Disturbance cues as a source of risk assessment information: Effects of sender identity and experience with risk on the response of Trinidadian guppies (*Poecilia reticulata*)

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ABSTRACT

Disturbance cues as a source of risk assessment information: Effects of sender identity and experience with risk on the response of Trinidadian guppies (*Poecilia reticulata*)

Jack A. Goldman

Chemical cues are commonly used by aquatic vertebrates to assess local threats and facilitate behavioural decision-making. Disturbance cues are 'early warning signals' common among aquatic vertebrates, that warn conspecific and heterospecific prey guild members of potential risks. Recent studies suggest that disturbance cues may play an important role in the predation sequence, providing prey with valuable information that affords the opportunity to interrupt the predation sequence and successfully avoid predator encounters. However, little is known about the informational content of disturbance cues and what factors influence the response patterns to these cues. Therefore, we assessed the role of species-specificity and prior experience with predation risk on the information content conveyed by disturbance cues. First, we tested whether receivers exhibit graded antipredator responses to cues from either 10 or 20 conspecific and heterospecific senders. Receivers increased the intensity of their antipredator response to cues from 20 compared to 10 conspecific donors. However, we found that while receivers respond to heterospecific disturbance cues, there was no effect of donor group size, suggesting that there may be a degree of species-specificity of disturbance cues. Second, using wild-caught and laboratory-reared fish, we tested whether senders and receiver responded differentially to disturbance cues if they had prior experience with predation risk. The response of high-risk fish was dependent upon the source of disturbance cue senders (high vs. low risk). However, guppies collected from a low-risk site exhibited similar responses to disturbance cues, regardless of the sender population. Combined, our results demonstrate that disturbance cues may serve as a more elaborate form of risk assessment information than previously thought, and that the response to these cues is, at least in part, dependent upon both the sender's and receiver's recent experience with predation.

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Contribution of Authors

Chapters 2 and 3 were written as manuscripts for peer-reviewed publication. JA Goldman contributed to the conception, design, collection of data, data analyses and wrote all chapters. GE Brown contributed to the conception, design, collection of data (Chapter 3 only), data analyses (Chapter 3 only) and editing. A Singh and EEM Demers contributed to the collection of data for Chapter 2. LEA Feyten contributed to the collection of data and editing for Chapters 2 and 3.

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Chapter 1: General Introduction

Predation risk is a pervasive selection pressure, shaping the behaviour of prey animals. In fact, in response to the nonconsumptive effects of predation (Preisser et al. 2005), prey are often forced to trade-off time and energy devoted to activities such as foraging, courtship and/or territorial defence in favour of increased vigilance and predator avoidance (Lima and Dill 1990). The ability of prey to successfully balance such trade-offs is heavily influenced by the availability of information and at various stages of the predation sequence. Although predation tactics vary considerably across species, predators must continually complete requisite steps in order to successfully consume prey. For prey to avoid consumption they must interrupt a predator's ability to complete the requisite steps. These steps are commonly referred to as the predation sequence (Figure 1.1) and generally consists of: (1) encounter or detection stage, followed by (2) attack, (3) capture and (4) death (Lima and Dill 1990). Risk assessment cues regarding local predators are a source of public information that allow prey to assess the characteristics of a threat (e.g. predator location, density). The acquisition of public information should allow prey to make appropriate behavioural decisions (McNamara and Dall 2010) and potentially increase survival during encounters with predators (Wisenden et al. 1999; Mizra and Chivers 2001; Crane et al. 2018). Prey gather public information that conveys local predation risk using multiple sensory modalities, of which visual and chemical cues are the most well studied (Hartman and Abrahams 2000).

The predation sequence can be simplified into two phases: pre-attack and post-attack (Figure 1.1, Bateman et al. 2014; Wisenden et al. 2015). At each phase, the cost and benefits of acquiring information and acting on that information differs considerably. Pre-attack information can be beneficial by affording prey the opportunity to make fine-scale behavioural adjustments early in the predation sequence. These early fine-scale adjustments help prey avoid predator encounters (e.g. effective avoidance, Lima and Dill 1990) or the consequences of responding too late (Ludwig and Rowe 1990). For example, in response to predator-related chemical and visual stimuli, prey select habitats to avoid predators (Idler et al. 1956; Werner et al. 1983; Mathis and Smith 1992). Terrestrial prey move towards open habitats to increase vigilance against ambush predators (Valeix et al. 2009). In contrast, aquatic prey move to shallower habitats (He and Kitchell 1990) or to habitats with dense cover (Werner et al. 1983). However, these fine-scale adjustments are generally short-term (Metcalfe et al. 1987; Gotceitas and Colgan 1990) as long-term adjustments would be costly, leading to lost opportunities, such as foraging (Vandolin 2006) or courtship (Michelangeli and Wong 2014). Therefore, prey may return to riskier areas but are more vigilant towards future threats. Consequently, pre-attack information can also prime the receivers (Ferrari et al. 2008; Zaguri et al. 2018), which will influence how prey respond to future information later in the predation sequence, such as responding with increased accuracy or intensity (Ferrari et al. 2008). For example, desert isopods (*Hemilepistus reaumuri*) approaching scorpion mounds respond defensively only when chemical odours are accompanied by visual cues (Zaguri et al. 2018).

Although beneficial, responding to pre-attack information can be costly because it is not as reliable, as prey are aware that there is a potential threat, but have not identified the specific threat. Therefore, it is possible that prey are responding to ecologically irrelevant threats leading to maladaptive behavioural decisions. However, the cost of making a maladaptive behavioural decision to information in the pre-attack phase is lower than making a ‘bad’ decision when responding to post-attack information. Prey failing to respond later in the predation sequence would experience higher capture and mortality rates compared to prey failing to response earlier in the predation sequence. Conversely, the greater intensity of the antipredator behaviours that prey exhibit later in the predation sequence (e.g. evasive maneuvers versus vigilance, Godin 1997) can lead to greater opportunity costs (Hemmi and Pfeil 2010). Another potential cost associated with post-attack information is that prey may have to learn that a threat is risky, whereas, with pre-attack information, prey fish do not have to learn that a threat is risky in order to respond. Although some species exhibit innate predator recognition (e.g. birds, Veen et al. 2000; fish, Berejikian et al. 2003, Hawkins et al. 2004; mammals, Fendt 2006), most species must learn to recognize a predator. Learning to recognize predators can be costly as prey must survive an initial encounter with a predator. However, from post-attack information, prey are better able to discern the severity of the threat (i.e. identity, density, where they are and when) and whether the predator is actively foraging. Pre-attack, on the other hand, provides prey with the information that there may be a predator present and they should be cautious or wary of a potential threat. Consequently, post-attack information generally elicits drastic evasive maneuvers and antipredator behaviours as it signals to prey that ‘something is here’.

The sources of information available to prey as both pre-attack and post-attack information span multiple sensory modalities. Among aquatic species, prey rely on a suite of cues to assess predation, including chemosensory assessment of risk, which is one of the most widespread information sources in aquatic environments (Kats and Dill 1998; Ferrari et al. 2010). For example, prey fish trade off foraging and other related activities for antipredator behaviours when exposed to chemosensory cues that indicate risk (Brown and Cowan 2000). However, there are numerous chemical cues that convey information about a potential predation threat (Ferrari et al. 2010), and their availability as sources of risk assessment information varies across the predation sequence (Wisenden 2015). To date, studies have primarily focused on post-attack sources of chemosensory information (e.g. damage-released alarm cues, Smith 1992; Chivers and Smith 1998). Therefore, sources of pre-attack chemosensory information are poorly understood. However, due to the increase in risk in the later stages of the predation sequence it would be more advantageous for prey to detect a potential predator threat at the earliest possible point of the predation sequence (Lima and Dill 1990; Endler et al. 1991). Success can be defined as a function of time at first detection, because the later a prey detects a predator, the less time a prey has to decide as whether to engage in antipredator behaviours. Thus, early detection allows prey to avoid predation risk sooner, as they engage in antipredator tactics which interrupt the predation sequence. This increases a prey's ability to make appropriate behavioural trade-offs to maximize fitness and their chance to escape (Endler et al. 1991). Therefore, a better understanding of sources of chemosensory information available early in the predation sequence will allow us to understand what shapes predator-prey interactions.

In the pre-attack phase, chemical information can come directly from the predator itself or from chemical cues emanating from conspecific and/or heterospecific prey-guild members (Berijikian et al. 2003; Vavrek and Brown 2009). As with the well-studied damage-released alarm cue system, predator odours have received more attention, while cues emanating from prey-guild members early in the predation sequence have received less attention (reviewed in Ferrari et al. 2010). The only known pre-attack chemical cue emanating from prey-guild members are disturbance cues. Disturbance cues are released from chemically and physically stressed or disturbed prey that warn both conspecific and heterospecific prey-guild members of potential risk (Wisenden et al 1995; Wisenden 2015; Abreu et al. 2016). These cues are commonly referred to as 'early-warning signals' (Wisenden et al. 1995) and allow prey to increase vigilance and prepare antipredator tactics prior to a predator encounter. Although they are referred to as 'early-warning signals' there is no evidence to suggest that they are actual signals. To be considered signals, disturbance cues must demonstrate a clear benefit to cue senders (Endler 1993) and it has only been shown that they benefit receivers (Wisenden et al. 1995; Vavrek et al. 2008; Vavrek and Brown 2009; Brown et al. 2012; Bairos-Novak et al. 2017). A clear receiver benefit is a pre-requisite for a cue (Endler 1993), and thus disturbance cues meet this definition. Disturbance cues are widely distributed across phyla, having been identified in a diverse range of species (Table 1.1), including arthropods (Hazlett 1985; Hazlett 1990a; Siepielski et al. 2016), echinoderms (Nishizaki and Ackerman 2005), amphibians (Kiesecker et al. 1999; Gonzalo et al. 2010; Crane and Mathis 2011), annelids (Ressler 1968; Hazlett et al. 1990b; Crane et al. 2013), freshwater fish (Wisenden et al. 1995; Bett et al. 2016; Goldman et al. 2019) and marine fish (Manassa et al. 2013; Fulton et al. 2017).

Due to the early-warning nature of disturbance cues within the predation sequence and the seemingly innate response by cue receivers, these cues have recently received increasing attention and it has been proposed that these cues may play a more important role in predator-prey interactions that previously thought (Wisenden et al. 2015; Bairos-Novak et al. 2017, 2018). Previous studies have demonstrated that prey exposed to disturbance cues early in the predation sequence increase the survival rate of prey during a staged predator encounter (Berejikian et al. 1999; Mirza and Chivers 2002; Gazdewich and Chivers 2003). Likewise, disturbance cues are implicated in learning, as they can 'prime' prey to respond to additional sources of information. For example, juvenile rainbow trout (*Oncorhynchus mykiss*) pre-exposed to disturbance cues prior to alarm cues learn faster and subsequently respond more intensely than individuals that were not pre-exposed to disturbance cues (Ferrari et al. 2009). Therefore, disturbance cues are sources of risk assessment information that can be used as a vehicle to assess potential predation risk at the earliest possible stages of the predation sequence without known costs associated with learning (Goldman et al. unpublished data).

Disturbance cues are thought to be metabolic by-products released in nitrogenous waste in urine of arthropods, fish and some amphibians (Kiesecker et al. 1999; Zulandt Schneider and Moore 2000; Vavrek et al. 2008; Brown et al. 2012), across the gill epithelium in fishes (Vavrek et al. 2008; Brown et al. 2012) and secreted through the skin in some amphibians (e.g. salamanders, Crane and Mathis 2011; Crane et al. 2013). Zulandt Schneider and Moore (2002) showed that red swamp crayfish (*Procambarus clarkia*) respond to urine of stressed conspecifics but not from cues collected from the gill epithelium. However, the active component is not yet known, and it seems as if it may differ across phyla. In amphibians and arthropods, early studies suggest that the primary chemical compound is ammonia (Hazlett et al. 1990; Kiesecker et al. 1999). For example, when disturbed, red-legged tadpoles (*Rana aurora*) increase ammonium excretion compared to undisturbed individuals, and, in subsequent tests, tadpoles exposed to low concentrations of ammonium increased their antipredator behaviours (Kiesecker et al. 1999). Conversely, in fish, the primary chemical compound is thought to be urea (Brown et al. 2012). When disturbed, juvenile convict cichlids (*Amatitlania nigrofasciata*) and rainbow trout (*Oncorhynchus mykiss*) do not release ammonia, and when exposed to ammonia at different concentrations, individuals do not increase antipredator behaviours (Vavrek et al. 2008). However, cichlids and rainbow trout respond with similar intensity to urea as they do to disturbance cues collected from stressed conspecifics (Brown et al. 2012). It has also been argued that since disturbance cues can be evoked from stressful encounters (e.g. physical chase), cortisol (a stress hormone) may be an active component of these cues. Cortisol can be released in nitrogenous wastes or across the gill epithelium, therefore it fits within the presumed release pathway. However, when exposed to water-borne cortisol, juvenile pink salmon (*Oncorhynchus gorbuscha*) and sockeye salmon (*O. nerka*) do not respond (Bett et al. 2016). Understanding the function of these cues in eliciting responses in receivers and the information it can convey will allow researchers to identify potential chemicals and pathways to investigate in future studies.

General goals

A goal of this thesis was to investigate how prey fish respond to disturbance cues and what factors influence perception of these cues by receivers. We investigated the response of Trinidadian guppies (*Poecilia retuculata*) to disturbance cues and the role of species-specificity and prior experience with risk in shaping how guppies respond to these cues. First, we investigated the role of species specificity and donor group size on the threat-sensitive responses to disturbance cues. If the quantity and quality of information conveyed by disturbance cues differs based on whether cue senders and receivers are conspecifics or heterospecifics, this would help us to better understand whether these cues are in fact generalized cues as previously suggested, or if they are species-specific. Likewise, if disturbance cues are to a degree species-specific, we would assume that the quality and quantity of information differs based on whether the cue comes from a conspecific or heterospecific sender. Information from heterospecific donors should be considered less reliable. It would be maladaptive for individuals to respond to heterospecific disturbance cues with similar intensity to disturbance cues from conspecifics. A predator threatening heterospecifics may specialize on that species and not pose a significant threat to others. Subsequently, cues from conspecifics are probably more reliable as a predator that predates upon the same species would pose more risk than predators that predate upon heterospecifics, which would pose less risk. Therefore, if a guppy were to respond to disturbance cues from heterospecifics with increased intensity they could be responding to an irrelevant threat and incur lost opportunity costs. Similar response patterns have been observed in guppies exposed to heterospecifics alarm cues. For example, guppies do not respond to heterospecific alarm cues from Hart's rivulus (*Rivulus harti*) if the donor is large, but do respond to cues from smaller donors (Elvidge and Brown 2015). This suggests that predators posing a threat to larger rivulus do not pose a threat to smaller prey (i.e. guppies). We predict that disturbance cues should present a degree of species-specificity, as individuals should not respond with increased intensity to heterospecific disturbance cues from 20 compared to 10 donors. We also predict that if these cues are species-specific, it would be more likely that disturbance cues could convey more complex information about the sender (i.e. contain a great number of pieces of information, Freeberg et al. 2012).

Second, we examined whether personal information influences how guppies respond to disturbance cues. We observed the effects of previous experience with risk of both senders and receivers and how they influenced the response to disturbance cues. In the presence of variable information, there should be a benefit for prey to exhibit different intensities of antipredator responses. Responding to differing information with the same intensity should lead to an increase in maladaptive behavioural decisions and enhance potential fitness costs. Therefore, in response to differences in quality and/or quantity of information that may arise based on previous experience, we predict that prey would exhibit varying intensities of antipredator responses. For example, if the quantity and/or quality of information conveyed by disturbance cues differs based on the experience of senders, then we expect receivers to benefit by responding with increased intensity to cues from high-risk vs. low-risk senders.

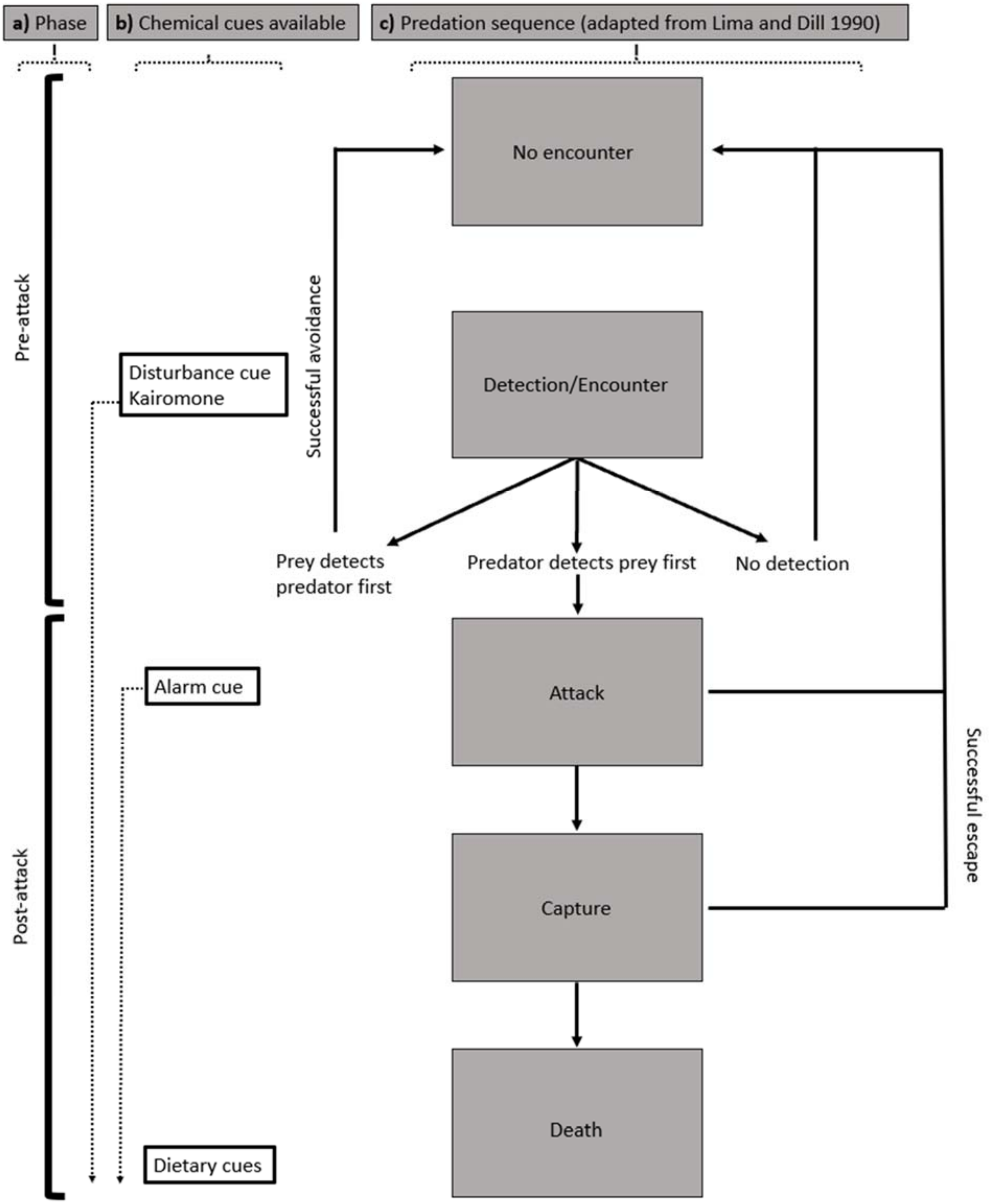

Figure 1.1: The predation sequence (adapted from Lima and Dill 1990) and chemosensory risk assessment cues (adapted from Wisenden 2015) available across the two distinct phases of the predation sequence: pre-attack and post-attack (column A). The four chemosensory cues (column

B) are disturbance cues and kairomones in pre-attack phase, and alarm and dietary cues in the post-attack phase. The phases of the predation sequence (column C) proceed from ‘no encounter’ to ‘death’. This graphic presentation highlights the requisite steps taken by predators to consume prey and the decisions made by prey to avoid predators that can be informed through the availability of specific cues at different stages and phases of the predation sequence.

Table 1.1: Species where the presence of disturbance cues has been confirmed.

Species	Response	Reference
Phylum Annelida		
Common earthworm *Lumbercus terrestris*	Sharp increase in latency to escape	Ressler 1968
Phylum Arthropoda		
Hermit crab *Calcinus laevimanus*	Increased watchful behaviour	Hazlett et al. 1985
Common yabby *Cherax destructor*	Adopted a raised posture	Hazlett and Lawler 2010
Murray crayfish *Euastacus armatus*	Increased in locomotion	Hazlett and Lawler 2010
Northern crayfish *Oronectes virilis*	Increased watchful behaviour	Hazlett et al. 1990
Signal crayfish *Pacificatus leniusculus*	Adopted a neutral posture (known antipredator behaviour)	Stebbing et al. 2010
Red swamp crayfish *Procambus clarkia*	Moved faster and farther away from signal source	Zulandt Schneider and Moore 2000
Damselfly *Ishnura cervula*	Missed foraging bouts	Siepielski et al. 2016
Mayfly *Paraleptophlebia adoptiva*	Increased avoidance of predator (swimming and crawling)	Ode and Wissinger 1993
Phylum Echinodermata		
Red sea urchin *Mesocentrotus francisanus*	Juveniles moved closer to adults for protection	Nishizaki and Ackerman 2005
Phylum Chordata		
Class Amphibia		
Wood frog *Lithobates sylvaticus*	Reduction in the number of lines crossed	Bairos-Novak et al. 2017, 2019a
Iberian green frog *Pelophylax perezi*	Reduction in the number of lines crossed	Gonzalo et al. 2010
Northern red-legged frog *Rana aurora*	Decreased activity, intensified the use of shelters and moved away from sender	Kiesecker et al. 1999
Splash-back poison frog *Ranitomeya variabilis*	Avoidance of area labelled with cue	Schulte and Rössler 2013
Hellbender *Cryptobranchus alleganiensis*	Decreased time spent moving	Crane and Mathis 2011

Class Amphibia (continued)		
Mountain dusky salamander *Desmognathus ochrophaeus*	Increased nose tapping behaviour (antipredator response)	Johnson and Sullivan 2014
Ozark zigzag salamander *Plethodon angusticlavius*	Reduced foraging rate	Watson et al 2004
Class Actinopterygii		
Convict cichlid *Amatitlania nigrofasciata*	Increased spatial occupation behaviour, spent less time moving, altered foraging rate	Jordão 2004; Vavrek et al. 2008; Vavrek and Brown 2009; Brown et al. 2012; Goldman et al. 2019
Orange clownfish *Amphiprion percula*	Reducted movement and foraging	Manassa et al. 2013
Slimy sculpin *Cottus cognatus*	Reduction in activity and movement to shelter	Bryer et al. 2001
Zebrafish *Danio rerio*	Higher amount of whole body cortisol level, avoided chemical cue source	Oliviera et al. 2013; Barcellos et al. 2014; Abreu et al. 2016
White seabream or sargo *Diplodus sargus*	Higher cortisol plasma levels	Olivotto et al. 2002
Iowa darter *Etheostoma exile*	Suppressed exploratory behaviour and exhibited alert posture	Wisenden et al. 1995
Rainbow trout *Oncorhynchus mykiss*	Higher cortisol plasma levels, intensification of response to secondary chemical cue, decreased foraging rate and time moving	Toa et al. 2004; Ferrari et al. 2008; Vavrek et al. 2008; Vavrek and Brown 2009; Brown et al. 2012
Sockeye salmon *Oncorhynchus nerka*	Avoided area labelled with cue	Bett et al. 2016
Gulf toadfish *Opsanus beta*	Pulsatile urea excretion when exposed to waterborne conspecific cues (antipredator behaviour)	Fulton et al. 2017
Nile tilapia *Oreochromis niloticus*	Higher amount of whole body cortisol	Barcellos et al. 2011
Small-scaled pacu *Piaractus mesopotamicus*	Moved away from cue source, altered dispersion	Jordão and Volpato 2000
Fathead minnow *Pimephales promelas*	Intensification of shoaling, dashing and freezing	Bairos-Novak et al. 2019b
Trinidadian guppy *Poecilia reticulate*	Increased shoaling and decreased area use, increased latency to inspect a model predator	Goldman et al. 2019; Goldman (submitted)

Class Actinopterygii (continued)		
Spotted sorubim *Pseudoplatystoma corruscans*	Stopped foraging and increased time spent swimming	Giaquinto and Hoffmann 2012
Jundiá catfish *Rhamdia quelen*	Spent more time in shelter and increased spatial occupation, higher amount of whole body cortisol	Barcellos et al. 2011; Vogel et al. 2017
Brook trout *Salvelinus fontinalis*	Avoided pike and increased survival during staged encounters	Mirza and Chivers 2002

Chapter 2: Does donor group size matter? The response of Trinidadian guppies and convict cichlids to disturbance cues from conspecific and heterospecific donors

Abstract

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

Introduction

Prey are often forced to trade off time and energy devoted to activities, such as foraging, courtship and territorial defence, in favour of increased vigilance and predator avoidance (Lima and Dill 1990). Publicly available information (risk assessment cues) regarding local predators should allow prey to better balance these trade-offs (McNamara and Dall 2010) and potentially increase survival during encounters with predators (Wisenden et al. 1999; Mirza and Chivers 2001; Crane et al. 2018). However, risk assessment cues may vary in reliability (Magrath et al. 2009), potentially restricting the ability to make correct choices (Dall et al. 2005) and leading to inappropriate or costly behavioural decisions.

Within aquatic ecosystems, prey are known to rely on at least two main forms of chemosensory information to assess local predation threats: damage-released chemical alarm cues and disturbance cues (Ferrari et al. 2010; Chivers et al. 2012). The well-studied damage-released alarm cues are released from the skin (or visceral tissue) of a wide range of taxonomically diverse aquatic prey following mechanical damage, as would occur during a predator attack (Chivers and Smith 1998; Chivers et al. 2012). Alarm cues are reliable and honest indicators of risk (Chivers et al. 2012) and can elicit dramatic short-term increases in predator-avoidance behaviours (Ferrari et al. 2010; Brown et al. 2011; Chivers et al. 2012). Disturbance cues are thought to be metabolic by-products that are released in the urine or across the gill epithelium (Kiesecker et al. 1999; Vavrek et al. 2008; Brown et al. 2012) by aquatic vertebrates when stressed or disturbed (Wisenden et al. 1995; Kiesecker et al. 1999; Abreu et al. 2016). When detected by nearby conspecific and heterospecific prey, disturbance cues may function as an ‘early warning’ cue of nearby predation threats (Wisenden et al. 1995; Kiesecker et al. 1999). Evidence suggests that disturbance cues play a vital role in the predation sequence, providing preliminary information used in the preparation of antipredator responses (Wisenden 2000, 2014). Like the damage-released alarm cues, disturbance cues have been reported in a wide variety of taxa (Hazlett 1985; Bryer et al. 2001; Jordão 2004; Nishizaki and Ackerman 2005; Gonzalo et al. 2010; Bett et al. 2016; Bairos-Novak et al. 2017).

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

Although the effect of concentration has been thoroughly investigated, little is known about how donor group size (the number of individuals that are emitting a cue) influences the response patterns of receivers independent of an increase in concentration. The greater the number of donors releasing a cue could lead to an increase in the quality or quantity of information. Similarly, there may be interacting effects of both the donor and receiver as receivers may be able to detect donor group size either through the qualitative or quantitative differences in the cue. We may then expect that the response pattern to changes in donor group size would be analogous to the concentration-dependent response pattern when exposed to conspecific and heterospecific disturbance cues. However, information from heterospecifics may differ in quality and should be considered less reliable. It would be maladaptive for individuals to respond to disturbance cues from heterospecifics with the same intensity as conspecifics as the type of predator threatening heterospecifics may be specialized and pose no threat. Therefore, an increase in donor group size may not change the reliability of information and we may not observe an increased response. Alternatively, when responding to cues from conspecifics, there may be additional information available based on the donor group size. Therefore, we may observe an increased response to conspecific disturbance cues when donor group size is increased from 10 to 20 donors.

We had three main goals in this study. First, we sought to confirm the presence of disturbance cues in guppies. Second, we tested if donor group size determines the strength of the behavioural response in guppies. Third, we tested if the cross-species response to disturbance cues is specific enough to observe an effect of donor group size. We exposed shoals of guppies to tank water from conspecifics collected before and after a simulated predator chase and tested for both a baseline response and an effect of donor group size on the intensity of the response. Given the likelihood that disturbance cues are not species-specific (Vavrek et al. 2008), there could be a cross-species effect of donor group size on antipredator responses; Vavrek and Brown (2009) have shown that, when controlling for concentration, prey fish elicited antipredator responses to disturbance cues collected from 10 donors but not five donors. To test this, we exposed independent shoals of guppies and convict cichlids to both conspecific and heterospecific disturbance cues at different donor group sizes.

Materials and Methods

Test fish

We used guppies (females) and convict cichlids (males and females) from our laboratory stock populations. We used these two species because they are phylogenetically distant, allowing us to avoid possible confounding effects of using closely-related species, and also because the predator avoidance behaviour of both species has been well studied (Brown and Godin 1999; Brown et al. 2006, 2009, 2010, 2013; Joyce et al. 2016; Bayani et al. 2017; Goldman et al. 2019). Although guppies and convict cichlids do not naturally co-exist in Trinidad, guppies are often sympatric with confamilial cichlids (e.g. blue acara, *Aequidens pulcher*; brown coscarob, *Cichlasoma taenia*). Prior to testing, guppies were held in 110 L glass aquaria (water temperature 26-27 °C) and cichlids in 37 L glass aquaria (22–23 °C); each aquarium was filled with continuously-filtered dechlorinated water and had a gravel substrate and artificial vegetation. Fish were exposed to a 12:12 h light:dark cycle. Guppies and cichlids were fed with commercial tropical fish flake food (Nutrafin) twice a day.

Stimulus preparation

To collect disturbance cues and water of undisturbed shoals, we placed either 10 fish in a 9.5 L tank (filled with 4.7 L of dechlorinated tap water) or 20 fish in a 35 L tank (filled with 9.5 L of dechlorinated tap water). This was done to ensure that the relative concentration of disturbance cue was not affected (i.e. there was a similar donor to water volume ratio) and thus we controlled for concentration. Two tank sizes (9.5 and 35 L) ensured that fish had enough room to move around and were not stressed as a result of the water level. Fish were placed in tanks 24 h prior to stimulus collection to allow an adequate time to acclimate; tanks were wrapped in black garbage bags to ensure that the fish would not be disturbed prior to stimulus collection. Air stones were placed in the rear-right corner of each tank, which were removed 1 h prior to stimulus collection. Before collecting the disturbance cue, we removed 125 mL of tank water to use as the control (i.e. water from undisturbed conspecifics). To collect disturbance cues (experimental variable), we passed a realistic predator model (15 cm in length and connected to a glass rod) through the tank for 60 s. It has been demonstrated that a simulated predator chase is required to observe antipredator responses to water from disturbed conspecifics (Abreu et al. 2016). We were careful not to contact the fish to avoid the introduction of damage-released alarm cues. After passing the model through the tank, we waited 60 s and carefully mixed the water around the tank and removed 125 mL of tank water to be used as a disturbance cue. This procedure has been used by others to successfully collect disturbance cues (Vavrek and Brown 2009; Brown et al. 2012). We collected all cues immediately before the experiment and used them within 1 h of collection, to ensure that the cue did not degrade over time (Vavrek and Brown 2009; Brown et al. 2012).

Test tanks

For both experiments, we used 37 L glass aquaria filled with 20 L of dechlorinated water as test tanks. A gravel substrate covered the bottom of our test tanks. Instead of continuously filtering water through the tanks, we placed an air stone against the back of the tank. Directly above the air stone we attached air tubing to the rear of the tank. This tubing was 1.5 m in length and was used to inject the stimulus into the tank (to disrupt the fish as little as possible). We wrapped each tank in blue opaque plastic to eliminate visual communication between fish in neighbouring tanks. This setup has previously been used in other experiments measuring the antipredator behaviour of both guppies (Brown et al. 2009, 2010, 2013) and cichlids (Brown et al. 2006; Joyce et al. 2016). To quantify area use, we drew three black horizontal lines on the exterior of the tanks, which divided the tank into three vertical sections. This is a well documented method of quantifying area use (Brown et al. 2006, 2009, 2010). To allow an adequate amount of time to acclimate, we placed focal fish into test tanks 1 h prior to testing. We tested guppies in shoals of three as this is a common method used to accurately quantify shoaling index and area use as smaller shoals tend to exhibit stereotypic stress responses (Brown et al. 2009, 2010, 2013). We tested cichlids in pairs, as prior experiments show that cichlids tested alone are not active (Brown et al. 2006). For both guppies and cichlids, we selected donors and test fish randomly from the same stock population. After testing, we removed fish and placed them back into the stock population. We used donors and focal fish only once. We fed the focal fish ad libitum up to 1 h before testing began. Therefore, food remained on the substrate during observations, allowing the quantification of foraging attempts without dispensing additional food (Brown et al. 2006).

Experimental protocol

Behavioural testing consisted of two observation periods: 5 min pre-stimulus and 5 min post-stimulus. Immediately following the pre-stimulus observation period, we withdrew 60 mL of tank water through the stimulus injection tube and discarded it to ensure no residual cues remained in the injection tube. We removed and retained an additional 60 mL to be used to slowly flush the stimulus into the tanks. We then introduced 10 mL of either disturbance cue (experimental variable) or undisturbed water (control). The stimulus was then slowly flushed into the test tank using the 60 mL of retained tank water. Post-stimulus injection observations began once the stimulus was fully introduced into the observation (test) tank. This protocol follows previous studies and has been used to accurately measure antipredator responses in guppies (Brown et al. 2009, 2010, 2013) and cichlids (Brown et al. 2006; Joyce et al. 2016).

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

The goal was to establish if guppies produce and respond to conspecific disturbance cues and to determine whether donor group size influences the form or intensity of the observed response pattern. We collected disturbance cues and the water from undisturbed conspecifics from groups of 10 or 20 fish (described above). Mean total mass (± SD) of donor group size was 4.61 ± 0.15 g (10 donors) and 10.08 ± 0.13 g (20 donors).

Behavioural observations consisted of paired 5 min pre-stimulus and 5 min post-stimulus injection observations and were conducted in real time. During both the pre-stimulus and post-stimulus observation periods, we scored shoaling index and area use. An increase in shoaling index and a reduction in area use (within shoals of three) is a reliable indicator of increased predator avoidance in guppies (Brown and Godin 1999; Brown et al. 2009, 2010, 2013, 2018). We scored shoaling index and area use every 15 s throughout the 5 min pre-stimulus and 5 min post-stimulus observation periods. Shoaling index was recorded as the position of each test fish in relation to one another. Scores ranged from 1 to 3 based on the proximity of the individuals being within one body length of each other (1 = no fish within one body length of each other; 3 = all fish within one body length of each other). Area-use scores ranged from 3 (all fish in the bottom third of the tank) to 9 (all fish in the top third of the tank). Both area-use score and shoaling-index score were summed and divided by the number of observations to obtain a mean value. Mean size (± SD) of test fish was 2.29 ± 0.17 cm standard length at the time of testing. Using the mean scores, we calculated the change in both shoaling index and area use (post-stimulus observation minus pre-stimulus observation) as difference scores. Mean difference scores have been used as an accurate metric of antipredator response (Brown et al. 2006, 2009, 2010, 2013).

We analyzed data using a linear mixed effects model (with mean scores used as response variables, stimulus treatment and donor group size as main effects, and tank as random effect) using the package nlme (Pinheiro et al. 2019) in R version 3.6.0 (R Core Team 2019). Figures were made using KaleidaGraph version 4.1.3.

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

The goal was to determine whether the response to heterospecific disturbance cues elicits a greater response as a result of exposure to different donor group sizes. We collected disturbance cues from heterospecifics and conspecifics, as well as the water from undisturbed heterospecifics and conspecifics from groups of 10 or 20 donors. Mean mass (± SD) of groups of 10 donors were 5.20 ± 0.36 g for guppies and 5.69 ± 0.95 g for cichlids; and for groups of 20 donors, 10.47 ± 0.89 g for guppies and 10.44 ± 0.87 g for cichlids. For guppies, we scored shoaling index and area use during pre-stimulus and post-stimulus observation periods (see above). For cichlids, we recorded time spent moving and rates of foraging and aggression. We measured time spent moving on one individual. For foraging, we measured the attempts per observation period, which we defined as pecking at the substrate at an angle greater than 45° relative to the gravel substrate. We quantified aggression as the number of times an individual bit another individual. Both foraging rate and aggression rate were quantified for all individuals. A decrease in time moving, aggression rate, and foraging rate (within shoals of two) is a reliable indicator of increased predator avoidance behaviour in cichlids (Brown et al. 2006; Vavrek et al. 2008; Vavrek and Brown 2009; Joyce et al. 2016; Bayani et al. 2017). At the time of testing, mean length (± SD) of guppies was 2.31 ± 0.19 cm and 2.18 ± 0.21 cm for cichlids. Similar to Experiment 1, we calculated the change in behavioural measures by subtracting the post-stimulus observation from the pre-stimulus observation and we used the difference scores as response variables in statistical analyses.

We analyzed data using a linear mixed effects model (with mean scores used as response variables, stimulus treatment and donor group size as main effects, and tank as random effect) using the package nlme (Pinheiro et al. 2019) in R version 3.6.0 (R Core Team 2019). Whether the donor was a conspecific or heterospecific was not used as a main effect because the data were subset into conspecific or heterospecific donors to simplify analyses. Figures were made using KaleidaGraph version 4.1.3.

Results

Experiment 1

There was a significant main effect of stimulus type on both the shoaling index ($F_{1,34}$ = 81.81, $P < 0.0001$; Fig. 2.1A) and area use ($F_{1,34}$ = 39.67, $P < 0.0001$; Fig. 2.1B) of guppies. Guppies exhibited antipredator behaviours by increasing shoaling index and decreasing area use when exposed to disturbance cues compared with water from undisturbed donors. Donor group size had a significant main effect on shoaling index, as we observed an increase in shoaling index with cues from 20 donors compared with 10 donors (shoaling index, $F_{1,34}$ = 17.64, $P = 0.0002$; Fig. 2.1A). However, there was no significant main effect of donor group size on area use ($F_{1,34}$ = 0.229, $P = 0.6351$; Fig. 2.1B). There was a significant interaction between the presence of disturbance cues and the number of donors for both shoaling index ($F_{1,34}$ = 7.52, $P = 0.0096$; Fig. 2.1A) and area use ($F_{1,34}$ = 5.130, $P = 0.03$; Fig. 2.1B).

Experiment 2

Guppy receivers significantly increased shoaling ($F_{1,56}$ = 112.40, $P < 0.0001$; Fig. 2.2A) and decreased area use ($F_{1, 56}$ = 62.37, $P < 0.0001$; Fig. 2.2B) when exposed to conspecific disturbance cues. Cichlid receivers significantly decreased time moving ($F_{1,56}$ = 52.65, $P < 0.0001$; Fig. 2.3A), aggression ($F_{1,56}$ = 11.72, $P = 0.0012$; Fig. 2.3B) and foraging rate ($F_{1,56}$ =

31.27, $P < 0.0001$; Fig. 2.3C) when exposed to conspecific disturbance cues. These results are indicative of an antipredator response. When exposed to conspecific disturbance cues from 10 compared to 20 donors, guppies significantly increased shoaling ($F_{1,56} = 7.08$, $P = 0.010$; Fig. 2.2A), but did not decrease area use ($F_{1,56} = 1.06$, $P = 0.305$; Fig. 2.2B). There was an interacting effect of donor group size times exposure to conspecific disturbance cues for both shoaling ($F_{1,56} = 16.49$, $P = 0.0002$; Fig. 2.2A) and area use ($F_{1,56} = 13.43$, $P = 0.0005$; Fig. 2.2B). Similarly, in response to changes in donor group size, cichlids decreased time moving ($F_{1,56} = 9.93$, $P = 0.003$; Fig. 2.3A) and foraging rate ($F_{1,56} = 6.15$, $P = 0.016$; Fig. 2.3C), but not aggression ($F_{1,56} = 2.29$, $P = 0.135$; Fig. 2.3B). We observed an interacting effect of donor group size times exposure to conspecific disturbance cues on time moving ($F_{1,56} = 7.74$, $P = 0.007$; Fig. 2.3A), but not aggression ($F_{1,56} = 0.21$, $P = 0.646$; Fig. 2.3B) and foraging rate ($F_{1,56} = 0.02$, $P = 0.88$; Fig. 2.3C).

In response to heterospecific donors, guppies significantly increased shoaling ($F_{1, 56} = 30.86$, $P < 0.0001$; Fig. 2.2A) and decreased area use ($F_{1,56} = 102.21$ $P < 0.0001$; Fig. 2.2B). However, when exposed to heterospecific disturbance cues from 10 compared to 20 donors, there was no significant change in shoaling ($F_{1,56} = 0.145$, $P = 0.70$; Fig. 2.2A) and area use ($F_{1,56} = 0.52$, $P = 0.472$; Fig. 2.2B). We did observe an interacting effect of donor group size times exposure to heterospecific disturbance cues on area use ($F_{1,56} = 6.22$, $P = 0.015$; Fig. 2.2B), but not shoaling ($F_{1,56} = 1.56$, $P = 0.215$; Fig. 2.2A). However, a post hoc analysis comparing the change in area use in response to disturbance cues from 10 compared to 20 heterospecific donors revealed no significant effect of group size ($F_{1,28} = 3.59$, $P = 0.068$; Fig. 2.2B). Similarly, in response to heterospecific donors, cichlids significantly decreased time moving ($F_{1,56} = 9.80$, $P = 0.002$; Fig. 2.3A) and aggression ($F_{1,56} = 6.76$, $P = 0.018$; Fig. 2.3B), but did not significantly decrease foraging rate ($F_{1,56} = 0.54$, $P = 0.462$; Fig. 2.3C). When exposed to heterospecific disturbance cues from 10 compared to 20 donors, cichlids did not significantly decrease time moving ($F_{1,56} = 0.79$, $P = 0.375$; Fig. 2.3A), aggression ($F_{1,56} = 0.20$, $P = 0.604$; Fig. 2.3B) and foraging rate ($F_{1,56} = 0.23$, $P = 0.631$; Fig. 2.3C). We did not observe interacting effects of donor group size and exposure to heterospecific disturbance cues on time moving ($F_{1,56} = 0.04$, $P = 0.826$; Fig. 2.3A), aggression ($F_{1,56} = 1.63$, $P = 0.206$; Fig. 2.3B) and foraging rate ($F_{1,56} = 0.54$, $P = 0.462$; Fig. 2.3C).

Discussion

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

Under natural conditions, shoal sizes of guppies vary greatly (Magurran and Seghers 1994). Thus, when disturbed, the density of donors may differ. Our results provide evidence that number of donors of early-warning disturbance cues influences the overall antipredator response of individuals. Similar results have been shown for juvenile convict cichlids and rainbow trout; Vavrek and Brown (2009) reported antipredator responses to disturbance cues collected from 10 but not 5 donors for both species independent of concentration. Our results show that guppies exhibited a marked increase in antipredator responses when exposed to cues from 20 versus 10 donors. Previous studies have shown analogous response patterns for damage-released chemical alarm cues and learned predator odours (Jachner and Rydz 2002; Dupuch et al. 2004; Zhao et al. 2006). However, these response patterns were concentration dependent. For example, the intensity of antipredator responses of guppies is dependent on the concentrations of alarm cues, as response intensity increased commensurately with concentration (Brown et al. 2009). Likewise, the adjustment of antipredator behaviours in northern redbelly dace (*Phoxinus eos*) is correlated to the concentration of chemical alarm cues (Dupuch et al. 2004).

Since our experiment controlled for concentration, our results suggest that, independent of concentration, the number of donors that release a cue influences the response patterns of receivers. There are two possible explanations as to why we observed a difference of donor group size in response to conspecific disturbance cues. First, donors may release qualitatively or quantitatively different cues. Larger groups perceive risk differently than smaller groups due to the safety advantages of larger group sizes and may release qualitatively or quantitatively different cues. Information coming from disturbance cues released by larger groups may be considered a more accurate indicator of risk and subsequently may also be more reputable. For example, the central assumption of the 'many-eyes' hypothesis is if one member of a group detects a threat, every individual in the group is alerted to an attack (Lima 1990). Therefore, the more individuals responding to a cue in a larger group may lead to receivers perceiving the cue as more relevant and, thus, receivers respond with increased intensity. In our experiment, the receivers may be detecting an increase in disturbance cue quantity as the more individuals that release the cue leads to an amplification of the cue through social facilitation (i.e. the performance of the cue is greater when there are more compared to less donors). This could also be supported by the function of disturbance cues, which is to increase vigilance towards a threat; it could be beneficial for larger groups to use disturbance cues to alert members and promote collective behaviours.

Second, there may be interacting effects of both the donor and receiver as receivers may be able to detect donor group size either through the qualitative or quantitative differences in the cue such as the density of donors. Since larger shoals mean greater safety the larger the shoal that releases a cue may suggest a more severe threat and the receivers should respond with increased intensity. Analogous group-size detecting mechanisms have been shown in prey fish. For example, fathead minnows (*Pimephales promelas*) can differentiate between the density of predators through predator odours, responding with increased intensity to the odour of 12 predators compared to two when concentration was controlled for (Ferrari et al. 2006). Therefore, receivers may actively discriminate between disturbance cues from two groups with different densities. This could explain why the response intensity is increased when exposed to cues from larger groups compared to smaller groups. According to the 'safety in numbers' hypothesis, prey fish will associate with larger shoals to reduce the chances of predation (Hager and Helfman 1991). For example, in the presence of a predatory fish, fathead minnows choose to associate with larger shoals than smaller ones (Hager and Helfman 1991). Likewise, guppies discriminate between shoal sizes and have preferences towards larger shoals (Lachlan et al. 1998). Therefore, disturbance cues from larger donor groups could signal greater safety, which leads to receivers responding with increased intensity.

In our experimental design, we changed the number of donors and size of the tank between the 10 and 20 donor treatments. Thus, there is a potential that there is a confounding effect of tank size. Therefore, we cannot definitively state that donor number is influencing the response of guppies to disturbance cues. It would be expected that the larger tank used by the 20-donor treatment will allow fish to swim faster and farther. Therefore, it is possible that the fish in the 20-donor treatments experienced increased metabolic rates compared to fish in the 10-donor treatment. Because disturbance cues are believed to be metabolic by-products (Wisenden et al. 1995; Kiesecker et al. 1999; Vavrek et al. 2008) this could influence the amount of cue released. However, even though we are increasing the tank size in the 20-donor treatment, when taking into consideration the ratio of the number of fishes to volume of water, there is a similar amount of space an individual fish to move around between the 10-donor and 20-donor treatments.

When exposed to the cues of heterospecific donors, we found no effect of donor group size in either guppies or cichlids. These results extend those of Vavrek and Brown (2009) who demonstrated significant cross-species responses to disturbance cues among juvenile convict cichlids and rainbow trout. The lack of donor group size effects in this study suggests some degree of species-specificity in disturbance cues that is similar to the well-studied damage-released chemical alarm cue, which does exhibit species-specificity to a certain degree (Brown et al. 2010; Chivers et al. 2012). It is possible that there is a chemical difference between disturbance cues from heterospecifics and conspecifics, as found in damage-released alarm cues (Kelly et al. 2006). It is argued that differences in metabolic mechanisms cause individuals to produce distinct chemical compounds (Chivers et al. 2012). Since disturbance cues are thought to be a by-product of metabolized protein (Vavrek and Brown 2009), variation in metabolic mechanisms between species can lead to the development of chemical signatures that are species-specific. Studies suggest that the composition of chemical cues could be a result of diet (Wisenden and Smith 1997); however, in our study, we controlled for diet (as discussed in *Materials and Methods*). Kelly et al. (2006) argued that purine compounds present in damage-released alarm cues are homologous in related species. Therefore, certain chemical compounds present in disturbance cues may be unique to conspecifics. In urine, fish are known to have unique mixtures of chemical compounds to trigger behavioural responses in conspecifics (Sorensen and Stacey 1999). Fish excrete a variety of nitrogenous wastes, including methylamines, taurine and imidazole (Van Waarde 1988), which if released simultaneously with urea may influence species-specific responses. Thus, a likely scenario for the cross-species response to disturbance cues could be that heterospecifics detect and respond to disturbance cues; however, the level of uniqueness in the mixture of chemical compounds leads to a limited response intensity. Alternatively, differences in species-specific olfactory sensitivity may likewise render heterospecific disturbance cues less effective. Regardless of the mechanism, our results do demonstrate a differential effect of donor group size between conspecific and heterospecific disturbance cues.

The observed response could also be a result of the reliability of public information from heterospecific prey-guild members. Since the relevance of public information plays a significant role in mediating antipredator behaviours (Dall et al. 2005), it could be that information from heterospecifics is less relevant. Individuals respond to the information, but these responses are limited or capped. Therefore, the quality of information outweighs the quantity and might explain why we did not observe a difference of donor group size. Our results provide evidence that conspecifics provide more relevant information compared with heterospecifics in terms of chemosensory cues. These findings support the idea that public information use is widespread among species (Valone and Templeton 2002). However, it also demonstrates that there is a relative scale of importance of public information in its use by fish, as conspecific information is more important than heterospecific information.

Our results can be attributed to the importance of information quantity or quality in making appropriate behavioural decisions (Dall et al. 2005). Increasing donor group size could be defined as proliferating the quantity or quality of public information, enriching the value of the disturbance cue and leading to an intensification of antipredator behaviour. It is well established that the amount of reliable public information influences predator avoidance (Dupuch et al. 2004; Schoeppner and Relyea 2005; Ferrari and Chivers 2009), thus potentially providing an explanation for the function of disturbance cues. This is because disturbance cues are early-warning signals to inform prey-guild members of a potential threat (Wisenden et al. 1995); increasing information quantity or quality leads to more detailed information about the imminence of a threat (i.e. the more information, the more imminent a threat). Likewise, guppies exposed to varying concentrations of damage-released alarm cues exhibit responses proportional to the amount of cue detected (Brown et al. 2009), responding accordingly based on the quantity of information. From this, we suggest that an increase in donor group size may be mechanistically the same as a difference in concentration by similarly increasing the quantity and the reliability the information.

Notably, in response to undisturbed donors, we showed that guppies decrease shoaling and increase area use and exhibited a graded response to donor group size. This unexpected result is contrary to our predictions because we would expect responses to undisturbed donor cues to be similar in both pre-stimulus and post-stimulus treatments, as reported in other species (Vavrek and Brown 2009). However, our results suggest the possibility of competitive effects from the undisturbed donor (control) on the behaviour of female guppies. This could be explained by an increase in perceived competition, leading prey to higher competition for resources. Guppies under low-risk conditions exhibit an increase in rates of competition (Chuard et al. 2016), thus it is possible that water from undisturbed conspecific donors influences competitive behaviours. Although undisturbed donors still release urea at low concentrations, we do not expect this to elicit a response. Fish responding to disturbance cues are believed to be responding to a sharp increase in urea beyond a given threshold of urea that naturally circulates within their surroundings (Brown et al. 2012).

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

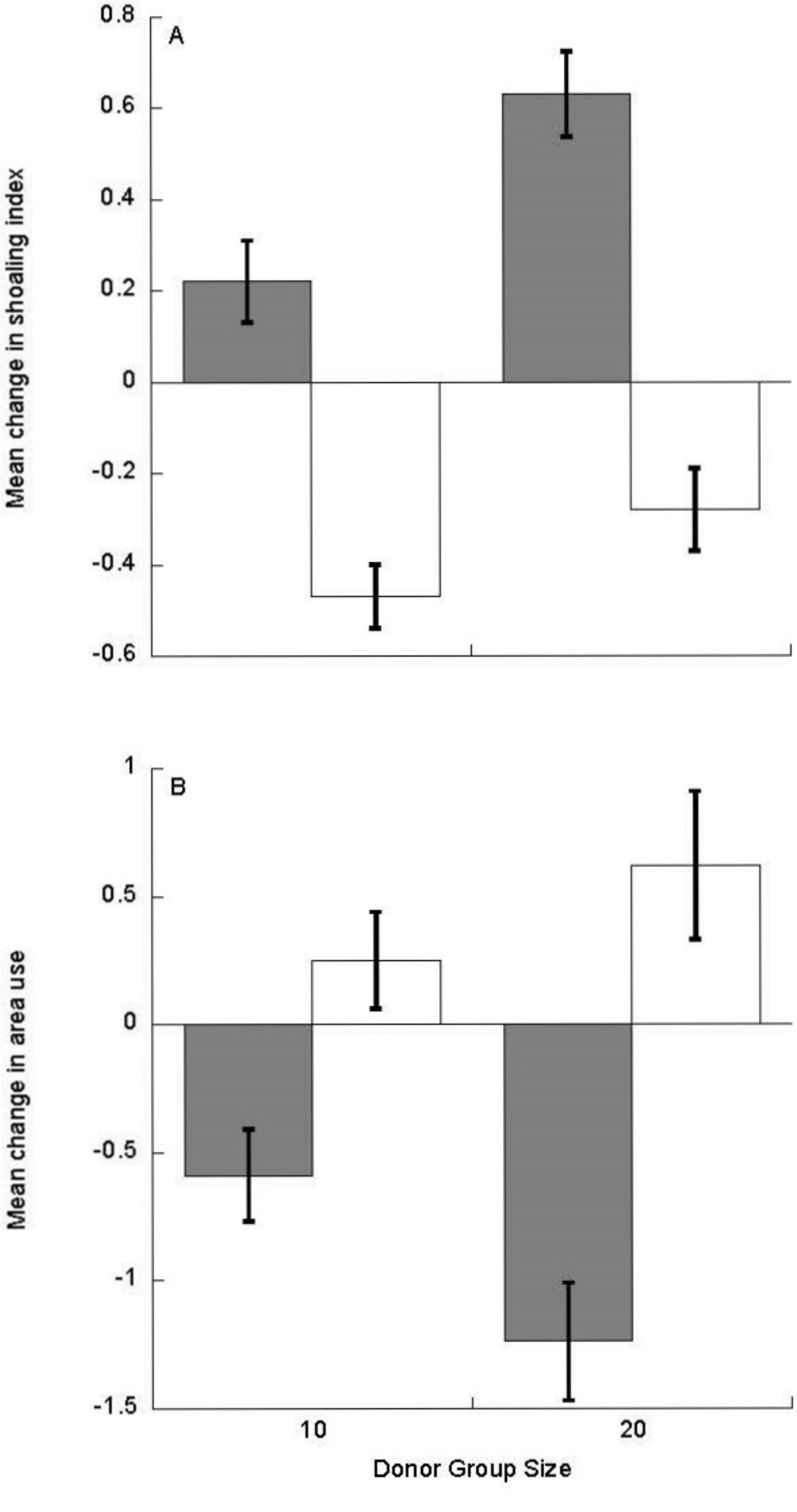

Figure 2.1: Mean change (± SE) in shoaling index (A) and area use (B) for guppies exposed to disturbance cues (solid bars) and water from undisturbed conspecifics (open bars) collected from groups of 10 or 20 conspecific donors. N = 15 for each treatment combination.

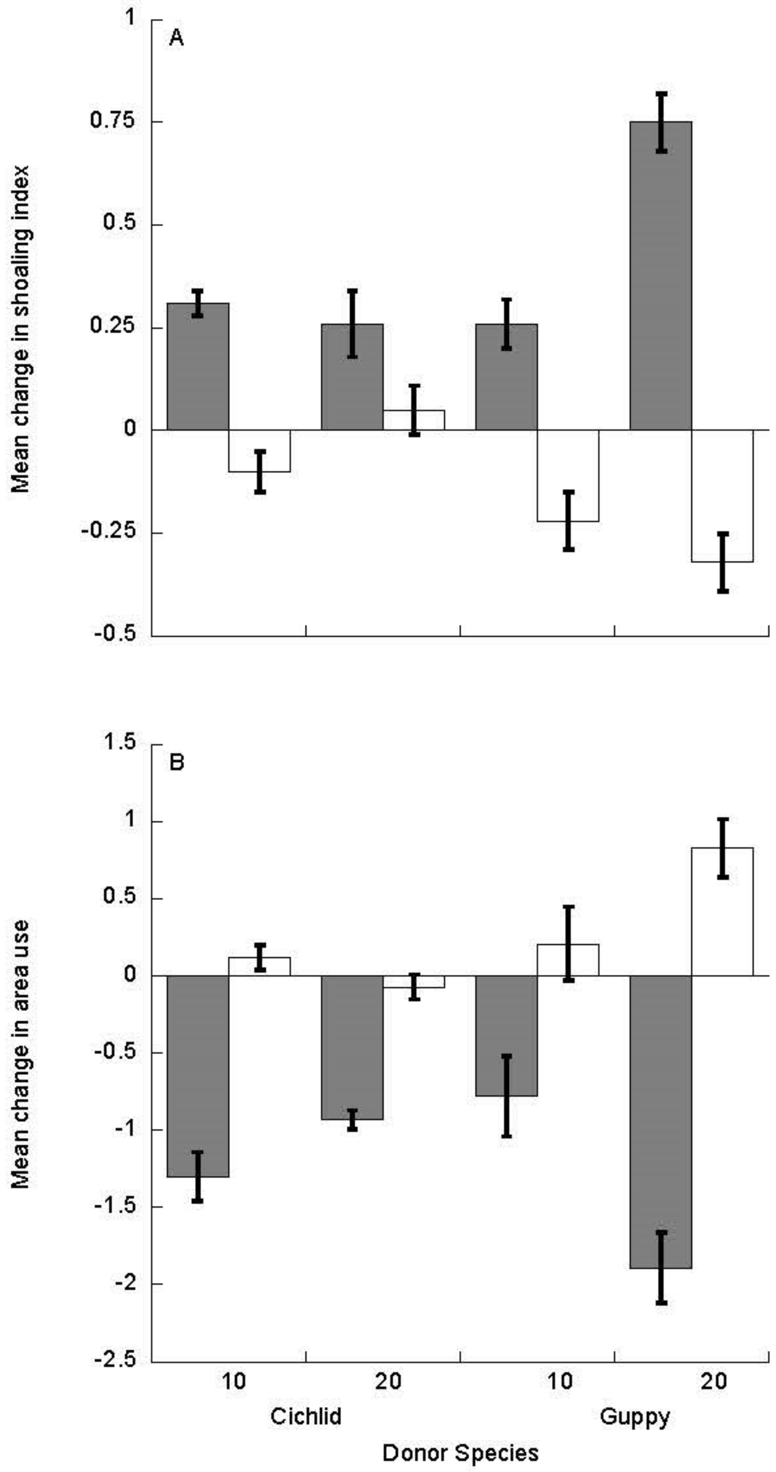

Figure 2.2: Mean change (± SE) in shoaling index (A) and area use (B) for guppies exposed to disturbance cues (solid bars) and water from undisturbed donors (open bars) collected from groups of 10 or 20 heterospecific (convict cichlid) and conspecific (guppy) donors. N = 15 for each treatment combination.

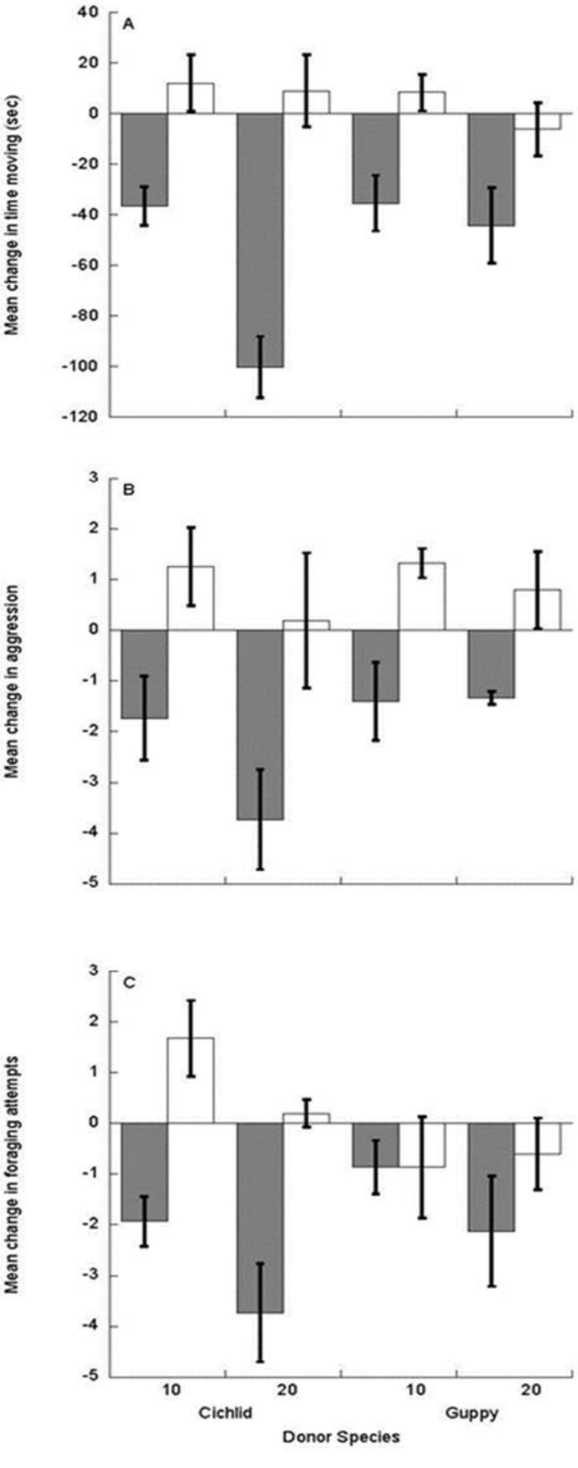

Figure 2.3: Mean change (± SE) in time moving (A), aggressive behaviours (B), and foraging attempts (C) for convict cichlids exposed to disturbance cues (solid bars) and water from undisturbed donors (open bars) collected from groups of 10 or 20 heterospecific (guppy) and conspecific (cichlid) donors. N = 15 for each treatment combination.

Chapter 3: Sender and receiver experience alters the response of Trinidadian guppies to disturbance cues

Abstract

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

Introduction

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

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

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

(Wisenden et al. 1995; Manassa et al. 2013; Bett et al. 2016; Fulton et al. 2017; Goldman et al. 2019).

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

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

We tested the effects of background predation risk on both the production of and response to disturbance cues in Trinidadian guppies in wild-caught guppies from populations with different background predation risk conditions (Experiment 1), and in a common laboratory population in which we manipulated background risk experimentally (Experiment 2). Initially, we predict that guppies from a high predation risk population should respond to disturbance cues more intensely and produce disturbance cues that elicit a stronger response compared to conspecifics from a low predation risk population. Secondly, if predicted responses of wild caught guppies are due to predation experience (and not population-specific differences), we predict the same response patterns in a laboratory strain of guppies experimentally exposed to high versus low background risk conditions.

Materials and Methods

Experiment 1: Effects of sender and receiver source population

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

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

Behavioural observations were conducted in a series of 20 L glass aquaria, filled with 18 L of dechlorinated tap water (~24°C, 12:12 h light:dark cycle). We affixed a single air stone to the back wall of the tank and attached an additional 1.5 m length of airline tubing to facilitate stimulus injection. Mean size (± SD) at testing was 23.50 ± 1.89 mm SL for Lopinot guppies, and 22.15 ± 1.79 mm SL for Upper Aripo guppies. Observations consisted of a 5 min pre-stimulus and a 5 min post-stimulus observation period. Immediately following the pre-stimulus observation period, we injected 10 ml of disturbance cue (experimental) or the odour of undisturbed conspecifics (control) and began the post-stimulus observation. During both the pre- and post-stimulus observations, we recorded an index of shoaling and vertical area use every 15 s. Shoaling index scores ranged between 1 (no fish within one body length of another) to 3 (all fish within one body length of each other). Area use scores were recorded as the position of each guppy within the tank (1 = bottom third; 3 = top third), aided by horizontal lines drawn on the exterior of the tank. Area use scores ranged from 3 (all fish near the bottom) to 9 (all fish near the water surface). An increase in shoaling index and a reduction in area use (within shoals of three) is a reliable indicator of increased predator avoidance in guppies (Brown and Godin 1999; Brown et al. 2009, 2010, 2013, 2018; Goldman et al. 2019). The observations were made blind to treatment and the order of testing was randomized throughout the experiment.

We calculated the proportional change ((post-pre)/pre) in shoaling index and area use scores for each replicate as dependent variables in all subsequent analyses (as per Mitchell et al. 2016). We used univariate ANOVAs (SPSS V24.0) to test the effects of disturbance cue (and undisturbed control) population, focal shoal population, and stimulus (disturbance cue vs. undisturbed cue) on the change in shoaling index and area use separately. All data met the assumptions of parametric tests. We conducted a total of N = 10 observations per treatment combination.

Experiment 2: Laboratory manipulation of risk

Using a laboratory population of Trinidadian guppies, we manipulated background predation risk to further investigate the effects of ambient risk on the production and response to conspecific disturbance cues. Guppies were ~ 10th generation descendants of wild-caught Upper Aripo guppies. Prior to testing, guppies were held in 110 L glass aquaria (~26°C, 12:12 h light:dark cycle). Guppies were fed with commercial tropical fish flake food (Nutrafin) twice a day. In order to manipulate background risk levels, we generated conspecific damage-released chemical alarm cues in order to manipulate background risk, as in Brown et al. (2013). We used a total of 95 non-gravid (visually assessed) female guppies as alarm cue senders (mean ± SD SL = 26.2 ± 0.57 mm, and body depth = 4.7 ± 0.14 mm). Senders were euthanized via cervical dislocation (in accordance with Concordia University Animal Research Ethics Protocol #30000255). We immediately removed the head, tail (at the caudal peduncle) and internal visceral tissues. The remaining tissue was placed into 100 mL of chilled distilled water and then homogenized and filtered through polyester filter floss. We added distilled water to achieve our desired final volume. We collected a total of 181.6 cm^2 of skin (diluted to a final volume of 1210 mL). Alarm cues were frozen in 40 mL aliquots at −20 °C until required. Damage-released chemical alarm cues at this concentration are known to elicit reliable increases in predator avoidance behaviour in guppies (Brown et al. 2009, 2013).

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

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

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

Results

Experiment 1

Our analyses reveal a significant main effect of focal population on the proportional change in shoaling index (Table 3.1; Fig 3.1A). Independent of stimulus type, guppies from the Lopinot (high predation) River increased shoal cohesion greater than guppies from the Upper Aripo (low predation) River (Fig 3.1A). However, we did not observe a significant effect of sender population on shoal cohesion (Table 3.1; Fig 3.1A) as guppies did not significantly alter shoal cohesion based on sender population alone. Regardless of treatment combination, guppies appeared to increase shoal cohesion in response to disturbance cues (Table 3.1; Fig. 3.1A). We observed a significant two-way interaction of focal population and stimulus. Independent of sender population, receivers from high-predation populations exposed to disturbance cues increase shoal cohesion greater than receivers from low-predation populations (Table 3.1; Fig. 31.A). We did not observe significant two-way interactions of sender population and stimulus or sender population and focal population on shoal cohesion (Table 3.1; Fig. 3.1A). However, we observed a significant three-way interaction of sender population, focal population and stimulus (Table 3.1). Both Lopinot (high predation) and Upper Aripo (low predation) River guppies exhibited greater shoal cohesion when exposed to the disturbance cues collected from Lopinot senders compared to Upper Aripo senders, with the greatest response in the 'Lopinot receivers – Lopinot sender' treatment combination (Fig. 3.1A). For the proportional change in area use, we found only a significant main effect of stimulus (disturbance cue vs. the odour of undisturbed conspecifics, Table 3.1; Fig. 3.1B). We observed similar reductions in area use regardless of disturbance cue sender or focal population (Table 3.1; Fig. 3.1B).

Experiment 2

We found no significant effect of pre-conditioning block (nesting factor, P > 0.05 for both shoaling index and area use; Table 3.2). As with wild caught guppies, laboratory guppies exhibited an increase in shoal cohesion in response to disturbance cues, regardless of background risk treatment (Table 3.2). Guppies increased shoal cohesion when exposed to disturbance cues compared to the cues of undisturbed shoals (Fig. 3.2A). Guppies significantly increased shoal cohesion based on the sender population (Table 3.2; Fig. 3.2A). We observed a significant two-way interaction of sender and stimulus (Table 3.2). Guppies increased shoal cohesion with a greater intensity when exposed to disturbance cues from high-risk compared to low-risk senders (Fig. 3.2A). Guppies also exhibited greater shoal cohesion when the sender population were high-risk and the receiver population were high-risk compared to both low-risk or a combination of the two, independent of stimulus type (Table 3.2; Fig. 3.2A). However, we found a significant three-way interaction for the proportional change in shoaling index as guppy receivers pre-exposed to the high-risk background treatment exhibited significantly greater increases in shoaling index when exposed to disturbance cues from high-risk versus low-risk senders compared to odour of undisturbed shoals (Fig. 3.2A; Table 3.2). As seen above for wild-caught guppies, we found a significant decrease in proportional area use in response to disturbance cues regardless of the background sender guppies (Table 3.2; Fig. 3.2B). However, guppies exhibited a greater decrease in proportional area use when focal fish had previous experience with a high-risk background compared to a low-risk background (Table. 3.2; Fig. 3.2B).

Discussion

Our first experiment shows the interacting effects of sender and receiver experience on the response of guppies when exposed to disturbance cues compared to the odour of undisturbed shoals. Guppies from the Lopinot (high-risk) River showed stronger overall responses compared to guppies from the Upper Aripo (low-risk) when exposed to disturbance cues. However, the response of Upper Aripo guppies did not differ based on the experience of the sender, whereas the response of Lopinot guppies differed based on sender experience. Lopinot guppies exhibited the greatest overall intensity to disturbance cues collected from Lopinot compared to Upper

Aripo guppies. Our results suggest that under high-risk conditions, there is an interacting effect of receivers and senders, but under low-risk conditions there is no effect of senders. The results of our second experiment compliment those of Experiment 1, demonstrating that when background risk is induced, we also observe similar interacting effects of sender and receiver experience.

It is interesting to note that the observed effects of sender and receiver experience were only seen in one of our behavioural measures (shoaling index). Given that disturbance cues are released earlier in the predation sequence, they may be perceived as a lower risk form of information (Wisenden et al. 1995; Ferrari et al. 2010). Alternatively, our measure of area use may not have been able to capture relatively subtle differences in behavioural response. Because we divided the tank into three sections to calculate area use, it is possible that the sections are too large and therefore cannot accurately measure potential subtle, but important, changes in area use. Future experiments under fully natural conditions would be required to validate our results.

Our results represent the first demonstration that prey fish can modify the intensity of their antipredator responses to chemosensory cues based on information provided by the prior experience of both cue senders and receivers. We cannot rule out population-specific differences in Experiment 1 due to potential differences in competition or diet. However, the results of Experiment 2, in which we induce risk in a single population, show analogous response patterns and provide support for our initial hypothesis. Alongside our results, recent studies show that background risk affects receivers of chemosensory cues (Brown et al. 2009; Bairos-Novack et al. 2017). Our results extend this, suggesting that experience also shapes how senders produce information. Previous studies show that background risk does not alter alarm cue production (Chivers et al. 2007). However, as disturbance cues are released earlier in the predation sequence than alarm cues (Wisenden et al. 1995), senders may have greater plasticity in disturbance cue production. Bairos-Novak et al. (2017) demonstrated interacting effects between background risk and response to disturbance cues in wood frog tadpoles, although the observed responses were all-or-nothing. Our results suggest that there is a graded response based on interacting experience. However, guppies increase the intensity of their antipredator responses based on sender experience only when the receivers were high-risk. Therefore, the effects of a sender’s experience only matter for high-risk receivers. This suggests that high-risk receivers may be more sensitive to information that is conveyed by the sender.

The benefits accrued by prey from responding to disturbance cues have already been demonstrated across a wide variety of taxa (Ferrari et al. 2010), and our results provide further evidence for the benefits of disturbance cues. Bairos-Novak et al. (2017) argued that disturbance cues could be social cues and used to increase collective behaviours. Because shoaling intensifies relative to the level of combined experience (e.g. whether it is high-high vs. high-low), we hypothesize that senders may benefit by recruiting shoalmates. When senders and receivers are in proximity to each other, they should benefit from shoaling together (e.g. confusion effect, Miller 1922). The 'confusion effect' purports that prey benefit from collective shoaling, a behaviour that is an adaptive response under predation threat and can increase survival (Krause and Godin 1995). The aggregation of prey would increase the amount of information a predator must process, potentially causing a decline in processing accuracy (Krakauer 1995), leading to a reduction in attack rate and success (Dukas 2002). Predator confusion has been suggested to be important early in the predation sequence (Endler 1991) and should allow prey to optimize antipredator behaviours according to the level of perceived risk. Considering that sender experience influences cue perception in receivers, it should be more beneficial for senders to alter the quantity and/or quality of the cue. Doing so would potentially increase benefits associated with increased shoaling (Endler 1991). By detecting predators early in the predator sequence, prey should have a better chance at survival. Likewise, senders could benefit by releasing the cue early in the predation sequence as it could act as an antipredator tactic. Thus, experience and the resulting modification of the cue could be a reliable indicator of the extent to which receivers should intensify antipredator responses (i.e. vigilance to escape tactics). If senders do benefit from the release of disturbance cues, this could meet the requirements for the designation as a signal according to the 'sensory drive hypothesis' (Endler 1992).

There is growing interest towards understanding the ecology of information in predator-prey interactions. Aquatic environments tend to be extremely heterogenous, which makes it important to quantify the degree to which variation within the environment shapes information. Our results demonstrate that predation risk alters the information conveyed by disturbance cues, and that prey benefit from responding to these cues and may also benefit from their release. Therefore, we highlight the importance of disturbances cues in predator-prey interactions and specifically, how they can play a crucial role in the predation sequence. Combined with recent studies, we provide evidence for the use of disturbance cues as a model to investigate the ecology of information in predator-prey interactions. Future studies should investigate whether these cues are in fact a signalling system to warn prey-guild members, whether they can also be utilized as an antipredator tactic by senders to increase survival and the degree to which other ecological factors shape their information content (e.g. competition or diet).

Table 3.1: Results of univariate GLMs on the proportional change in shoaling index and area use for guppies tested in Experiment 1. Sender population (high vs. low predation risk), focal population (high vs. low predation risk) and stimulus (disturbance cue vs. odour of undisturbed conspecifics) were included as independent variables. N = 10 per treatment combination.

	F	df	P
Shoaling index			
Sender	0.34	1, 72	= 0.56
Focal	4.09	1, 72	**= 0.047**
Stimulus	48.33	1, 72	**< 0.001**
Sender x Stimulus	1.87	1, 72	= 0.18
Focal x Stimulus	8.04	1, 72	**= 0.006**
Sender x Focal	0.04	1, 72	= 0.84
Sender x Focal x Stimulus	5.78	1, 72	**= 0.019**
Area use			
Sender	1.05	1, 72	= 0.31
Focal	0.33	1, 72	= 0.57
Stimulus	37.21	1, 72	**< 0.001**
Sender x Stimulus	0.11	1, 72	= 0.92
Focal x Stimulus	0.94	1, 72	= 0.34
Sender x Focal	0.19	1, 72	= 0.67
Sender x Focal x Stimulus	0.27	1, 72	= 0.61

Table 3.2: Results of nested univariate ANOVAs for the proportional change in shoaling index and area use for guppies tested in Experiment 2. Sender background risk (high vs. low), focal background risk (high vs. low) and stimulus (disturbance cue vs. odour of undisturbed conspecifics) were included as independent variables. N = 9 to 12 per treatment combination.

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

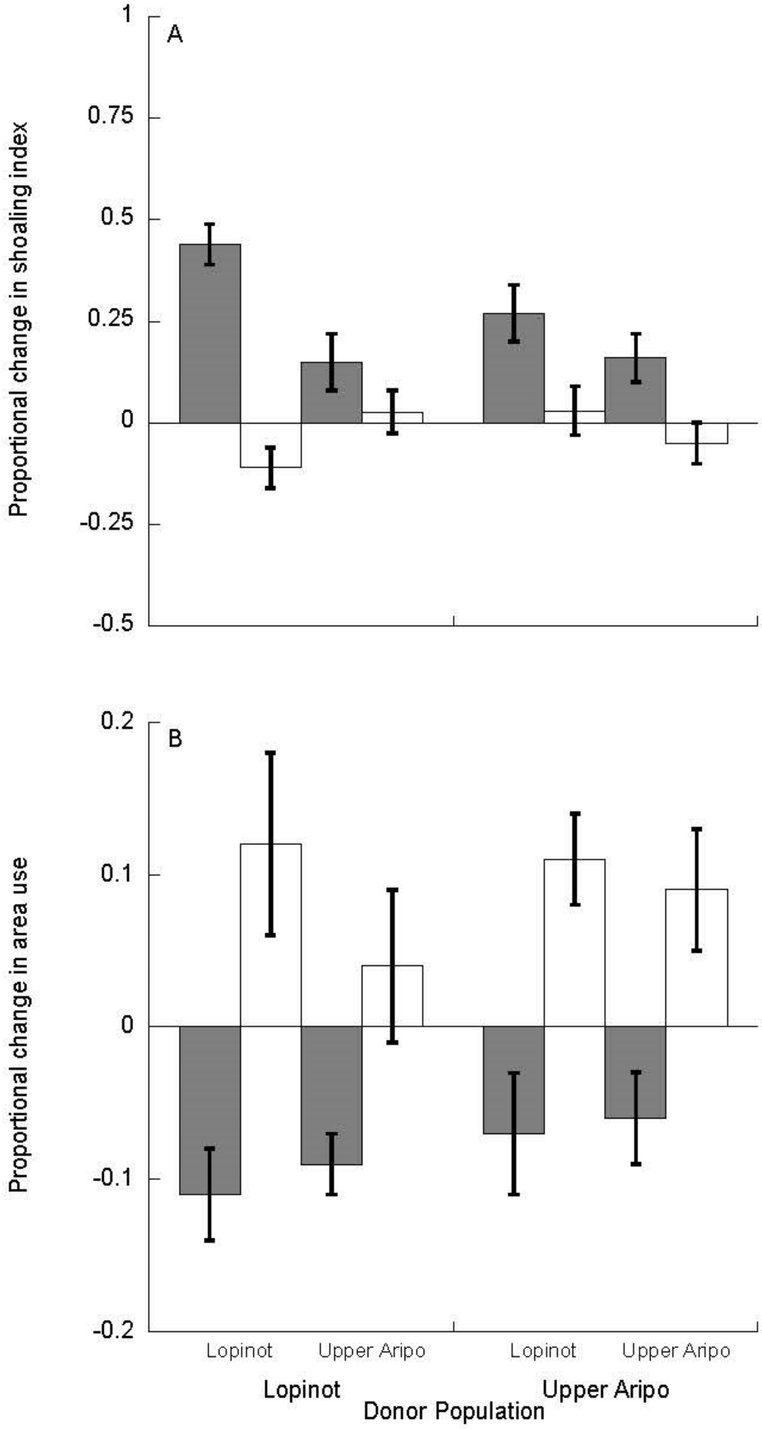

Figure 3.1: Mean proportional change (± SE) in shoaling index (A) and proportional change in area use (B) for guppies collected from high-predation (Lopinot) and low-predation (Upper Aripo) rivers and exposed to the disturbance cue (shaded bars) or odour of undisturbed guppies (open bars) from Lopinot or Upper Aripo senders. N = 10 per treatment combination.

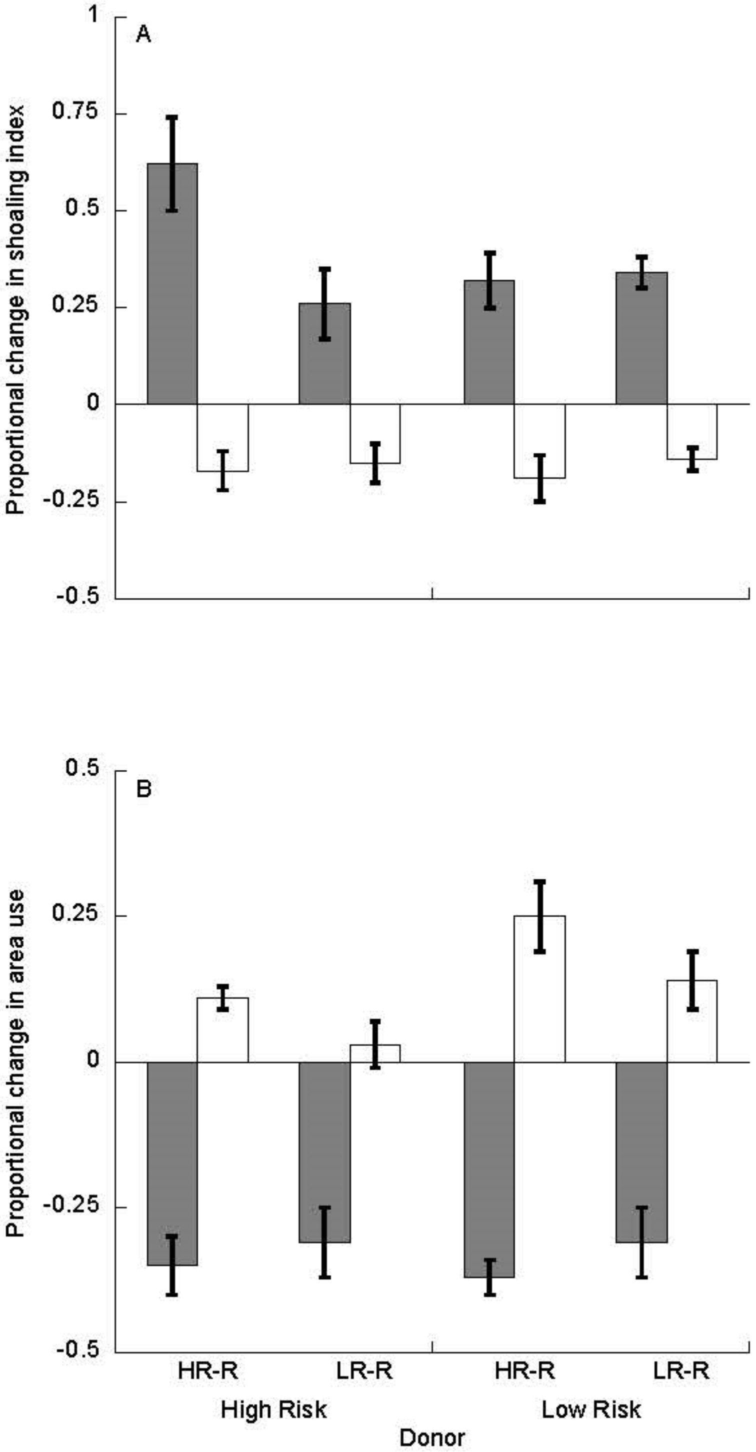

Figure 3.2: Mean proportional change (± SE) in shoaling index (A) and proportional change in area use (B) for guppies preconditioned to high vs. low predation risk and exposed to the disturbance cue (shaded bars) or odour of undisturbed guppies (open bars) from high- vs. low-risk senders. N = 9 to 12 per treatment combination.

Chapter 4: General Discussion

The main goal of this work was to examine how receivers respond to disturbance cues and the factors that influence risk assessment based on these cues. Understanding how receivers respond to disturbance cues and what shapes their response is central to elucidating the important role that these cues can play in the predation sequence and whether these cues are simplified secondary 'signals' (i.e. alert or 'early warning' cues) or more complex true signals. Previously, research concerning chemosensory risk assessment in aquatic prey have focused on other cues, therefore our understanding of disturbance cues is limited. The data presented in this thesis demonstrate that disturbance cues are to a degree species-specific rather than simply generalized cues as previously thought (Hazlett et al. 1990b; Vavrek and Brown 2008). We show that the response to an increase in the number of heterospecific disturbance-cue donors did not result in an increase in the intensification of antipredator behaviour (Chapter 2). These results suggest that heterospecific disturbance cues do not elicit threat-sensitive responses. This supports our initial hypothesis that how receivers respond to disturbance cues depends on the identity of the sender. Furthermore, in both wild and laboratory-reared populations, receivers can adjust their antipredator behaviour based on the sender's previous experience with predation risk. High-risk receivers responded with increased intensity to cues from high-risk compared to low-risk senders, demonstrating that both sender and receiver experience matters in the response to disturbance cues (Chapter 3). Additionally, we show that individuals who have experienced a high-risk environment increase their perception of risk. This further supports our hypothesis, as these results suggest that senders release different cues, either quantitatively or qualitatively.

Presumably, the more information prey can acquire earlier in the predation sequence, the more equipped they should be to make context appropriate behavioural decisions, allowing prey to optimize fitness related trade-offs (Endler 1991). Therefore, the quantity and/or quality of information acquired early in the predation sequence should influence the ability of prey to avoid predators and make context appropriate behavioural decisions. Our results highlight the important role that disturbance cues play in the predation sequence by relaying specific information to other prey-guild members that can be used to optimize antipredator responses. Unlike disturbance cues, kairomones are unable to convey a similar degree of specificity in their information. The information conveyed by kairomones are restricted to the identity, size and number of the predator (Mitchell et al. 2017), whereas disturbance cues can relay the identity and prior experience of the sender and the relatedness between sender and receiver (Bairos-Novak et al. 2019a). Therefore, disturbance cues allow prey to make the most informed decision concerning a potential predator threat.

Apart from elucidating the potential role that disturbance cues can play in the predation sequence by relaying more complex information, the results from Chapter 3 have broader implications for understanding the effect of background risk on prey behaviours. Experience with varying levels of background risk has been shown to shape the behavioural phenotypes of prey species (Cote et al. 2013; Abbey-Lee et al. 2016). Under natural conditions, predation risk is extremely heterogeneous, subsequently the level of background risk perceived by prey exhibits strong spatio-temporal dynamics (Sih et al. 2000). Therefore, the behavioural response patterns of prey in response to chemosensory cues differs based on their prior experience. However, research has focused on receiver experience as it has not been demonstrated that sender experience shapes the response to chemosensory cues by receivers. Our study provides the first support that the background risk experienced by prey fish alters the production or release of chemosensory cues. These results demonstrate the complexity of predator-prey interactions and how important communication systems can be influenced by the conditions experienced over both short- and long-term timescales.

Prospectus

Previously, it had been suggested that disturbance cues may function as alert cues or 'signals' (Wisenden et al. 1995). Alert cues are simplified cues detected by receivers that draws their attention but does not relay specific information. Considered to be secondary signals that are released more rapidly and over a shorter duration than primary signals (Endler 1993), these cues are by-products of senders and provide no benefit to senders. In predator-prey interactions, alert cues would subsequently be considered as simple cues that do not relay differential information and serve only to make prey attentive toward potential risks. However, due to the presumption that these cues are voluntarily released (Wisenden et al. 1995; Ferrari et al. 2008: Bairos-Novak et al. 2019b) and that they function as early-warning cues, it is possible that disturbance cues can be highly context-dependent (i.e. can be shaped by different circumstances and surroundings) and relay specific information about the sender. Therefore, the sender's identity, motivational state (state of desire to preform a behaviour) or prior experiences may influence the quantity and/or quality of information conveyed by disturbance cues. However, we can not completely rule out if the degree of risk perceived by senders is simply driving differences in quantity or quality of information, and further experiments are needed to test this hypothesis. Although, when combined, our results and those of Bairos-Novak et al. (2019a,b) provide evidence that disturbance cues contain information beyond just indicating a potential predator threat. Disturbance cues can indicate the relationship between sender and receiver (i.e. conspecifics/heterospecific, kin/non-kin or familiar/unfamiliar) and their previous experience with risk (Bairos-Novak et al. 2017; Goldman et al. 2019). If the information conveyed by disturbance cues can change based on ecological factors (e.g. predation, competition, food availability, temperature), these cues should be considered a more integral part of the predation sequence. This is because compared to other cues, disturbance cues could impart more specific information, which would provide increased fitness benefits by affording prey the opportunity to make more informed decisions. Our results provide evidence that disturbance cues may not be simple alert/secondary cues as they can convey information to receivers that the cue originated from either a conspecific or heterospecific and that the experience of both the receiver and the sender dictates how receivers use that information. Instead, we argue that disturbance cues may possibly function as primary cues which serve not to only alert prey, but to provide them with important information that allows receivers to make appropriate behavioural decisions. This affords prey a greater opportunity to avoid the costs of making maladaptive responses, which can result from the non-complex information content conveyed by the simpler alert cues. An important result of our work is that it suggests that the ability of prey to be successful in avoiding predators during the predation sequence can be shaped not just by their personal experience but also the experience of other conspecifics.

However, it is not yet known whether these cues are in fact true signals. In order to be considered a true signal there must be a clearly demonstrated benefit to the sender. To date, there has been no definitive evidence that senders benefit from releasing disturbance cues. Bairos-Novak et al. (2019b) suggested that releasing these cues may benefit the sender by providing evidence that senders release these cues voluntarily. Likewise, it is plausible that our results demonstrating that senders may release either quantitatively or qualitatively different cues (Chapter 3) indicate potential sender benefits. Combined, these studies help establish a baseline that warrants further investigation into the potential that disturbance cues are true signals.

Although this thesis builds upon pre-existing knowledge of disturbance cues by providing new insights into a previously understudied cue, a gap remains in our knowledge and understanding of these cues. One of the most significant gaps exists in our knowledge of the mechanisms controlling the release and production of these cues, therefore studies are necessary to identify the chemical design and release pathways of disturbance cues. While studies suggest that the active chemical component is urea in fish (Brown et al. 2012), and ammonia in amphibians (Kiesecker et al. 1999), a chemical analysis of these cues has yet to be conducted. If these cues are released as nitrogenous waste, there may be a suite of chemicals that function as active components of these cues. Understanding the chemical design of these cues will further allow researchers to test to what degree a prey's surrounding environment shapes the release and response to these cues. Zulandt Schneider and Moore (2000) identified urine as a release pathway of disturbance cues in arthropods, however, due to the excretion of nitrogenous waste occurring through different orifices in fish (e.g. gills, Smith 1929), there is a need to confirm whether these cues are excreted across the gills and/or through the urine in prey fish.

Along with understanding the chemical design and release pathways, there is a need to test the function of disturbance cues at different life stages. Due to their size, we could expect that more mature individual may release a higher concentration of the cue, therefore the quantity of information may lead to an intensification in receivers that are juveniles. However, for more mature individuals the risk posed by potential predators may be less risky. Therefore, it is plausible that mature individuals may release less disturbance cues, if any. Alternatively, there may be an allometric relationship between the response of mature individuals to predators, as their response may be size-dependent (size of predator compared to prey). Likewise, the costs associated with responding to a threat are lower for larger-bodied compared to smaller-bodied organisms (Preisser and Orrock 2012). Therefore, mature individuals may also respond differently to the cues from juvenile prey and vice versa. It is also plausible that mature species stop responding to disturbance cues when they reach a given size due to lost fitness-related opportunities associated with responding to these cues. However, there are also conflicts when prey species become reproductively active. Reproductively active male and female fish are known to release chemical sexual signals (pheromones) during courtship (Liley 1982). For example, reproductively active male round gobies (*Neogobius melanostomus*) use a pheromone to attract females (Corkum et al. 2006). Likewise, female goldfish (*Carassius auratus*) also voluntarily release pheromones in urine in the presence of male conspecifics (Appelt and Sorensen 2007). Therefore, if these cues are released using the same pathways, disturbance cues may be suppressed during courtship, or their function lost entirely, when prey become reproductively active. The response of these cues may also be shaped by the degree of sociality of a species.

Bairos-Novak et al. (2017) argued that disturbance cues may be true signals and that these cues may be more prevalent in highly social species compared to non-social species. Since fish are known to modify the production and pulsatile release of urine in social contexts (Almeida et al. 2005; Barata et al. 2007, 2008; Martinovic-Weigelt et al. 2012; Maruska and Fernald 2012; Bayani et al. 2017), highly social species may utilize these cues more frequently than non-social species. It has also been proposed that disturbance cues are a more honest indicator of risk in social compared to non-social species (Bairos-Novak et al. 2017). Therefore, highly social species may respond differently to these cues, responding with increased intensity when exposed to disturbance cues in predation sequence compare to non-social species, who may not respond with a similar intensity. According to the 'costly information hypothesis', prey in high risk environments may rely more heavily on social information compared to prey in low risk environments due to the costs of acquiring personal information in risky environments (Webster and Laland 2008). However, cues released in urine are known to mediate multiple interactions in highly social species (Derby and Sorensen 2008). Therefore, the amount of interactions that are mediated by disturbance cues may limit the function of disturbance cues as an 'early-warning signal' in the predation sequence. Subsequently, testing the function of disturbance cues in highly social vs. non-social species would be important to understand how the role of these cues differs across species and whether its function may be impaired by the degree of a sociality of a species.

Impairment of the olfactory system and the degradation of chemosensory cues upon release into the environment as a result of human-induced environmental change is a prevalent issue when dealing with the impacts of these changes on the predation sequence (Leduc et al. 2013). *In situ* experiments in freshwater environments have shown that acute exposure to weakly acidic conditions impairs the ability of juvenile brook trout (*Salvenius fontinalis*) and Atlantic salmon (*Salmo salar*) to detect and subsequently respond to damage-released alarm cues (Leduc et al. 2004). The impairment of chemosensory risk assessment in acidified streams alters behavioural patterns (Elvidge and Brown 2015) and reduces survival of juvenile Atlantic salmon (Elvidge and Brown 2013). Likewise, in marine environments, exposure to elevated CO_2 and reduced pH levels results in predators avoiding alarm cues from injured prey (Cripps et al. 2011). Therefore, testing the impairment of the olfactory system along with the degradation of disturbance cues due to pollution could provide significant insights into the role that these cues may play in the predation sequence. We would expect that if disturbance cues play an important role in the predation sequence, prey would experience increased capture rates and subsequently reduced survival, if disturbances cues are indeed impacted by environmental change. However, if urea does function as the active component in disturbance cues, degradation may not occur in impacted environments. Therefore, the ability of disturbance cues to persist in environments impacted by change is pertinent to elucidating the relative scale of importance of the role that disturbance may play in the predation sequence. If disturbance cues still function under weakly acidic or elevated CO_2 conditions, the importance of these cues may exceed that of more reliable chemosensory risk assessment cues (i.e. alarm cues) in the predation sequence.

Aside from understanding the chemical design of disturbance cues and the environmental factors that shape their production and response, it is worth exploring potential alternate functions of these cues in terms of mediating other social interactions. One potential adaptive role for the release of disturbance cues by senders would be to deceive receivers. Our results suggest that senders may release quantitatively or qualitatively different cues based on their prior experience with risk. Subsequently, we hypothesize that during the predation sequence, tactical deception (Whiten and Byrne 1988) could be used by senders of disturbance cues to under-exaggerate the level of risk. By under-exaggerating the level of risk, senders could benefit from receivers disregarding the severity of the threat. This could lead to a maladaptive behavioural response by a receiver in the face of a legitimate predation threat, giving senders a better chance to avoid predation. Also, due to the trade-offs between exhibiting antipredator responses and lost fitness-related activities, we hypothesize that senders may release disturbance cues in a competitive context to deceive competitors. This would allow senders to monopolize a resource. Since it has been suggested that disturbance cues are voluntarily released (Bairos-Novak et al. 2019b), senders could use these cues to signal to nearby competitors that a predation threat is imminent. This could cause receivers to trade-off foraging for antipredator behaviours. Likewise, during courtship contests where multiple males are forced to compete for females, releasing disturbance cues may increase courting opportunity and success for the sender. Future studies should be conducted that examine alternate uses of disturbance cues by senders, and whether these cues can be used to deceive receivers.

Conclusion

Prey species exhibit a wide array of antipredator strategies with the intended purpose of interrupting the predation sequence and avoiding consumption. However, the ability to employ such strategies relies heavily on the acquisition of information. Without information, prey are at an increased cost of exhibiting maladaptive behavioural responses. Therefore, the availability of information is a major determinant of successful avoidance. Disturbance cues provide prey with valuable information early in the predation sequence that afford prey the opportunity to engage in antipredator tactics. The value of the information conveyed by chemosensory cues relies heavily on both its quality and quantity, thus shaping the importance of a given cue within the predation sequence. This thesis provides evidence for the importance of disturbance cues within the predation sequence by demonstrating that these cues can relay complex information about sender identity and experience.

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