The influence of chemical alarm cue concentration and temporal variation on the threat-sensitive foraging decisions of juvenile convict cichlids.

Patricia E. Foam

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Abstract

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Patricia E. Foam

At the individual level, prey are faced with a series of trade-offs between the benefits of predator avoidance and those of a suite of other behaviour patterns. The threat-sensitivity hypothesis, however, assumes that the antipredator behavioural responses shown by prey are graded in intensity in a manner that corresponds with the level of perceived risk. Previous studies examining threat sensitive responses to chemical alarm cues have provided mixed results at best. The first experiment of this thesis examined the influence of subthreshold chemical cues on the threat sensitive decision of foraging posture. Juvenile convict cichlids (*Archocentrus nigrofasciatus*) were given a choice of head-down or head-up foraging posture, and exposed to sub-threshold or suprathreshold alarm cues. Cichlids exposed to the subthreshold cue did not exhibit an overt antipredator response, but did significantly alter their foraging posture. Likewise, cichlids exposed to the odour of a piscivorous predator exhibited a similar threat-sensitive shift in foraging posture. The second experiment examined the influence of temporal variation in predation risk on antipredator and foraging behaviour. Convict cichlids were exposed to one of four conditioning regimes, differing in concentration and frequency of alarm cue, and subsequently exposed to conspecific alarm cue or a control. Cichlids initially conditioned to high frequency cues, regardless of cue concentration, increased foraging effort during 'safe' periods and exhibited reduced antipredator responses during 'risky' periods. Together, these results suggest that juvenile cichlids do not respond to chemical cues with the quantitatively graded responses assumed by the threat-sensitivity hypothesis, but rather exhibit threat-sensitivity by making qualitative changes in antipredator and foraging behaviour.

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Table of Contents

Chapter One: General Introduction

Chapter Two: Juvenile convict cichlids switch to threat-sensitive foraging tactics based on chemosensory information.

Chapter Three: Juvenile convict cichlids allocate foraging in response to temporal variation in predation risk

List of Tables

List of Figures

Chapter 1. Introduction

In the presence of a predation threat, prey exhibit a suite of antipredator responses intended to aid avoidance and escape. When engaged in antipredator behaviour patterns, other fitness related activities (e.g. foraging, searching for mates, territorial defense) are usually reduced. Hence, predator avoidance is likely costly (Werner and Anholt 1996; Werner and Peacor 2003), since it would reduce time and energy available for other fitness related activities. As a result, the response to predation pressure, at the individual level, is shaped by a series of trade-offs between the benefits of predator avoidance and those of a suite of other fitness related behaviour patterns (Lima and Dill, 1990; Lima and Bednekoff 1999, Werner and Peacor 2003). Individuals should be at a selective advantage if they are capable of reliably assessing local predation risk, and adjusting the intensity of their anti-predator behaviour modalities, to match their current risk (Lima and Dill, 1990; Lima and Bednekoff, 1999).

Threat Sensitivity

The level of threat presented by a predator may vary. This variation can be due to the size of the predator, hunger of the predator, foraging strategy, and other factors (Helfman 1989). Due to the necessary trade-off between antipredator and other fitness-related behaviour patterns, it is not advantageous for prey to exhibit overly intense antipredator responses, or to respond to a non-threatening predator (Lima and Dill 1990). Prey must meet certain energy requirements for survival, and prey that respond to non-threatening predators may miss foraging opportunities, as well as the prospect of other fitness-related activities (Brown in press).

Variation in the level of threat a predator presents is addressed by the threat-sensitivity hypothesis. The threat-sensitivity hypothesis predicts that prey should alter their antipredator response; in a manner that reflects the magnitude of the predatory threat (Helfman 1989). The hypothesis makes the assumptions that (1) animals are faced with the need to balance time and energy, and therefore are (2) faced with a trade-off between antipredator behaviour modalities and other fitness-related activities, and (3) must resolve these trade-offs in a graded manner appropriate to the threat. This predicts that for a given magnitude of predation risk, prey will respond with antipredator responses of a corresponding intensity. Helfman (1989) examined the antipredator behaviour patterns of threespot damselfish (*Stegastes planiformes*) when presented with models of a predatory Atlantic trumpetfish (*Aulostomus maculates*). Damselfish exhibited more intense antipredator behaviour patterns, when the model of the predator was closer, larger, or in a strike pose. Helfman and Winkleman (1997) demonstrated that bicolor damselfish (*Pomacentrus partitus*) exhibited less threat-sensitivity than threespot damselfish. This difference between the two species may be due to increased sociality in the bicolor damselfish, demonstrating that even closely related species may experience predation risk differently, and hence show different levels of threat-sensitivity. Bishop and Brown (1992) found that larval threespine sticklebacks (*Gasterosteus aculeatus*) increased their foraging as they increased in size relative to the predator. This demonstrates that larval sticklebacks can adjust their behaviour patterns in a threat-sensitive manner, in response to visual cues of predator size.

While several studies (see above), have demonstrated threat sensitivity in response to visual predator cues, few studies involving threat-sensitivity have exclusively used chemical cues as indicators of predation risk. Chivers *et al.* (2001) found that small (<42 mm total length) and medium (42-66 mm total length) sized slimy sculpins (*Cottus cognatus*), responded with threat sensitive predator avoidance when they were able to assess the predator visually. However, large sculpins (>66 mm) did not differ in their predator avoidance patterns, regardless of the size of the predator. However, when the odour of predatory brook trout (*Salvelinus fontinalis*) was presented to sculpins in the absence of visual cues, all three of the size classes responded to this chemical cue.

Recent studies involving chemical cues (see below) as indicators of predation risk, suggest that graded responses are not seen with chemical cues, such as alarm or predator odour cues. Instead a non-graded response to increasing concentrations of the chemical cue is seen (Brown in press). In response to varying concentrations of chemical alarm cue, receivers often show an all or nothing rather than a graded response. Chivers *et al.* (2001) have proposed that the lack of graded responses they observed in slimy sculpins might be due to the early warning role played by predator odour cues, the information provided by these cues can be acted upon even before the predator is present. Alternatively, when slimy sculpins could assess the predator visually they showed threat-sensitive predator avoidance. This suggests that the graded responses assumed by the threat-sensitivity hypothesis may require that the predator be visible (Chivers *et al.* 2001).

Many antipredator studies testing for graded responses in freshwater prey fish have focused on overt behaviour patterns such as shoaling, area use, and aggression (Brown in press). Furthermore, the fact that those studies examining graded responses have obtained mixed results, may be due to focusing solely on overt antipredator responses. It may be that prey fish will display subtle changes in response to variation in risk and subthreshold concentrations of chemical cues, such as changes in foraging patterns. When studies focus their observations on overt, more explicit behavioural modalities, they may miss these subtle changes in behaviour patterns.

Chemical Alarm Cues

In order to make a more informed decision about their antipredator response, individuals require reliable information about the risk of predation in their environment. A wide range of terrestrial and aquatic prey species rely on chemical cues to assess local predation risk. Root voles (*Microtus oeconomus*, Borowski 2002), and lizards (*Podarcis sicula*, Downes and Bauwens 2002) use the odours of predators in risk assessment, while harvestmen spiders (*Goniosoma proximum*) can assess predation risk using damage released chemical alarm cues (Machado *et al.* 2002). The use of chemical alarm cues to assess local predation risk is also common among both aquatic invertebrates and vertebrates (Chivers and Smith 1998; Summey and Mathis 1998; Vadas and Elner 2003; Brown 2003).

A wide variety of taxonomically diverse freshwater prey fishes use damage-released chemical alarm cues to assess their risk of predation. Chemical alarm cues are typically stored in the epidermis, and released through mechanical damage to the skin such as would occur during a predation event. Upon detecting these alarm cues, receivers respond with a range of antipredator behavioural responses, such as decreased foraging activity, decreased activity, decreased aggression, and increased group cohesion and area avoidance (Chivers and Smith 1998; Smith 1999). Responding to these cues has been shown to confer a measurable survival benefit to the cue receivers (Mirza and Chivers 2003a, b).

Subthreshold Alarm Cues

As the concentration of chemical alarm cue decreases, there comes a point at which overt responses are no longer immediately observable. This point is referred to as the minimum behavioural threshold (Lawrence and Smith 1989; Brown *et al.* 2001). Recently, several authors have shown that chemical alarm cues at concentrations below that required to elicit a visible antipredator response (sensu Smith, 1999), can provide valuable information to prey individuals (Brown *et al.* 2001; Marcus and Brown, 2003; Mirza and Chivers, 2003a; Brown *et al.* 2004a). For example, Brown *et al.* (2001) have demonstrated that fathead minnows (*Pimephales promelas*) are able to detect and learn to recognize the chemical identity of a novel predator, paired with concentrations of chemical alarm cues well below the threshold required to elicit a visible antipredator response. Mirza and Chivers (2003a) demonstrated that juvenile rainbow trout (*Oncorhynchus mykiss*) gain significant survival benefits during predatory encounters, when exposed to conspecific alarm cues at concentrations well below the minimum behavioural threshold. Finally, Brown *et al.* (2004b) demonstrated that subthreshold concentrations of chemical alarm cues increased vigilance towards conspecific visual alarm displays in glowlight tetras (*Hemigrammus erythrozonus*). Taken together, these studies demonstrate that prey fishes attend to conspecific chemical alarm cues, at concentrations below some minimum response threshold. Prey can use the presence of these subthreshold cues to make threat-sensitive decisions about antipredator response intensity, duration, or foraging postures (see Chapter 2). Thus, both subthreshold and suprathreshold concentrations of alarm cue, provide information used to assess local risk of predation and to respond with behaviour appropriate to the magnitude of perceived risk.

Temporal Variation in Predation Risk

In addition to the presence of sub- or suprathreshold chemical alarm cues, a prey fish's threat-sensitive decisions can be influenced by their prior experience with risk. Predation risk can vary on many time scales, as well as on geographic scales. The time scale experienced can vary from a moment-to-moment basis, to a daily basis or even a seasonal basis (Sih *et al.* 2000). As a result, prey individuals must constantly assess their environment for risk, and will use their prior experience to make predictions about future behavioural decisions. The antipredator and foraging decisions of prey will be sensitive to not only their present level of risk, but also to their prior experience with predation threats (Lima and Bednekoff, 1999). For a prey individual, the pattern of risk they experience is as important as the intensity of that risk in the shaping of their behavioural decisions.

While studies have examined antipredator responses to subthreshold concentrations of chemical alarm cues (see above), little work has focused on the influence of subthreshold concentrations on foraging decisions. In addition, although variation in predation risk has been examined in a few studies (see Chapter 3), little research has been done on the influence of temporal variation in subthreshold alarm cues on both foraging and antipredator decisions. I conducted this study to: 1) examine the influence that predator odours would have on foraging decisions, 2) examine the influence of subthreshold chemical alarm cues on foraging and antipredator decisions and 3) determine what influence, if any, temporal variation in risk would have on foraging and antipredator decisions.

In Chapter 2, I used juvenile convict cichlids (*Archocentrus nigrofasciatus*) to examine the role that chemosensory information plays in a threat-sensitive behavioural decision, such as that of foraging posture. I designed a two-part laboratory study in order to test the hypothesis that predator odour, a subthreshold concentration of alarm cue, and a suprathreshold concentration of alarm cue, should all have an effect on the threat-sensitive decision of the test fish to forage in a head-down or head-up position.

In Chapter 3, I used juvenile convict cichlids in a laboratory study to determine the role that temporal variation in predation risk plays on the threat-sensitive decisions of foraging and antipredator behaviour. I also wanted to examine the effect that subthreshold concentrations of alarm cues would have on foraging behaviour.

Chapter 2. Juvenile convict cichlids switch to threat-sensitive foraging tactics based on chemosensory information.

Introduction

While an animal needs to be aware of, and react to predators, it must also meet its daily energy requirements (Houston *et al.* 1993). Foraging can be a costly activity in terms of predation risk because a prey animal's body posture while foraging or its foraging tactic may affect its ability to detect potential predators (Elgar 1989; Lima and Dill 1990; Krause and Godin 1996). Prey feeding on or near the substrate may be at a greater risk of predation when foraging in a head down position, due to a reduced ability to detect the presence of potential predators (Krause and Godin 1996; Bednekoff and Lima 2002). Moreover, predators can assess the vigilance level of their prey (Elgar 1989) and may selectively avoid attacking vigilant prey (FitzGibbon 1989; Krause and Godin 1996). For terrestrial vertebrates, which forage primarily on the ground (e.g. grainivorous birds, small mammals or herbivores), individuals must frequently raise their heads to scan for potential predators, resulting in a decrease in overall foraging rate (Lima and Bednekoff 1999; Bednekoff and Lima 2002). For prey foraging in three-dimensional space (e.g. substrate-feeding prey fishes), a shift in foraging posture may allow individuals to reduce their risk of predation, while limiting any potential loss of foraging benefits (Krause and Godin 1996). As such, foraging posture can be viewed as a trade-off between foraging and the ability to detect potential predators. Individuals capable of reliably assessing local predation risk should be at an advantage, as it would allow them to adjust the intensity of predator avoidance in a graded fashion, according to perceived risk (i.e. threat-sensitive trade-offs; Helfman 1989, Helfman and Winkelman

1997; Chivers *et al.* 2001). Such threat sensitive trade-offs allow individuals to maintain some foraging benefits while reducing their risk of predation (Helfman 1989; Lima and Dill, 1990).

Benthic foraging fishes such as juvenile convict cichlids (*Archocentrus nigrofasciatus*) display a variety of body postures when foraging, the most common being to actively forage on the substrate (head-down posture) or vertical surfaces (head-up posture) (Conkel 1993; Barlow 2000). While feeding on a horizontal food patch (i.e. on the substrate), the cichlid's body is typically inclined towards the substrate at an angle greater than 45°. While feeding on a vertical food patch (i.e. rock faces), its body is usually parallel with the substrate. In juvenile convict cichlids, foraging in a 'head-down' position is the preferred foraging posture, mainly due to the location of food items, and is thought to be more energetically efficient (Conkel 1993; Barlow 2000). Thus, juvenile convict cichlids might be expected to forage primarily in a head-down position and shift to the potentially less profitable head-up position under conditions of increased perceived predation risk.

Recent evidence suggests that freshwater prey fishes can use chemosensory information to make threat-sensitive behavioural decisions (Chivers *et al.* 2001; Brown and Magnavacca 2003; Marcus and Brown 2003; Brown *et al.* 2004a; Kim *et al.* in press). A wide range of taxonomically diverse aquatic vertebrates and invertebrates rely on chemosensory cues to assess local predation risk and to respond with context appropriate behaviour patterns (Chivers and Smith 1998; Kats and Dill 1998; Wisenden 2000; Brown 2003). Damage-released chemical alarm cues (see Chapter 1) elicit dramatic short-term increases in species-typical antipredator behavioural modalities (i.e., an overt antipredator response, Smith 1999; Mirza and Chivers 2001, see also Chapter 4). The relative concentration of chemical alarm cues can also provide relevant information regarding local predation risk (Brown *et al.* 2001; Mirza and Chivers 2003a). Given that risk should be inversely related to distance to predation threats (Lawrence and Smith 1989), lower concentrations could indicate lower risk (Marcus and Brown, 2003; Dupuch *et al.* 2004). In addition, it is known that prey fishes can use alarm cues at subthreshold concentrations. For example, glowlight tetras (*Hemigrammus erythrozonus*) increase vigilance towards visual predator cues in the presence of chemical alarm cues below the concentration required to elicit an overt antipredator response (Brown *et al.* 2004a, see also Chapter 4).

Prey fishes may also gain information about predation risk from predator diet cues (reviewed in Chivers and Mirza 2001). The presence of conspecific and/or heterospecific prey in the diet of a predator may chemically label the predator as dangerous (Mathis and Smith 1993a; Brown *et al.* 1995). Individuals exposed to the chemical cues of a novel predator fed a diet containing either the prey's conspecifics or sympatric heterospecifics exhibit increased antipredator responses (Brown *et al.* 1995) and are able to acquire the recognition of the predator (Mathis and Smith 1993a; Mirza and Chivers 2003b). Responding to both damage-released chemical alarm cues and recognizable predator odours is known to confer a significant survival benefit (Mathis and Smith 1993b; Mirza and Chivers 2000).

While previous studies have demonstrated threat sensitive (i.e. graded) trade-offs by prey fishes towards visual predator cues (Helfman 1989; Bishop and Brown 1992; Chivers *et al.* 2001), similar graded responses towards chemosensory cues are not found in aquatic prey species (Chivers *et al.* 2001; Brown *et al.* 2001; Mirza and Chivers 2003a). In this experiment, I tested the influence of chemical alarm cues on foraging posture by exposing juvenile convict cichlids to supra and sub-threshold levels of damage-released alarm cues (Experiment 1) and various predator diet cues (Experiment 2) while offering them a choice of two foraging positions (vertical or horizontal). I predicted that cichlids exposed to subthreshold alarm cues or predator odour cues would choose to change their foraging posture rather than overtly decrease it, thereby attempting to mitigate their risk while still maintaining some level of foraging. Specifically, I predicted that convict cichlids exposed to concentrations of alarm cues below the threshold required to elicit an overt antipredator response or the odour of a predator fed a prey fish diet would adopt a threat sensitive head up foraging posture with no overall change in foraging rate.

Methods

Experiment 1: concentration effects

Test fish

Juvenile convict cichlids originated from laboratory stock populations. Prior to the study, cichlids were held in 110 L aquaria, filled with continuously filtered, dechlorinated tap water (27°C, pH 7.2, 12:12 light:dark cycle) and were fed *ad libitum*, twice daily, with commercial flake food. Green swordtails (*Xiphophorus hellerii*), used as a diet cue (see below), were obtained from a commercial supplier and held under the same conditions as the cichlids. Adult cichlids, used as predator odour donors (see below), were also from laboratory populations and were held individually in 37 L aquaria, under the same conditions as the juvenile cichlids.

Stimulus preparation

I prepared skin extract for the alarm cue stimulus from 25 juvenile convict cichlids (Mean standard length ± S.E. = 5.18 ± 0.10 cm); the donor fish were sacrificed with a blow to the head (in accordance with Concordia University's Animal Care Protocol AC-2002-BROW). Skin was removed from both sides of the fish and placed immediately in 50 ml of chilled, glass-distilled water. I collected a total of 184.15 cm^2 of skin from the cichlids, homogenized and filtered the extracts through filter floss to remove any tissue and adjusted the final volume to 2080 ml with glass distilled water. The resultant concentration was similar to that used in previous studies (Lawrence and Smith 1989; Brown *et al.* 2004a). I froze the resultant solution at -20°C in 40 ml aliquots until required, and diluted the cichlid skin extract to 10% as needed for use in the treatments and the trials, by adding 1ml of thawed full concentration skin extract to 9 ml of distilled water. I discarded any unused portion of the skin extract. 10% was chosen as the sub-threshold concentration because my preliminary tests revealed that there was no significant difference in foraging rate or aggressive interactions between this low concentration of skin extract and distilled water. Previously, Wisenden and Sargent (1997) and Brown *et al.* (2004b) demonstrated that a reduction in foraging and aggression (see below) are reliable indicators of an antipredator response in juvenile convict cichlids under laboratory conditions. As a control, I froze distilled water in 40 ml aliquots at –20 °C as well.

Experimental protocol

My experimental food patches consisted of standard glass microscope slides (2.6 cm X 7.6 cm) dipped in gelatin and covered with Kyowa^{TM} food pellets (1.0 mm in diameter). The amount of food on each slide was more than could be consumed during an observation, in an attempt to control for competition between the test fish. For two days prior to testing, I conditioned the test fish in the communal holding tanks to the presence of the experimental food patches by allowing the cichlids to feed *ad libitum* on the slides.

I chose fish of similar size (Mean standard length ± S.E. = 2.80 ± 0.05 cm) from the communal tank and randomly assigned them to test tanks. They were placed into the 37L test tanks in pairs and allowed a 24 hr acclimation period prior to observations. The set-up of the test tanks was identical to that of the holding tanks and I fed all fish equal amounts of food the evening before the test day and the morning of the test day (at least 2 hours before testing). During observations, the test fish were allowed to freely forage on paired microscope slides (patches) positioned near the front of the test tanks. I positioned one slide horizontally on the gravel, and one vertically (> 80° angle) leaning on the side of the tank; the two slides were approximately 5 cm from each other. This arrangement was done to eliminate the possible bias of cichlids simply foraging on the first available patch and both slides were easily accessible to the fish. I alternated the location of the slides to avoid possible location preferences. Each pair of cichlids was tested once and I performed 10 replicates of each treatment.

Trials were videotaped and lasted six minutes, the tape was started during the first minute and the two food slides were placed on the gravel on one side of the tank. Once the slides were in place, I injected 10 ml of either 10% (sub-threshold) or 100% (full strength) concentration of skin extract, or a control of 10 ml of distilled water into the tank using a syringe and a length of silicone air tubing. The trial was then videotaped for a further five minutes. From the videotapes I measured per capita foraging rates (horizontal versus vertical) and aggression, defined as the frequency of chasing and biting. Foraging was defined as 'pecking at a food item while hovering above either patch' (Grant *et al.* 2002). Chasing was defined as 'an accelerated swim towards another cichild' (Keeley and Grant 1993). A bite was defined as 'one fish striking another with its mouth' (Keeley and Grant 1993). An increase in shoal cohesion and a decrease in aggressive interactions are indicative of an antipredator response in juvenile cichlids (Wisenden and Sargent 1997; Alemadi and Wisenden 2002; Brown *et al.* 2004a,b). I collected data for five minutes following the complete injection of the stimulus into the tank. The data were normally distributed. I made comparisons on per capita foraging and aggression rates across the three treatments using one-way ANOVAs. I made subsequent post-hoc comparisons using the Fisher's Protected Least Square Differences test (Sokal and Rohlf 1995).

Experiment 2: effects of predator diet cues

Stimulus preparation

I held adult convict cichlids (Mean standard length ± S.E. = 9.28 ± 0.15 cm) individually in 37L aquaria, equipped with a gravel substrate and filter. They were fed juvenile cichlid or swordtail, chopped into appropriately sized pieces and measured as 4.0-4.5 ml by volumetric displacement in water, or similar volumes of vegetable based commercial food pellets (Wards^{TM}) for four days (two cichlids per diet treatment, N = 6). After four days of feeding, the predators were transferred to identical tanks, which were aerated, but not filtered. Predators were not fed once transferred to the new tanks. Two days later, I collected 500 ml of water from each tank, filtered it through polyester floss (to remove any particles), and froze it in 20 ml aliquots at -20°C until needed. As a control, I also froze 20 ml aliquots of distilled water.

Experimental protocol

All of the set up for the tanks and the trials was identical to that for Experiment 1. Fish were exposed to diet cues of either 1) cichlid fed cichlid, 2) cichlid fed swordtails, or 3) cichlid fed vegetable pellets. I chose pairs of fish of similar size (Mean standard length ± S.E. = 2.96 ± 0.106 cm) from a general population tank and randomly assigned them to test tanks. I performed ten replicates of each treatment in which fish were used only once. The data were normally distributed and I collected and analyzed the data as in Experiment 1.

Results

Experiment 1: concentration effects

I found a significant treatment effect on both mean rates of aggressive interactions (ANOVA: $F_{2,27} = 3.51$, $P = 0.04$; Figure 1B) and foraging (ANOVA: $F_{2,27} = 4.61$, $P = 0.02$; Figure 1A). Cichlids exposed to the full strength skin extract stimulus exhibited significantly lower rates of both aggressive interactions (Figure 1A) and foraging (Figure 1B), while those exposed to the sub-threshold skin extract stimulus were not significantly different from the distilled water controls (FPLSD, Figures 1A,B). Decreases in aggression and foraging behaviour are typically seen as overt antipredator responses that are not present with subthreshold concentrations of alarm cue.

Likewise, I found a significant treatment effect on the proportion of foraging attempts directed towards the horizontal versus vertical food patches (ANOVA: $F_{2,27} = 5.74$, $P < 0.01$; Figure 1C). Cichlids exposed to the distilled water control directed a significantly greater proportion of foraging attempts at the horizontal versus vertical food patch, compared to those exposed to the full strength or sub-threshold skin extract stimuli. There was no significant difference in the proportion of foraging attempts directed at the horizontal versus vertical patch for cichlids exposed to either full strength or sub-threshold skin extract stimuli (Figure 1C).

Experiment 2: effects of predator diet cues

I found no significant treatment effect on either mean rate of aggression (ANOVA: $F_{3,36} = 0.90$, $P = 0.45$, Figure 2B) or foraging (ANOVA: $F_{3,36} = 0.70$, $P = 0.56$, Figure 2A). I did, however, find a significant effect of treatment on the proportion of foraging attempts directed towards horizontal versus vertical foraging patches (ANOVA: $F_{3,36} = 4.99$, $P = 0.01$, Figure 2C). Juvenile cichlids exposed to the odour of adult conspecifics fed either the cichlid or swordtail diets directed a significantly greater proportion of foraging attempts at the vertical food patch (FPLSD, Figure 2C) compared to those exposed to distilled water or a vegetable diet cue.

Discussion

My results demonstrate that juvenile convict cichlids rely on chemosensory cues to assess local predation risk and make threat-sensitive trade-offs between foraging and antipredator behaviour patterns. The results of Experiment 1 demonstrate that in the presence suprathreshold concentrations of conspecific chemical alarm cues, cichlids exhibited a significant decrease in foraging and aggressive interactions. These responses are overt antipredator behaviour patterns that are typical of exposure to suprathreshold alarm cues (Wisenden and Sargent 1997; Brown *et al.* 2004b). In addition, individuals directed a significantly higher proportion of foraging attempts towards the less risky vertical foraging patch (i.e. foraged in a head-up posture). Individuals exposed to the subthreshold concentration of alarm cues did not exhibit an overt antipredator response (i.e., foraging rate and frequency of aggressive interactions were not different from the distilled water control), however they did display caution by switching foraging to the vertical patch. The results of Experiment 2 demonstrated that while predator dietary cues did not elicit an overt antipredator response, cichlids exposed to the odour of predators fed prey fishes (swordtail or conspecifics) directed more foraging attempts towards the less risky vertical foraging patch, but not when exposed to the odour of a predator fed a vegetable diet or a distilled water control. Taken together, my data strongly suggest that juvenile convict cichlids use ambient chemosensory information to assess local predation risk and make threat-sensitive decisions regarding the trade-off between foraging and predator avoidance. Individuals capable of making such threat-sensitive trade-offs should be at a selective advantage, as it would allow them to maintain some foraging benefits while reducing their risk of predation.

Recent studies have demonstrated that a variety of prey fishes are able to detect conspecific chemical alarm cues at concentrations below the level required to elicit an overt (observable) behavioural response, and that such sub-threshold cues provide information regarding local predation risk. For example, Brown *et al.* (2001) demonstrated that fathead minnows could learn to recognize the odour of a novel predator when the predator cue is paired with hypoxanthine-3-*N*-oxide (the active component of the Ostariophysan alarm cue, Brown *et al.* 2000; 2003) at concentrations well below that required to elicit an overt antipredator response. Mirza and Chivers (2003a) have recently shown that juvenile rainbow trout (*Oncorhynchus mykiss*) gain measurable survival benefits during staged encounters with live predators when exposed to conspecific skin extracts well below the minimum behavioural response threshold. Finally, glowlight tetras increase their use of secondary (visual) cues when exposed to sub-threshold concentrations of hypoxanthine-3-*N*-oxide (Brown *et al.* 2004a). In response to these sub-threshold cues, tetras responded faster and more intensely to a visually displaying conspecific compared to those treated with a distilled water control (Brown *et al.* 2004a). It has been previously argued (Brown *et al.* 2001; 2004b; Mirza and Chivers 2003b) that increasing vigilance towards secondary information (e.g. visual cues) in the presence of low concentrations of chemical alarm cues would allow individuals to maximize the threat-sensitive trade-offs between antipredator and other fitness related activities such as mating, territorial defense and foraging. My current results demonstrate that juvenile cichlids can gauge current risk based on ambient chemosensory information and switch from the profitable ‘head-down’ foraging tactic to a potentially less profitable but more risk aversive foraging pattern. By doing so, individuals can continue to actively forage while still gaining some antipredator benefit.

The results of Experiment 2 demonstrate that the presence of predator diet cues elicited a threat-sensitive shift in foraging patterns, similar to that seen for conspecific alarm cues. While I did not see an overt antipredator response, I did find a significant increase in the proportion of foraging attempts directed towards the vertical versus the horizontal food patch by cichlids exposed to the odour of an adult cichlid fed either juvenile cichlids or swordtails. Previous work has shown similar threat-sensitive changes in predator inspection behaviour by prey fishes exposed to the odour of piscivorous predators (Brown and Godin 1999; Brown and Magnavacca 2003; Brown 2003). The observation that juvenile cichlids did not differentiate between the odours of a predator fed cichlids versus swordtails is contrary to previous findings in other prey fishes (reviewed in Chivers and Mirza 2001) and suggests that juvenile cichlids perceive any piscivorous predators as risky. Recent studies by Berejikian *et al.* (2003) and Vilhunen and Hirvonen (2003) found similar results for juvenile salmonids exposed to the odours of predators fed piscivorous diets.

Threat-sensitivity implies a trade-off between the threat a predator imposes and the needs of the prey (Lima and Dill 1990). While I did not directly measure the energetic efficiency of foraging in a head-up versus head-down posture, it is likely that foraging on vertical surfaces represents an energy cost to juvenile cichlids. Juvenile convict cichlids are usually considered to be primarily benthic foragers (Conkel 1993) and the zoobenthos they eat is not typically found on rock faces but rather on the substrate and other horizontal surfaces. Therefore, for juveniles, foraging in a head-up position is likely to reduce the quality and/or quantity of food available.

The presence of conspecific alarm cues (both supra and sub-threshold) or predator diet cues strongly influenced juvenile cichlid foraging posture. My results suggest that under conditions of increased perceived predation risk, juvenile cichlids modify their foraging posture. Similar results have been demonstrated for birds (Lima and Bednekoff 1999, Guillemain *et al.* 2001; Bednekoff and Lima 2002) and mammals (Arnez and Leger 1997a,b). By doing so, individuals should be better able to maximize the threat-sensitive trade-off between foraging gains and antipredator benefits. This research serves to highlight the intricacies of predation risk assessment and the sophistication with which prey use chemical information in their environment to gain a survival advantage.

Figure 2.1 Mean (± standard error) per capita foraging rate/minute (A), per capita rate of aggression/minute (B), and proportion of foraging attempts directed towards vertical food patch (head-up posture)/minute (C) for cichlids exposed to: distilled water (DW), sub-threshold concentration of skin extract (10% SE), and full concentration skin extract (100% SE).

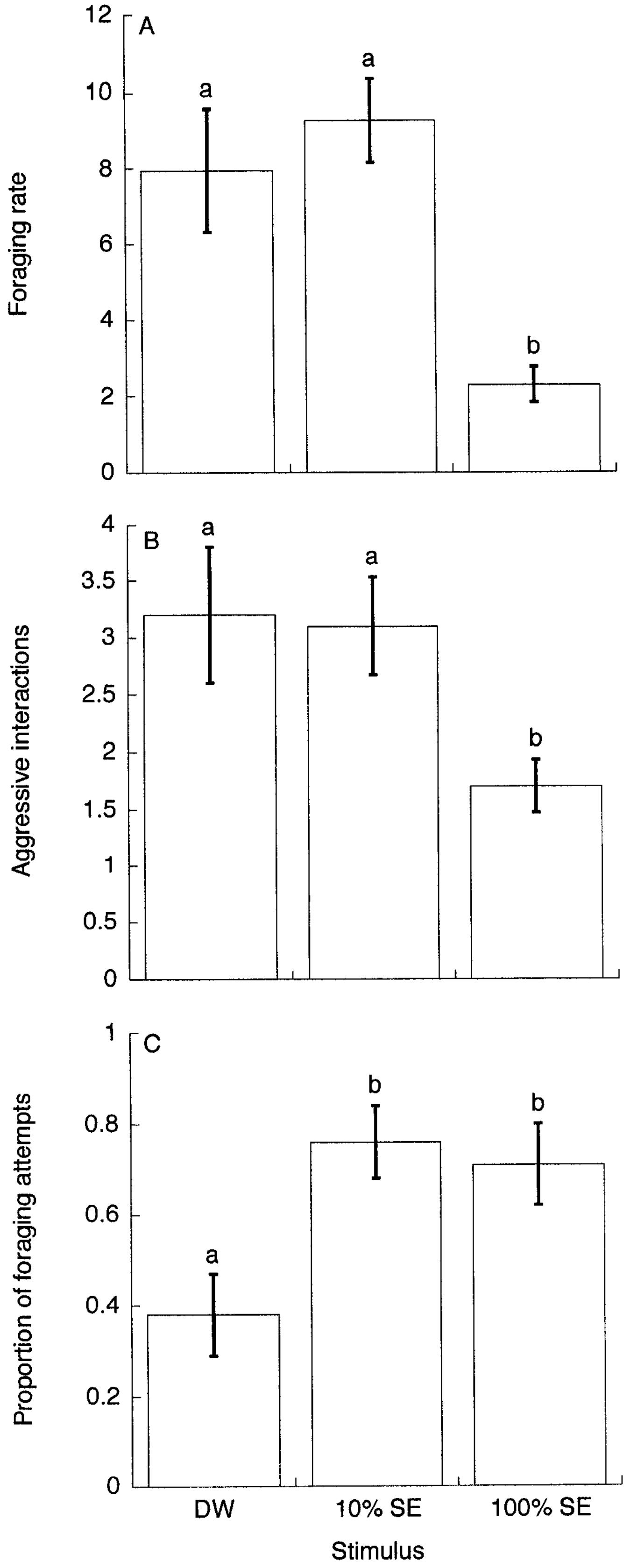
A
Foraging rate
0
2
4
6
8
10
12
a
a
b
B
Aggressive interactions
0
0.5
1
1.5
2
2.5
3
3.5
4
a
a
b
C
Proportion of foraging attempts
0
0.2
0.4
0.6
0.8
1
a
b
b
DW
10% SE
100% SE
Stimulus

Figure 2.1

Figure 2.2 Mean (± standard error) per capita foraging rate/minute (A), per capita rate of aggression/minute (B), and proportion of foraging attempts directed towards vertical food patch (head-up posture) /minute (C) for cichlids exposed to: distilled water (DW), predators fed vegetable diet (VEG-diet), predators fed juvenile cichlids (CC-diet) or predators fed swordtails (SWT-diet).

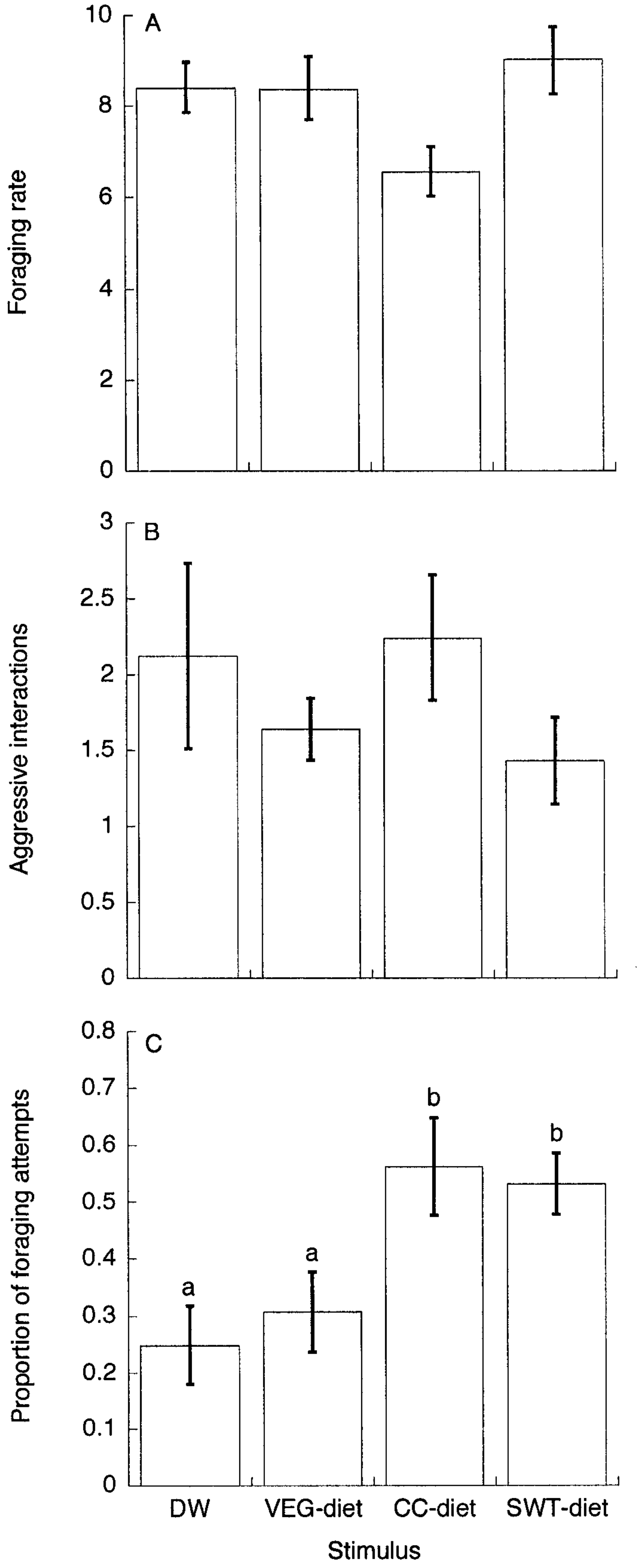
A
Foraging rate
10
8
6
4
2
0
B
Aggressive interactions
3
2.5
2
1.5
1
0.5
0
C
Proportion of foraging attempts
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
a
b
b
DW
VEG-diet
CC-diet
SWT-diet
Stimulus

Figure 2.2

Chapter 3. Juvenile convict cichlids allocate foraging in response to temporal variation in predation risk

Introduction

Prey individuals are continually faced with the daily conflict between foraging and predator avoidance (Lima and Dill 1990; Houston *et al.* 1993). This conflict is due to the fact that foraging activity leading to maximal food intake often leaves prey vulnerable to predators (Sih 1992; Sinclair and Arcese 1995; Elgar 1989; Werner and Anholt 1996, Werner and Peacor 2003). Antipredator behaviour can be considered costly, since it may reduce potential fitness benefits associated with foraging, mating or other behaviour patterns (Lima 1998). Individuals capable of reliably assessing local predation risk would be able to adjust the intensity of their antipredator behaviour accordingly (Lima and Dill 1990; Wisenden 2000). Thus, the response to predation pressure, at the individual level, is shaped by a series of threat-sensitive trade-offs between the benefits of predator avoidance and those of a suite of other fitness related behavioural modalities such as foraging (Lima and Dill 1990; Krause and Godin 1996; Werner and Peacor 2003). Individuals will be at a selective advantage if they are capable of optimizing this energetic trade-off, for which they need to be able to accurately assess local predation risk.

Convict cichlids can use damage-released chemical alarm cues, as well as predator odour and visual cues, as a means of assessing local predation risk. Upon detecting these chemical alarm cues cichlids exhibit the antipredator responses of decreased foraging activity, decreased activity, increased group cohesion, and area avoidance (Chivers and Smith 1998). As the concentration of chemical alarm cue decreases past the minimum behavioural threshold (Lawrence and Smith 1989; Brown *et al.* 2001), the above behavioural responses are no longer visible. Recently, studies have demonstrated that prey fishes can use these low concentration cues as a source of information in order to make threat-sensitive decisions about antipredator response intensity, duration, or foraging postures, see Chapter 1. (Brown *et al.* 2001; Marcus and Brown, 2003; Mirza and Chivers, 2003b; Brown *et al.* 2004a).

The level of predation risk in an individual's natural environment can vary over time, from year to year, season to season or even moment to moment due to changes in local prey and predator guild membership, prey movements through varying microhabitats and/or movement of potential predators (Sih *et al.* 2000). This temporal variation in predation risk can influence a prey individual's antipredator and foraging behaviour (Lima and Bednekoff 1999). The distribution of foraging and antipredator effort should reflect the frequency of risky and safe periods experienced rather than the absolute level of risk *per se*. If risky periods are infrequent then prey should exhibit higher intensity antipredator behaviour and greatly reduced feeding during those rare periods of risk, and during periods of safety they should forage at some moderate rate necessary to meet their energy requirements. When periods of risk are longer or more frequent prey should still exhibit antipredator behaviour during periods of risk, however, they should forage relatively more intensely during rare safe periods in order to meet their minimum energy requirements (Lima and Bednekoff, 1999; Sih and McCarthy, 2002). This prediction represents the reality that when the risk of predation is constantly high, antipredator behaviour becomes more costly if the individual is to meet its minimum energetic requirement or find a mate.

The majority of predator-prey studies have viewed a prey's response to a predator as a single snapshot in time (Lima and Dill 1990; Chivers and Smith 1998; Chivers and Mirza 2001), when in fact both prior experience and temporal variation can influence a prey's response. Predator-prey studies have generally not taken temporal variation in predation risk into account. Temporal variation in predation risk has been examined in only a handful of studies within the context of the predation risk allocation hypothesis. Hamilton and Heithaus (2001), using marine snails (*Littorina* spp.) as prey and crabs (*Cancer productus* and *C. magister*) as predators, found that with increased time spent at risk a greater number of snails were active during the risky periods, but showed no influence of time spent at risk on activity during low risk periods. Sih and McCarthy (2002), using freshwater physid snails (*Physa gyrina*) as prey and crayfish (*Orconectes juvenilis*) as predators, found that with increased time spent at risk snails were more active (interpreted as foraging) during safe periods, but when less time was spent at risk the snails showed only weak response to predation risk. Similarly, Gude (2004) demonstrated that in areas where frequency of predation risk from wolves was high, elk (*Cervus elaphus*) had a more dynamic distribution and tended to move during periods of safety following wolf encounters in those areas. Other studies have found no influence of temporal variability in predation risk, in pool frog tadpoles (*Rana lessonae*), crayfish (*Orconectes* spp.), and bank voles (*Clethrionomys glareolus*) (Van Buskirk *et al.* 2002; Pecor and Hazlett 2003; Sundell *et al.* 2004, respectively). Given the varying results in previous studies the relative importance of temporal variability in risk is still unclear and needs to be investigated (Sih *et al.* 2000).

This experiment consisted of two hypotheses. My first hypothesis was that temporal variability in perceived predation risk would influence foraging and antipredator behaviour. I tested this by creating treatments that differed in frequency of predation risk and concentration of alarm cue. I conditioned juvenile convict cichlids (*Archocentrus nigrofasciatus*) to high (suprathreshold) or low (subthreshold) concentrations of conspecific alarm cue given at a high or low frequency. The theory behind temporal variation of predation risk predicts that under high frequency of risk fish should exhibit less intense antipredator behaviour and reduced foraging during risky periods while foraging intensely during safe periods. Alternatively, fish conditioned to low frequency of predation risk should display intense antipredator behaviour and reduced foraging during periods of risk and moderate levels of foraging during periods of safety.

My second hypothesis was that an increase in temporal variation of perceived predation risk might result in the fish habituating to the conditioning cue.
I tested this by comparing the foraging levels during both risky and safe periods that resulted from the experimental setup mentioned above. The habituation hypothesis predicts that habituation is having a strong influence if fish under a high frequency of risk exhibit less intense antipredator behaviour and only slightly reduced foraging during risky periods, compared to fish under low frequency. The hypothesis also predicts that if fish are exhibiting habituation to the conditioning cue their foraging levels should be similar during a safe period regardless of frequency. My design gave me the ability to distinguish between these predictions.

The conditioning was done with both suprathreshold and subthreshold concentrations of alarm cue in order to test the hypothesis that subthreshold cues would provide usable information in the same manner as suprathreshold cues. If fish perceived the subthreshold alarm cues as differing in predation risk from the suprathreshold cues, I predicted they would respond with higher levels of antipredator behaviour and foraging to the suprathreshold versus the subthreshold cues. If the two concentrations of alarm cue were perceived as similar levels of risk and gave similar information I predicted the fish would exhibit similar levels of antipredator behaviour and foraging activity with cues of both concentrations.

Methods

Juvenile convict cichlids originated from a laboratory stock population, and green swordtails were obtained from a commercial supplier. Cichlids and swordtails were held, separately, in 110 L aerated aquaria, and filled with continuously filtered de-chlorinated tap water (26° C, pH 7.2) under a 12:12 light: dark cycle. Fish were fed *ad libitum* twice daily with brine shrimp (*Artemia* spp.) and commercial flake food.

Stimulus preparation

I collected skin extract from 25 juvenile convict cichlid donors (mean ± SD length = 5.10 ± 0.47 cm) and 6 swordtail donors (5.05 ± 0.29 cm). Donor fish were sacrificed with a blow to the head (in accordance with Concordia University's Animal Care Protocol AC-2002-BROW). Skin fillets were collected from both sides of donors and immediately placed into 50 ml of chilled, glass-distilled water. I then homogenized the samples, filtered them through polyester floss (to remove any particulate matter) and adjusted the final volume with the addition of distilled water. I collected a total of 184.15 cm^2 (in 2080 ml) and 44.44 cm^2 (in 875 ml) of skin for cichlid and swordtail, respectively. This skin extract was utilized as the full concentration (100%) cichlid alarm cue; I produced the 20% cue by diluting this with distilled water. According to the preliminary observations I conducted and those of Roh *et al.* (in press), my 20% alarm cue was just below the response threshold for juvenile convict cichlids (~ 25%). Swordtail alarm cue served as my control for the odour of any injured prey fish because while they possess an analogous chemical alarm cue system (Mirza *et al.* 2001), it is generally not recognized by juvenile cichlids (Brown *et al.* 2004a, b).

Conditioning

My conditioning tanks consisted of a series of 48 L opaque plastic basins (48 x 38 x 20 cm), filled with 23.0 L of dechlorinated tap water. Conditions were the same as described above with the exception that the water was not filtered. An overflow valve was positioned approximately 5 cm from the upper edge of the basin to facilitate water changes.

The conditioning phase consisted of exposing groups of 25 fish to one of four conditioning regimes (1) 100% alarm cue administered three times (3X) per day; (2) 100% alarm cue administered once (1X) per day; (3) 20% alarm cue administered three times (3X) per day; and (4) 20% alarm cue administered once (1X) per day. I conditioned fish for three days and on the fourth day I tested them in pairs with a high-risk cue (100% convict cichlid alarm cue) or a control (100% swordtail alarm cue).

I injected stimuli next to the air stone in series of three pulses, each separated by ten minutes, at 12:00 in the 1X treatments and at 7:30, 12:00, and 16:30 in the 3X treatment. The injections were given in bursts (a series of three 10 ml injections of alarm cue) to better simulate natural patterns of risk, individuals will generally experience risk in pulses and multiple exposures (convict cichlids, Ian Hamilton personal communication). Ten minutes after the last injection I slowly introduced 18L of de-chlorinated water into the basin to flush out most of the alarm cue between injections. These water changes were conducted 3X per day for all bins regardless of treatment frequency to control for any disturbance effects. I never gave conditioning injections in combination with feeding. Fish were fed morning and afternoon and were all fed similar amounts of commercial flake food.

Testing phase

My test tanks consisted of a series of 37 L aquarium tanks, equipped with a single air stone and an additional length of tubing to allow for the injection of control and experimental stimuli from a distance of at least 2 m. Tanks contained a gravel substrate and were filled with dechlorinated tap water, but were not filtered. Temperature and lighting were identical to the holding tanks. I positioned the test tanks behind a black plastic viewing blind. All observations were videotaped for later behavioural analysis.

Twenty-four hours prior to testing, I placed pairs of juvenile convict cichlids, matched for size (2.76 ± 0.16 cm) from one of the conditioning bins into the test tanks.

Test fish were fed similar amounts of food the evening before the test day as well as the morning of testing. They were fed enough to ensure that some flakes once saturated with water would sink to the substrate, providing foraging opportunities during the trials. The trials consisted of a 10-minute pre-stimulus period and a 10-minute post-stimulus observation period, at the start of the post-stimulus period I injected 10 ml of 100% cichlid skin extract or swordtail skin extract into the test tank near the air stone. Cichlids were tested only once. My pre-stimulus period represented a period of safety for the test fish, as it was the longest length of time before the introduction of an alarm cue, while my post-stimulus period represented a risky period. I conducted 10 replicates for each of the four treatment regimes, 1 X 100%, 1 X 20%, 3 X 100%, 3 X 20%.

The trials were videotaped and to test my antipredator predictions, I obtained per capita (per fish) vertical area use, distance to neighbour (shoaling), and time spent moving every 15 sec, from both the pre-stimulus and post-stimulus periods of the trials. Vertical area use was scored as the position of each cichlid within the tank, and ranged from 1 (near substrate) to 4 (near water surface), shoaling was measured as distance in mm between the two fish, and time spent moving was recorded as the total time in seconds spent swimming. These behavioural patterns are typical of convict cichlids in response to predation risk, particularly increases in shoaling, and decreases in time spent moving and area use (Wisenden and Sargent 1997).

To test my foraging prediction, I obtained the number of per capita substrate foraging attempts from the pre-stimulus periods of the trials; I also recorded foraging activity in the post-stimulus periods to determine whether the cichlids had become habituated to the alarm cue during the conditioning period. I defined foraging rate as the number of food items (leftover flake remains that had sunk to the bottom from the morning feeding) taken from the substrate per minute.

Statistics

For time spent moving, distance to neighbour, and area use, I calculated the difference between the pre-and post-stimulus observation periods. I then used these difference scores as dependant variables in a MANOVA, with stimulus type (cichlid versus swordtail skin extract), concentration (100% vs. 20%), and frequency (1X versus 3X) as the independent variables. This was followed by subsequent 3-way ANOVAs on all significant response variables.

I calculated the per capita foraging attempts during both the pre-stimulus (safe) and post-stimulus (risky) observation periods. To determine foraging activity for t he temporal variation and habituation hypothesis, I used foraging observations from the pre-stimulus period in an ANOVA with stimulus type, concentration and frequency as independent variables. As a further test for habituation to the alarm cue I compared foraging during the safe period versus the risky period, for the conditioning regimes of 3 X 100% and 3 X 20%, using paired t-tests with alpha set at 0.0125 (Bonferonni method: Sokal and Rohlf 1995) to control for increasing Type 1 error rates.

Results

I found significant main effects of both test stimulus and conditioning frequency on the antipredator behaviour of cichlids, but no significant main effect of conditioning concentration and no significant interactions (MANOVA, Table 3.1).

For time spent moving, shoaling and area use I found a significant effect of frequency. Fish conditioned three times per day spent significantly more time moving, stayed significantly farther apart, and used a significantly greater area of the tank than those conditioned once per day. I also found a significant effect of test stimulus on time spent moving and shoaling. Fish exposed to cichlid alarm cue spent significantly less time moving and spent more time significantly closer together than those exposed to the control cue (ANOVA, Table 3.1, Fig. 3.1).

During periods of safety, fish conditioned three times per day made significantly more foraging attempts compared to those conditioned once per day, regardless of the concentration of alarm cue during conditioning or the test stimulus used (conspecific versus swordtail control) (ANOVA, Table 3.2, Fig. 3.2 – Safe period). During periods of risk, fish conditioned three times per day foraged significantly more than fish conditioned once per day. Also, fish that were exposed to conspecific test stimulus significantly reduced their foraging rate compared to those exposed to the swordtail test stimulus.

Fish conditioned with suprathreshold alarm cue three times per day significantly decreased their foraging attempts upon introduction of the cichlid test stimulus (t_9=3.209, P=0.011; Fig. 3.2), however fish exposed to the control stimulus of swordtail did not (t_9=-0.363, P=0.725; Fig. 3.2). Similarly, fish conditioned three times per day with subthreshold alarm cue significantly decreased their foraging attempts in response to the test stimulus (t_9=3.184, P=0.011; Fig. 3.2) but not the control stimulus (t_9=0.284, P=0.783; Fig. 3.2).

Discussion

My results demonstrate that temporal variation in predation risk influences both antipredator behaviour and foraging effort in juvenile convict cichlids. During the testing phase, cichlids conditioned three times per day allocated significantly more foraging attempts to safe periods (pre-stimulus) compared to fish conditioned once per day, regardless of the concentration of alarm cue during conditioning. No matter what conditioning treatment cichlids were given, they exhibited an antipredator response when exposed to conspecific alarm cues versus control (swordtail). However, this response was significantly less intense for cichlids conditioned three times per day than for those conditioned once per day.

Conditioning concentration had no significant effect on the antipredator or foraging responses of my test fish, when it came to acquisition of information during the conditioning period they responded to both 100% and 20% in the same manner. Past studies have found that sub-threshold alarm cue concentrations elicit more imperceptible behavioural responses, but still provide usable information about predation risk (Brown *et al.* 2001; Brown, in press; Mirza and Chivers, 2003b; also see Chapter 2). My results demonstrate that when risk is varied temporally, fish conditioned with subthreshold alarm cues will still react in a manner similar to previous studies with single exposures of subthreshold cues.

The majority of studies that have examined temporal variation have done so within a risk allocation framework. The Predation Risk Allocation Hypothesis (RAH) asserts that recent temporal variation in perceived predation risk has an underlying influence on present antipredator and foraging decisions (Lima and Bednekoff, 1999).

Via the RAH, influences of temporal variation have been found on only foraging behaviour (Hamilton and Heithaus, 2001), on activity level (Sih and McCarthy, 2002), and on distribution and movement (Gude, 2004). Other studies have found no influence of temporal variation (Van Buskirk *et al.* 2002; Koivisto and Pusenius, 2003; Pecor and Hazlett, 2003; Sundell *et al.* 2004). I have found that temporal variability (indicated in my study by a change in frequency of predation risk) influences both foraging and antipredator behaviour. However more research involving temporal variation is needed. While temporal variation in predation risk had an influence in my study, it may not in all systems due to different life histories or foraging methods.

A criticism has been raised suggesting that when risk is elevated in a high-risk environment, any decreases in activity may be due to habituation rather than a behavioural decision (Hamilton and Heithaus, 2001; Sih and McCarthy, 2002; Pecor and Hazlett, 2003). Although this is what I observed in terms of general activity, if cichlids had become habituated to conspecific alarm cues, then I should have seen similar levels of foraging activity between the safe and risky periods. However, foraging attempts during safe periods differed significantly from risky periods for those cichlids that had been conditioned three times per day. Since my fish responded to the conspecific alarm cue with an antipredator response, I believe that habituation is not the primary mechanism here. My results can be attributed to allocation of effort to foraging and antipredator behaviour in response to perceived levels of risk.

Temporal variation in predation risk in the real world environment of prey is a result of many factors, some environmental and others behavioural in nature. The variation in risk can be due to predators varying in seasonal abundance, in location, or even in species composition (Sih *et al.* 1985). Variation in risk can also vary due to the movements of prey themselves. These aspects can, in turn, fluctuate from year-to-year, season-to-season or even moment-to-moment. Thus prey must constantly assess their environment for risk and as a result an individual's prior experience can be used to make predictions about future behavioural decisions. To some extent the plasticity of behavioural responses seen is a reflection of this changing risk, enabling prey to make more informed, more threat-sensitive choices for each predator encounter. If at some point behavioural responses are no longer adequate over longer periods of time, then animals may develop morphological defenses or exhibit shifts in life-history traits to avoid predation (Harvell and Tollrian, 1999).

There have been many laboratory experiments performed to test the effect of predation risk on antipredator behavioural patterns and other fitness related behavioural traits. Traditionally these studies have shown that prey individuals have strong responses to the presence of a predator and/or risk of predation, and have often proven difficult to translate to the field. The source of some of this difficulty may lie in the standard research protocols employed in most behavioural research laboratories, where research subjects are traditionally kept under constant low predation risk, and are more than adequately fed at a constant rate. Furthermore, animals are often housed in barren environments lacking in structural complexity. This practice may result in an over estimation of antipredator behaviour in laboratory studies (Sih *et al.* 2000). While much of this protocol has been established along animal care guidelines, I would suggest that an effort should be made to incorporate patterns of temporal variability into future behavioural predator/prey studies.

In contrast with other studies, I have found an influence of temporal variation on both antipredator behaviour and foraging effort. Even when exposed to subthreshold alarm cues, I observed fish exhibiting allocation of foraging effort and threat sensitivity in a similar manner to that seen when exposed to suprathreshold cues. My results and other recent studies examining risk allocation confirm the need to be cautious when extrapolating the results of laboratory predation experiments to the field (Sih *et al.*, 2000). In this experiment the conditioning cue at the same time each day and held the amount of food constant. It would be interesting in future studies to vary the time between conditioning cues or the amount of food given. Temporal variation should be of significant importance to research involving threat-sensitive decisions made under the threat of predation, and trade-offs between antipredator and foraging behaviour. Prior experience with predation risk and temporal variation are deciding factors in the foraging and antipredator decisions prey make when under threat. Future studies should take this into account.

Table 3.1 Influences of test stimulus type (swordtail control versus conspecific alarm cue), conditioning concentration (100% versus 20%), and conditioning frequency (1X versus 3X) on time spent moving, shoaling, and area use. Juvenile cichlids were tested in pairs and all values are expressed as per capita. N=10 per treatment combination, with significant p's in bold.

Table 3.1

	F	df	p
Manova	Wilks' Lambda		
Test stimulus	9.561	3, 70	**<0.0001**
Frequency	3.250	3, 70	**= 0.027**
Concentration	0.488	3, 70	= 0.691
Test Stimulus x Frequency	1.602	3, 70	= 0.197
Test Stimulus x Concentration	0.723	3, 70	= 0.541
Frequency x Concentration	0.163	3, 70	= 0.921
Test Stimulus x Concentration x Frequency	0.498	3, 70	= 0.685
Anova			
Time spent moving			
Test stimulus	17.59	1, 72	**<0.0001**
Frequency	5.01	1, 72	**= 0.028**
Concentration	0.049	1, 72	= 0.825
Test Stimulus x Frequency	0.911	1, 72	= 0.343
Test Stimulus x Concentration	0.798	1, 72	= 0.375
Frequency x Concentration	0.266	1, 72	= 0.608
Test Stimulus x Concentration x Frequency	0.581	1, 72	= 0.448
Shoaling			
Test stimulus	14.58	1, 72	**<0.0001**
Frequency	4.159	1, 72	**= 0.045**
Concentration	0.326	1, 72	= 0.570
Test Stimulus x Frequency	2.378	1, 72	= 0.127
Test Stimulus x Concentration	0.110	1, 72	= 0.741
Frequency x Concentration	0.058	1, 72	= 0.811
Test Stimulus x Concentration x Frequency	0.809	1, 72	= 0.371
Area use			
Test stimulus	2.18	1, 72	= 0.144
Frequency	4.161	1, 72	**= 0.045**
Concentration	1.25	1, 72	= 0.267
Test Stimulus x Frequency	2.887	1, 72	= 0.094
Test Stimulus x Concentration	1.987	1, 72	= 0.163
Frequency x Concentration	0.270	1, 72	= 0.605
Test Stimulus x Concentration x Frequency	0.070	1, 72	= 0.792

Table 3.2 Test for foraging activity during safe and risky periods showing influence of stimulus type, (swordtail control versus conspecific alarm cue), conditioning concentration (100% versus 20%), and conditioning frequency (1X versus 3X) on foraging attempts (ANOVA). Juvenile cichlids were tested in pairs and all values are expressed as per capita. N=10 per treatment combination with significant p's in bold.

Table 3.2

ANOVA	F	df	p
Safe period			
Test stimulus	0.015	1, 72	= 0.904
Frequency	4.364	1, 72	**= 0.040**
Concentration	0.021	1, 72	= 0.885
Test Stimulus x Frequency	0.196	1, 72	= 0.659
Test Stimulus x Concentration	0.001	1, 72	= 0.982
Frequency x Concentration	0.462	1, 72	= 0.499
Test Stimulus x Concentration x Frequency	0.041	1, 72	= 0.841
Risky period			
Test stimulus	0.015	1, 72	= **0.003**
Frequency	4.364	1, 72	**= 0.003**
Concentration	0.021	1, 72	= 0.896
Test Stimulus x Frequency	0.196	1, 72	= 0.416
Test Stimulus x Concentration	0.001	1, 72	= 0.799
Frequency x Concentration	0.462	1, 72	= 0.203
Test Stimulus x Concentration x Frequency	0.041	1, 72	= 0.542

Figure 3.1 Mean (± S.E.) changes in: time spent moving per minute (A), shoaling per minute (B), and vertical area use per minute (C) for cichlids conditioned with suprathreshold, 100%, cichlid skin extract given once or three times per day (full1X or full3X), and subthreshold, 20%, cichlid skin extract given once or three times per day (sub1X or sub3X). Control stimulus was swordtail skin extract (☐); test stimulus was cichlid skin extract (■). Fish were tested in pairs and all values are expressed as per capita. N=10 per treatment combination.

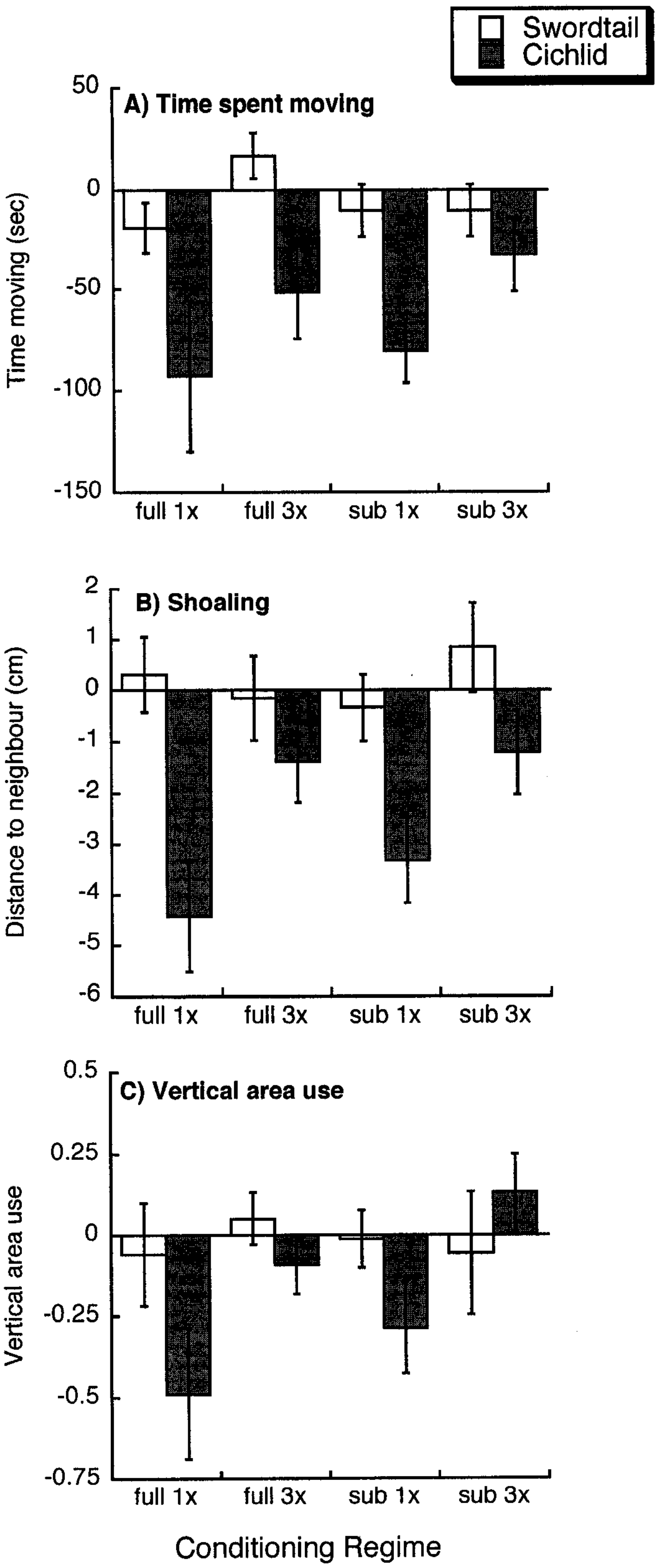
□ Swordtail
▨ Cichlid
A) Time spent moving
Time moving (sec)
50
0
-50
-100
-150
full 1x
full 3x
sub 1x
sub 3x
B) Shoaling
Distance to neighbour (cm)
2
1
0
-1
-2
-3
-4
-5
-6
full 1x
full 3x
sub 1x
sub 3x
C) Vertical area use
Vertical area use
0.5
0.25
0
-0.25
-0.5
-0.75
full 1x
full 3x
sub 1x
sub 3x
Conditioning Regime

Figure 3.1

Figure 3.2 Mean (± S.E.) number of foraging attempts during safe periods and risky periods, for cichlids conditioned with suprathreshold, 100%, cichlid skin extract given once or three times per day (full1X or full3X), and subthreshold, 20%, cichlid skin extract given once or three times per day (sub1X or sub3X). Control stimulus was swordtail skin extract (□); test stimulus was cichlid skin extract (■). Fish were tested in pairs and all values are expressed as per capita. N=10 per treatment combination.

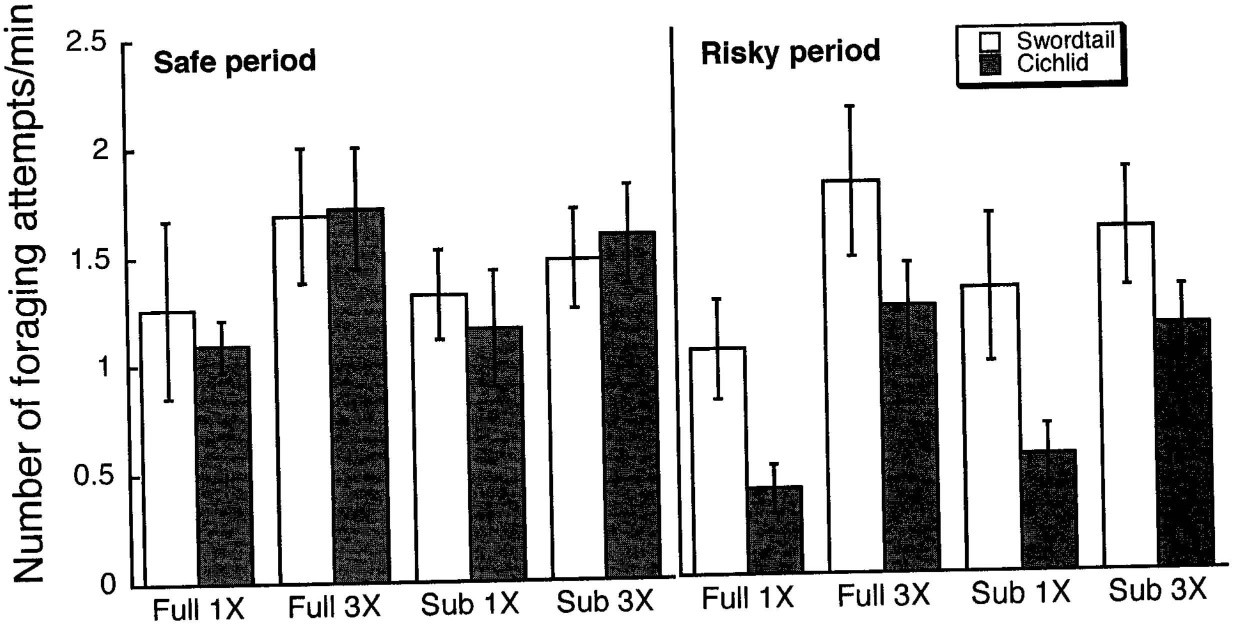
Number of foraging attempts/min
2.5
2
1.5
1
0.5
0
Safe period
Risky period
☐ Swordtail
■ Cichlid
Full 1X
Full 3X
Sub 1X
Sub 3X
Full 1X
Full 3X
Sub 1X
Sub 3X

Figure 3.2

Chapter 4. General discussion and conclusions

While other studies have demonstrated that freshwater prey fish will show threat-sensitive responses to visual cues of predation risk, my results indicate that this is also the case for their responses to damage-released chemical alarm cues. Chapters 2 and 3 indicate that juvenile convict cichlids will trade-off antipredator and foraging behaviour in a threat-sensitive manner in response to chemical alarm cues. In both chapters, the decisions made by fish in response to chemical cues differing in concentration and/or frequency, demonstrated an ability to subtly alter their behavioural responses in a manner that was appropriate to the pattern of predation risk they were experiencing.

Helfman (1989) proposed the threat-sensitivity hypothesis, which applies to a situation where prey are faced with a series of predation threats differing in magnitude or intensity. In both chapters 2 and 3, the predation threats differed in frequency of exposure and concentration of the cue used. Two of the assumptions inherent in threat-sensitivity are that 1) fish must have conflicting demands on their energy and time, and 2) there must be a trade-off between antipredator and other fitness-related behaviours. In both chapters 2 and 3, cichlids had conflicting demands of antipredator and foraging behaviour, and were forced to trade-off between them. A prey individual's experience with predation risk is likely quite complex, as the risk presented by a predator can change even within the same encounter (Helfman 1989).

The threat-sensitive hypothesis assumes that prey will respond to different magnitudes of predation risk with graded responses. The expectation is that with visual cues differing in intensity, prey will show different intensities of the same pattern of antipredator behaviour. For example, threespot damselfish exhibited increasingly stronger antipredator responses as the magnitude of risk was increased (Helfman 1989). Pacific treefrogs (*Hyla regilla*) also demonstrate threat-sensitivity in their antipredator responses (Puttlitz *et al.* 1999), as do American lobsters (*Homarus americanus*: Wahle 1992). The common element in these studies is that predation risk has been simulated using visual cues, and/or the presence of a live predator.

When examining threat-sensitivity to chemical alarm cues, an alternative to graded responses may be to have different behaviour patterns, rather than different intensities of the same behaviour patterns. My results indicated that juvenile convict cichlids chose to forage in a head up foraging posture, in response to both supra and subthreshold concentrations of alarm cue, rather than decreasing their foraging intensity. Furthermore, cichlids demonstrated a subtle change in foraging behaviour, by changing when they foraged, rather than reducing overall foraging in response to a high frequency of predation risk. These patterns are qualitatively different rather than quantitatively different, indicating that when chemical cues are present, prey may show subtle changes in behaviour rather than gradation in magnitude of the same behaviour.

Since chemical and visual cues provide information that can differ in quality and content, a prey fish's response to predation threats under natural conditions likely involves the use of both chemical and visual cues of predation risk. While visual cues are spatially and temporally reliable, they are inherently more risky due the close proximity of the predator (Kats and Dill 1998; Brown and Magnavacca 2003). Alternatively, chemical cues while being less reliable than visual cues, have lower associated risk because they do not require the presence of the predator (Kats and Dill 1998; Brown and

Magnavacca 2003). It has been suggested that chemical cues may function to increase vigilance towards secondary (visual) cues; having been warned in advance that a predation event has taken place in the area, prey may increase their vigilance (Brown *et al.* 2004b). It may be adaptive for prey to have a strategy involving different patterns of response for visual and chemical cues. My results indicated that changes in foraging patterns rather than overall foraging, are indeed seen in response to perceived predation risk, and these foraging decisions are likely to be adaptive.

Smith (1999) suggested the terms overt and covert to refer to the difference in behavioural responses between fish exposed to suprathreshold and subthreshold alarm cues. An individual's response to the presence of a chemical alarm cue had two elements, an overt readily visible response, and a covert response. Overt responses often took place within a few seconds of sensing the alarm cue, and usually consisted of the species-typical suite of antipredator behavioural responses (Smith 1999). Covert responses were more imperceptible and included recognition of novel predators, morphological changes, and the assessment of local predation risk (Smith 1999; Brown 2003).

My results provide new insight into the use of the terms overt and covert. Between the overt responses to suprathreshold cues and the covert responses to subthreshold cues, may lie a third behavioural option for prey. This option is best referred to as "threat-modulated behaviour". In response to chemical alarm cues that differ in concentration, prey can respond with threat-modulation by changing their behaviour pattern to one more appropriate to the magnitude of the threat. In addition, perhaps the behavioural responses of prey may be more realistically defined as overt and non-overt.

In the end it may be more useful to define covert behaviour as any antipredator behavioural response that cannot be classified as overt or threat-sensitive.

Rather than prey individuals exhibiting an all or nothing behavioural response to perceived predation risk, it now appears that there may be a whole range of possible behaviour modalities. This research serves to highlight the complex nature of chemosensory risk assessment and it's resulting behavioural responses.

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