive a fitness benefit from the detection and appropriate response to the cue (Ferrari et al. 2010). Signals evolve from a mutual selection, where receivers receive a fitness benefit from detection and response to the cue, and the receiver response confers a fitness benefit to the sender, therefore influencing the evolution of the signal itself (Wisenden 2015). Pheromones are communication chemicals with similar conditions of mutual selection, but are additionally species-specific (Wyatt 2010).

Both disturbance and alarm cues have been shown to elicit responses in heterospecifics, and are therefore not pheromones (Mirza and Chivers 2001, Brown et al. 2012, Goldman et al. 2019). Alarm cues are thought of as a true cue, despite previous hypotheses that have suggested ways for receiver responses to benefit alarm cue donors, it is more likely that alarm cue donors do not benefit, as the release of alarm cue requires the injury or death of the donor (Ferrari et al. 2010, Chivers et al. 2012). The most well-understood alarm cue system is that of the members of the large teleost clade Ostariophysi, who possess epidermal club cells that contain the alarm substance (Chivers and Smith 1998). These cells, however, likely evolved as an immune defense against parasites/pathogens entering through the epidermis (Chivers et al. 2012). Selection would have favoured receiver detection of alarm/immune substance released from mechanical damage to the club cells, resulting in a genetically fixed secondary function of this system as an honest risk cue.

Disturbance cues have traditionally been thought to similarly benefit receivers but not senders (Wisenden 2015), though not as overtly as through the damage-released property unique to alarm cues. However, Bairos-Novak et al. (2019) found that fathead minnows (*Pimephales promelas*) may voluntarily control their release of disturbance cue to release more in the presence of familiar conspecifics. Crane et al. (2020) later found that in guppies, the effect of familiarity in donor shoals on disturbance cues is highly mediated by donor background predation risk. Disturbance cue may therefore fulfill the criteria of functioning as a true signal and a source of social information pertaining to predation risk. The receiver response that benefits the sender may simply be the coordination of shoaling and other predator avoidant behaviours, but this should be explored on a species basis. Bairos-Novak et al. (2020) found no effect of interdonor familiarity or kinship in wood frog tadpoles, who responded to disturbance cue regardless of audience factors. This suggests that disturbance cues are still interpreted as an acute indicator of risk, but the donors, who were captive-raised in absence of predation risk, did not invest in releasing more disturbance cue based on audience factors. The concept of disturbance cue as a true signal of predation risk voluntarily released by senders may help us understand previous findings pertaining to background risk, and to ask better questions about what other factors may mediate when and how senders control the release of disturbance cue. This may also help put our results on depletion into context – if interdonor familiarity and high background risk cause donors to release more disturbance cues, we may expect familiar high-risk donors to deplete their disturbance cues quicker than low-risk and unfamiliar donors. By strengthening our understanding of disturbance cues, we gain insight into one of the key modalities by which aquatic prey species assess risk in their environment. Our results supported the hypothesis that disturbance cue is a metabolic byproduct by showing that under high predation risk, receivers do not respond to different donor body sizes when diet and biomass to water volume are controlled for. We also showed that disturbance cue release is constrained from being released in perpetuity when predator stimuli are intense and frequent, supporting the metabolic byproduct hypothesis. Future experiments will be required to generalize this hypothesis across different aquatic taxa, across different gradients of sender/receiver experience, and across metabolic constraints such as temperature. More field studies on disturbance cues are also needed. Currently there is one published paper showing fishes responding to disturbance cue in situ (Goldman et al. 2020a). More studies will be required to rigidify our understanding of the ecological relevance of disturbance cues. A better understanding of its identity as a nitrogenous waste byproduct of metabolized protein will also help us understand how anthropogenic impacts may interfere with a key source of acute risk information.

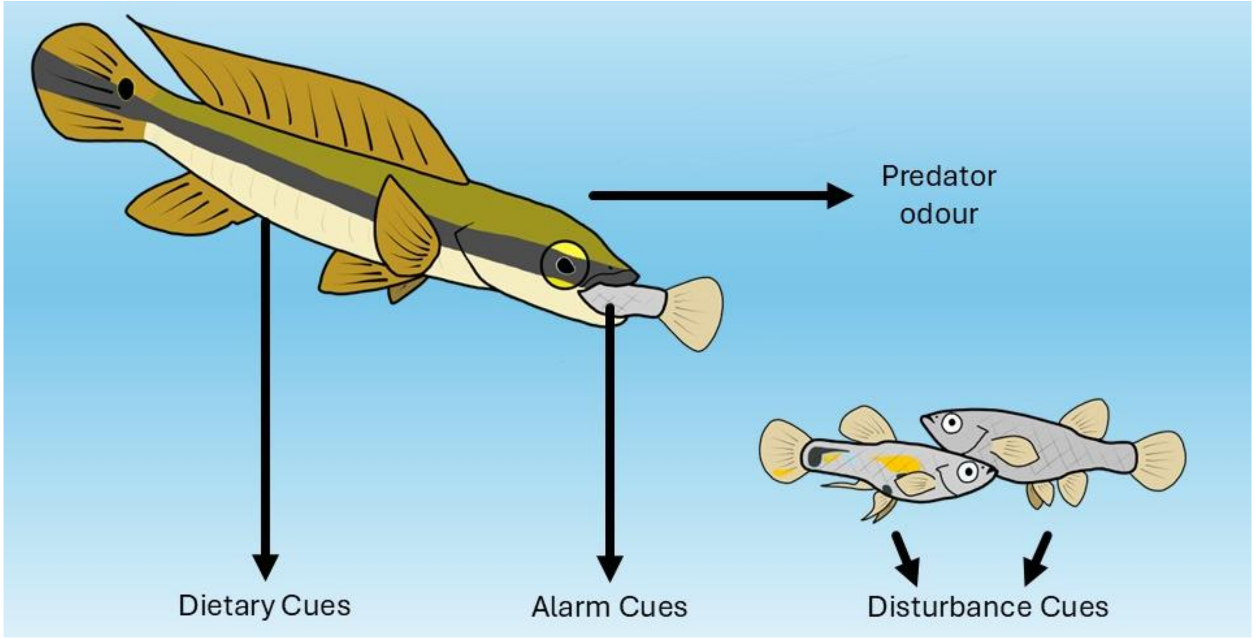

Figure 1. Typical chemical cues available during the predation sequence. A predator (ringtail pike cichlid, *Saxatilia saxatilis*) constantly emits predator odour (kairomones). As it consumes a guppy (Trinidadian guppy, *Poecilia reticulata*) alarm cues are released from mechanical damage to its skin and visceral tissues. Dietary cues are excreted by the predator, indicating to nearby receivers it had previously consumed other conspecifics. Nearby conspecifics, acutely disturbed by the predator's presence, release disturbance cues.

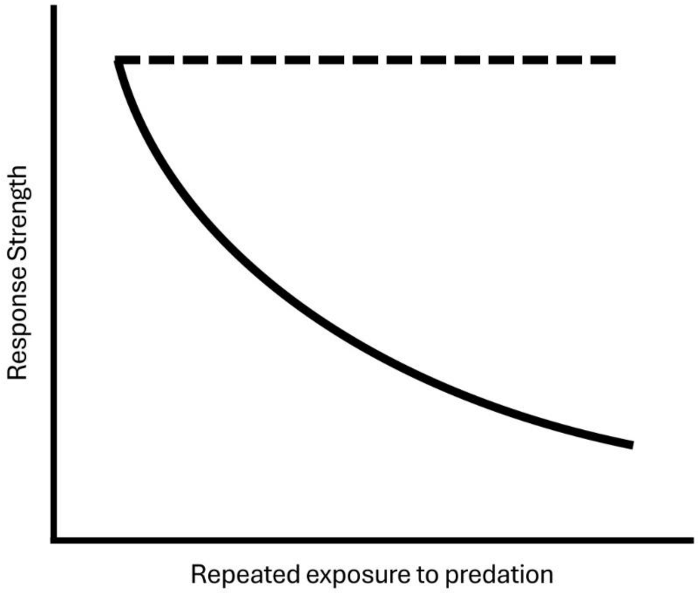

Figure 2. Prediction of disturbance cue depletion experiments (experiments 2 and 3). If disturbance cue is being depleted, the response strength should decrease across repeated predator stimuli (solid line). If depletion is not occurring the response strength should not change (dashed line).

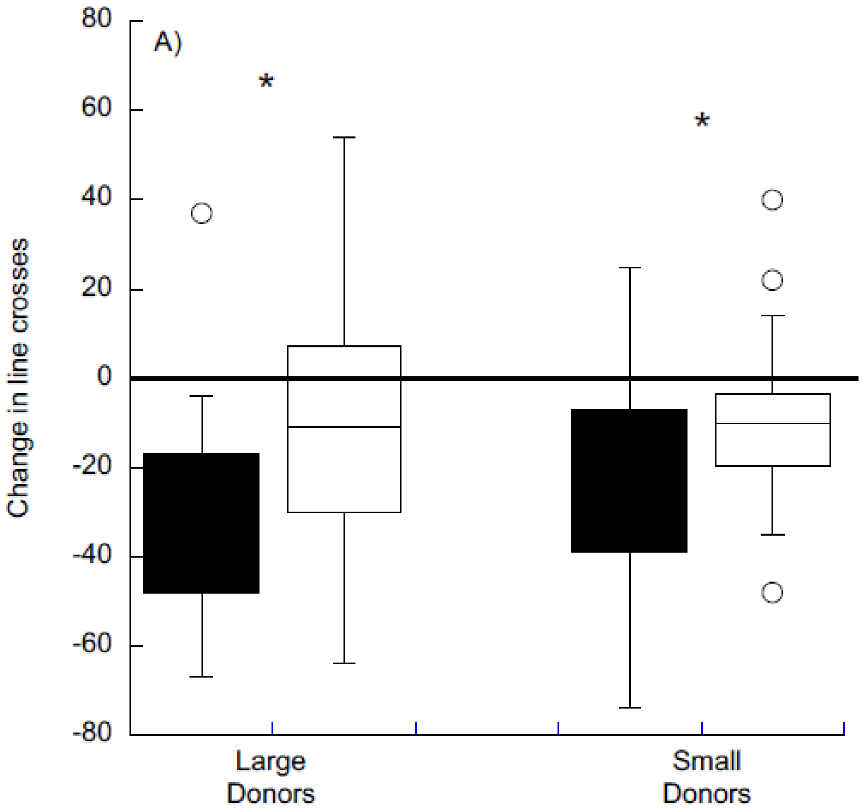

Figure 3. Mean (±SE) change in lines crossed for high-risk receivers in experiment 1. Receivers were conditioned to high background predation via regular exposures to conspecific alarm cues over a three-day period. They were then exposed to disturbance cues (black bars) or undisturbed cues (open bars) from large or small donors. Asterisks represent a statistically significant difference ($P < 0.05$), and NS = nonsignificant.

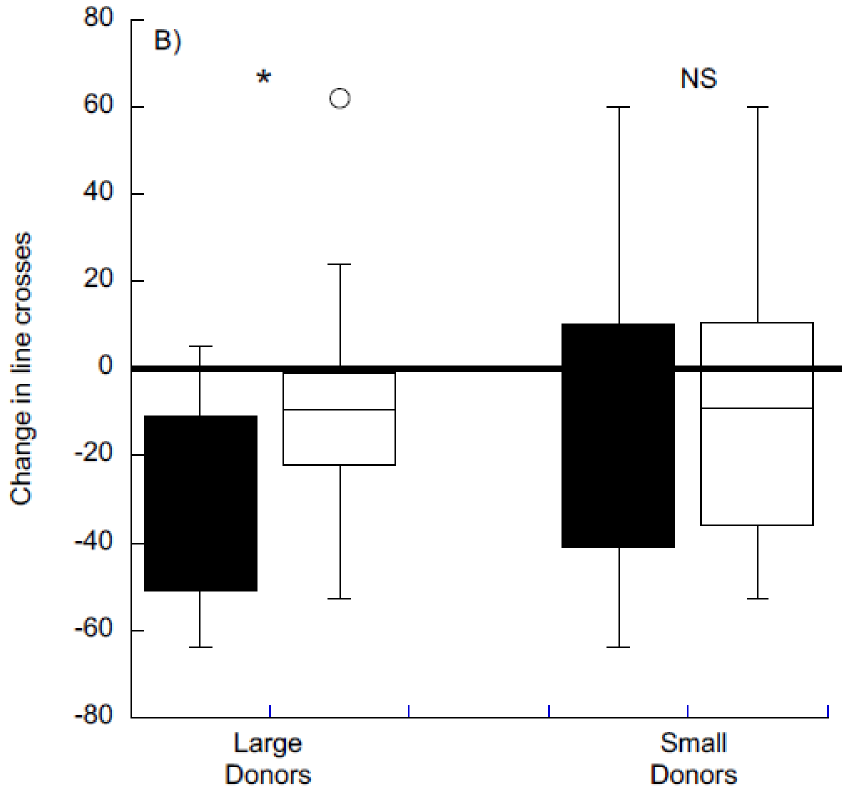

Figure 4. Mean (±SE) change in lines crossed for low-risk receivers in experiment 1. Receivers were conditioned to low background predation via regular exposures to dechlorinated water over a three-day period. They were then exposed to disturbance cues (black bars) or undisturbed cues (open bars) from large or small donors. Asterisks represent a statistically significant difference ($P < 0.05$), and NS = nonsignificant.

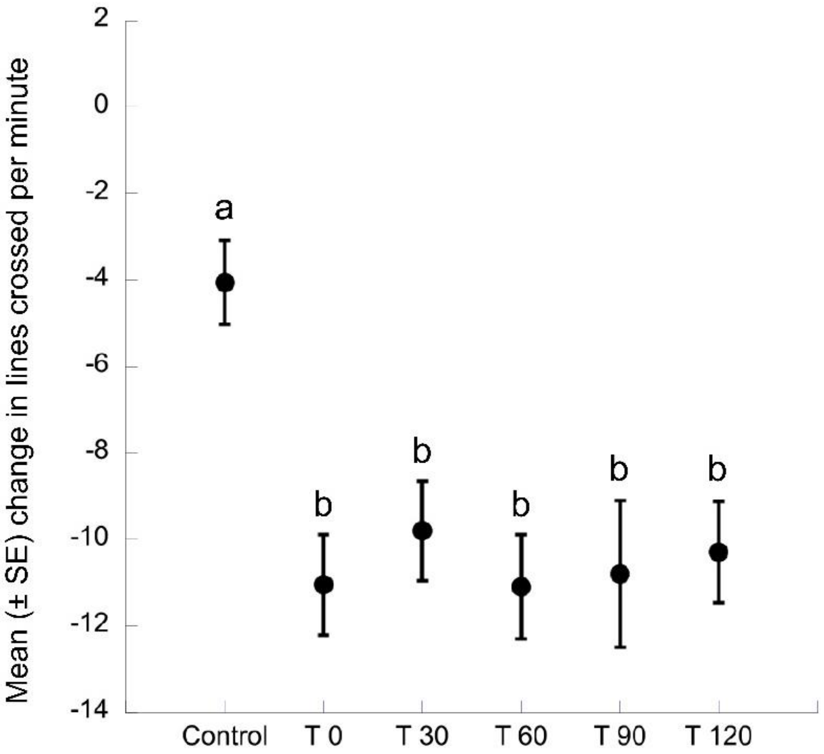

Figure 5. Mean (±SE) change in lines crossed per minute for experiment 2. The control treatment was dechlorinated water. Treatments T0 – T120 were disturbance cues collected from the same donor pool at 30-minute increments. Different letters denote significant differences ($P < 0.05$).

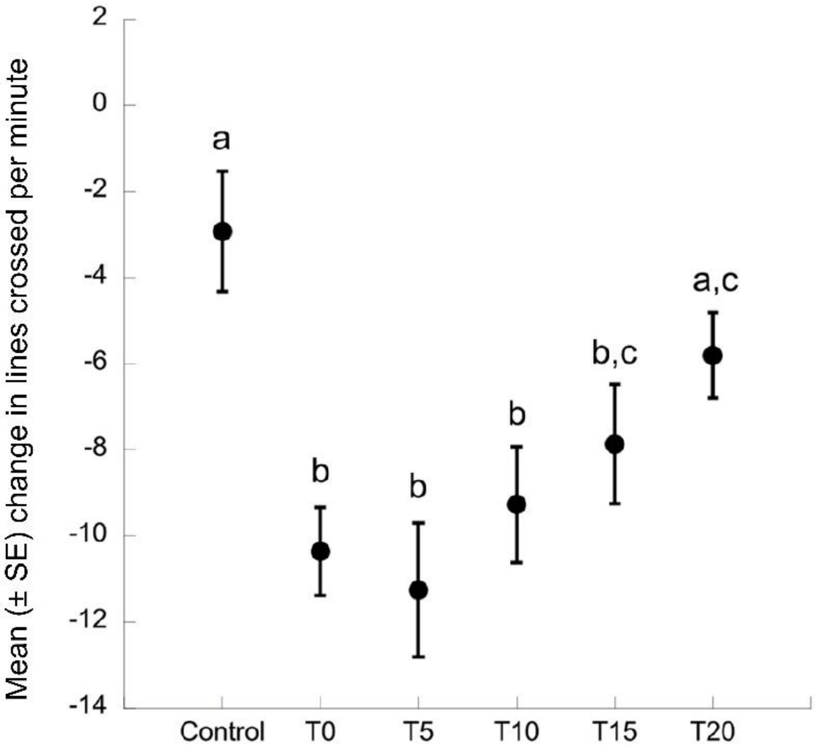

Figure 6. Mean (±SE) difference in lines crossed per minute for experiment 3. The control treatment was dechlorinated water. Treatments T0 – T20 were disturbance cues collected from the same donor pool at 5-minute increments. Different letters denote significant differences ($P < 0.05$).

Table 1. GLM output for experiment 1, testing the effects of donor body size, cue (disturbed/undisturbed), receiver background risk, and their interactions, on change in line crosses.

	F	*Df*	*P*
Risk	0.70	1	0.40
Body size	1.20	1	0.28
Cue	15.32	1	**<.001**
Risk × Body size	0.17	1	0.68
Risk × Cue	0.55	1	0.46
Body Size × Cue	0.97	1	0.33
Risk × Body Size × Cue	0.33	1	0.57

Bibliography

Achtymichuk, G. H., A. L. Crane, T. E. Wrynn, and M. C. O. Ferrari. 2025. Exploring the potency and replenishment of woodfrog disturbance cues, a nonspecific communication system in aquatic species. Animal Behaviour 219:123034.

Allgeier, J. E., S. J. Wenger, A. D. Rosemond, D. E. Schindler, and C. A. Layman. 2015. Metabolic theory and taxonomic identity predict nutrient recycling in a diverse food web. Proceedings of the National Academy of Sciences of the United States of America 112:E2640–E2647.

Bairos-Novak, K. R., A. L. Crane, G. H. Achtymichuk, J. Hsin, I. A. E. Rivera-Hernández, O. M. Simko, T. E. Wrynn, D. P. Chivers, and M. C. O. Ferrari. 2020. Forget the audience: tadpoles release similar disturbance cues regardless of kinship or familiarity. Behavioral Ecology and Sociobiology 74:1–10.

Bairos-Novak, K. R., M. C. O. Ferrari, and D. P. Chivers. 2019. A novel alarm signal in aquatic prey: Familiar minnows coordinate group defences against predators through chemical disturbance cues. Journal of Animal Ecology 88:1281–1290.

Bairos-Novak, K. R., M. D. Mitchell, A. L. Crane, D. P. Chivers, and M. C. O. Ferrari. 2017. Trust thy neighbour in times of trouble: background risk alters how tadpoles release and respond to disturbance cues. Proceedings of the Royal Society B: Biological Sciences 284:1–7.

Brönmark, C., and J. G. Miner. 1992. Predator-Induced Phenotypical Change in Body Morphology in Crucian Carp. Science 258:1348–1350.

Brown, G. E., T. Bongiorno, D. M. DiCapua, L. I. Ivan, and E. Roh. 2006a. Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. Canadian Journal of Zoology 84:1–8.

Brown, G. E., C. K. Elvidge, C. J. Macnaughton, I. Ramnarine, and J.-G. J. Godin. 2010. Cross-population responses to conspecific chemical alarm cues in wild Trinidadian guppies, Poecilia reticulata: evidence for local conservation of cue production. Canadian Journal of Zoology 88:139–147.

Brown, G. E., M. C. O. Ferrari, C. K. Elvidge, I. Ramnarine, and D. P. Chivers. 2013. Phenotypically plastic neophobia: a response to variable predation risk. Proceedings of the Royal Society B: Biological Sciences.

Brown, G. E., C. D. Jackson, P. H. Malka, É. Jacques, and M.-A. Couturier. 2012. Disturbance cues in freshwater prey fishes: Does urea function as an ‘early warning cue’ in juvenile convict cichlids and rainbow trout? Current Zoology 58:250–259.

Brown, G. E., C. J. Macnaughton, C. K. Elvidge, I. Ramnarine, and J.-G. J. Godin. 2009. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. Behavioral Ecology and Sociobiology 63:699–706.

Brown, G. E., J.-F. Poirier, and J. C. Adrian Jr. 2004. Assessment of local predation risk: the role of subthreshold concentrations of chemical alarm cues. Behavioral Ecology 15:810–815.

Brown, G. E., A. C. Rive, M. C. O. Ferrari, and D. P. Chivers. 2006b. The dynamic nature of antipredator behavior: prey fish integrate threat-sensitive antipredator responses within background levels of predation risk. Behavioral Ecology and Sociobiology 61:9–16.

Brusseau, A. J. P., L. E. A. Feyten, V. Groves, M. E. L. Felismino, D. Cao Van Truong, A. L. Crane, I. W. Ramnarine, and G. E. Brown. 2023. Sex and background risk influence responses to acute predation risk in Trinidadian guppies. Behavioral Ecology 34:898–906.

Chivers, D. P., G. E. Brown, and M. C. O. Ferrari. 2012. The evolution of alarm substances and disturbance cues in aquatic animals. Pages 127–139 *in* C. Brönmark and A. Hansson, editors. Chemical Ecology in Aquatic Systems.

Chivers, D. P., and R. J. F. Smith. 1994. The role of experience and chemical alarm signalling in predator recognition by fathead minnows, Pimephales promelas. Journal of Fish Biology 44:273–285.

Chivers, D. P., and R. J. F. Smith. 1998. Chemical alarm signalling in aquatic predator-prey systems: A review and prospectus. Écoscience 5:338–352.

Crane, A. L., K. R. Bairos-Novak, J. A. Goldman, and G. E. Brown. 2022. Chemical disturbance cues in aquatic systems: a review and prospectus. Ecological Monographs 92:e01487.

Crane, A. L., L. E. A. Feyten, A. A. Preagola, M. C. O. Ferrari, and G. E. Brown. 2024. Uncertainty about predation risk: a conceptual review. Biological Reviews 99:238–252.

Crane, A. L., L. E. A. Feyten, I. W. Ramnarine, and G. E. Brown. 2020. High-risk environments promote chemical disturbance signalling among socially familiar Trinidadian guppies. Oecologia 193:89–95.

Dugatkin, L. A., and J. J. Godin. 1992a. Reversal of female mate choice by copying in the guppy (Poecilia reticulata). Proceedings of the Royal Society B: Biological Sciences 249:179–184.

Dugatkin, L. A., and J.-G. J. Godin. 1992b. Prey approaching predators: a cost-benefit perspective. Annales Zoologici Fennici 29:233–252.

Dumaresq Synnott, F., A. J. P. Brusseau, A. L. Crane, and G. E. Brown. 2025. Response of juvenile male and female guppies to acute predation cues.

Ferrari, M. C. O., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology 88:698–724.

Foam, P. E., R. S. Mirza, D. P. Chivers, and G. E. Brown. 2005. Juvenile Convict Cichlids (Archocentrus nigrofasciatus) Allocate Foraging and Antipredator Behaviour in Response to Temporal Variation in Predation Risk. Behaviour 142:129–144.

Giaquinto, P. C., and G. L. Volpato. 2005. Chemical cues related to conspecific size in pintado catfish, Pseudoplatystoma coruscans. acta ethologica 8:65–69.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of Size and Temperature on Metabolic Rate. Science 293:2248–2251.

Goldman, J. A., A. L. Crane, L. E. A. Feyten, E. Collins, and G. E. Brown. 2022. Disturbance cue communication is shaped by emitter diet and receiver background risk in Trinidadian guppies. Current Zoology 68:433–440.

Goldman, J. A., I. S. Désormeaux, and G. E. Brown. 2020a. Disturbance cues as a source of risk assessment information under natural conditions. Freshwater Biology 65:981–986.

Goldman, J. A., L. E. A. Feyten, I. W. Ramnarine, and G. E. Brown. 2020b. Sender and receiver experience alters the response of fish to disturbance cues. Current Zoology 66:255–261.

Goldman, J. A., A. Singh, E. E. M. Demers, L. E. A. Feyten, and G. E. Brown. 2019. Does donor group size matter? The response of guppies (Poecilia reticulata) and convict cichlids (Amatitlania nigrofasciata) to disturbance cues from conspecific and heterospecific donors. Canadian Journal of Zoology 97:319–325.

Hazlett, B. A. 1990. Source and nature of disturbance-chemical system in crayfish. Journal of Chemical Ecology 16:2263–2275.

Helfman, G. S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. Behavioral Ecology and Sociobiology 24:47–58.

Helfman, G. S., and D. L. Winkelman. 1997. Threat Sensitivity in Bicolor Damselfish: Effects of Sociality and Body Size. Ethology 103:369–383.

Kats, L. B., and L. M. Dill. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. Écoscience 5:361–394.

Kiesecker, J. M., D. P. Chivers, A. Marco, C. Quilchano, M. T. Anderson, and A. R. Blaustein. 1999. Identification of a disturbance signal in larval red-legged frogs, Rana aurora. Animal Behaviour 57:1295–1300.

Kusch, R. C., R. S. Mirza, and D. P. Chivers. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. Behavioral Ecology and Sociobiology 55:551–555.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619–640.

Magurran, A. E. 1990. The inheritance and development of minnow anti-predator behaviour. Animal Behaviour 39:834–842.

Mirza, R. S., and D. P. Chivers. 2001. Are Chemical Alarm Cues Conserved Within Salmonid Fishes? Journal of Chemical Ecology 27:1641–1655.

Mirza, R. S., and D. P. Chivers. 2002. Brook Char (Salvelinus fontinalis) Can Differentiate Chemical Alarm Cues Produced by Different Age/Size Classes of Conspecifics. Journal of Chemical Ecology 28:555–564.

Oliveira-Cunha, P., P. B. McIntyre, V. Neres-Lima, A. Caliman, B. Moreira-Ferreira, and E. Zandonà. 2022. Body size has primacy over stoichiometric variables in nutrient excretion by a tropical stream fish community. Scientific Reports 12:1–9.

Sih, A. 2005. Predator-prey space use as an emergent outcome of a behavioral response race. Pages 240–255 *in* I. Castellanos and P. Barbosa, editors. Ecology of Predator-Prey Interactions. Oxford University Press, USA.

Swaney, W., J. Kendal, H. Capon, C. Brown, and K. N. Laland. 2001. Familiarity facilitates social learning of foraging behaviour in the guppy. Animal Behaviour 62:591–598.

Vavrek, M. A., and G. E. Brown. 2009. Threat-Sensitive Responses to Disturbance Cues in Juvenile Convict Cichlids and Rainbow Trout. Annales Zoologici Fennici 46:171–180.

Vavrek, M. A., C. K. Elvidge, R. DeCaire, B. Belland, C. D. Jackson, and G. E. Brown. 2008. Disturbance cues in freshwater prey fishes: do juvenile convict cichlids and rainbow trout respond to ammonium as an 'early warning' signal? Chemoecology 18:255–261.

Wilkie, M. P. 2002. Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. Journal of Experimental Zoology 293:284–301.

Wisenden, B. 2015. Chemical Cues That Indicate Risk of Predation. Pages 131–148 Fish Pheromones and Related Cues.

Wisenden, B., and D. Chivers. 2006. The Role of Public Chemical Information in Antipredator Behaviour.

Wisenden, B. D., D. P. Chivers, and R. J. F. Smith. 1995. Early warning in the predation sequence: A disturbance pheromone in Iowa darters (Etheostoma exile). Journal of Chemical Ecology 21:1469–1480.

Wyatt, T. D. 2010. Pheromones and signature mixtures: defining species-wide signals and variable cues for identity in both invertebrates and vertebrates. Journal of Comparative Physiology A 196:685–700.