It's how you use it: Environmental constraints on the threat-sensitive response to alarm cues during the juvenile to sub-adult life history shift in centrarchids.

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ABSTRACT

It's how you use it: Environmental constraints on the threat-sensitive response to alarm cues during the juvenile to sub-adult life history shift in centrarchids.

Justin L. Golub

Recent studies have shown that juvenile centrarchids undergo significant ontogenetic shifts in habitat use, foraging strategies and perceived predation risk, as well as in the use of conspecific and heterospecific damage-released chemical alarm cues. Microhabitat characteristics, such as habitat complexity and light availability, limit the use of visual cues, which increase perceived risk and can delay the shift to chemical alarm cues. The first experiment explores the behavioural responses of largemouth bass (*Micropterus salmoides*) between 40 and 65 mm SL to heterospecific or conspecific alarm cues in both high and low light conditions, or complex and open habitats. Individuals were exposed to the same chemical cue under both visually limited and unlimited conditions to study the plasticity of behavioural decisions by an individual. The second study was conducted in the field and used three focal size classes of pumpkinseed sunfish (*Lepomis gibbosus*) exposed to conspecific and heterospecific alarm cues in varying levels of habitat complexity. The results demonstrate significantly threat-sensitive behavioural decisions. With low habitat complexity or high light availability perceived risk was low and more significantly individuals demonstrated a foraging response. In high habitat complexity and low light availability perceived risk was high and individuals showed a greater anti-predator response. These results confirm that the shift from anti-predator to foraging responses is a threat-sensitive response and individuals are constantly making behavioural decisions based on the perceived risk and reliability of information available to them.

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General Introduction

THREAT-SENSITIVE TRADE-OFFS

An individual's fitness depends on its ability to successfully forage, mate and escape predation (Werner & Gilliam, 1984; Lima & Dill, 1990; Pettersson & Brönmark, 1993). Typically, when an individual is engaged in one of these behaviours, it is unable to engage successfully in the other two, and can potentially lose valuable opportunities to increase its fitness (Lima & Dill, 1990). As such, an individual's response to predation can be seen as a series of threat-sensitive (Helfman 1989) trade-offs between the benefits associated with predator avoidance and other fitness related activities (Lima & Dill, 1990). Many freshwater fishes undergo size specific behavioural shifts in ontogeny, that allow them to maximize their perceived benefits from these threat-sensitive trade-offs (Werner & Gilliam, 1984; Werner & Hall, 1988). Individuals that are able to reliably assess local predation risk should be able to optimize these trade-offs and hence be at a selective advantage (Helfman, 1989; Smith, 1992; Chivers & Smith, 1998; Brown, in press).

ALARM CUES

Damage-release alarm cues are found in many freshwater vertebrate and invertebrate taxa (Smith, 1992; Chivers & Smith, 1998). Alarm cues are released through mechanical damage, for example a predation event, and provide valuable information to both potential prey and predators. Alarm cues provide prey with important information regarding local predation risk (Mathis & Smith, 1993; Smith, 1999), as well as provide predators with a cue to locate potential foraging opportunities (Mathis *et al.*, 1995; Chivers *et al.*, 1996).

Upon detection of an alarm cue, cue receivers will respond with either anti-predator or foraging behaviour. Prey and predators respond differently to these alarm cues, each to maximize their potential benefit from these cues. Prey respond with anti-predator behaviours that decrease their probability of being prey upon (Mathis & Smith, 1993; Mirza & Chivers, 2001a; 2003), while predators respond with foraging behaviour, to increase the probability of finding and catching prey (Chivers & Smith, 1998). Anti-predator responses include a variety of overt behaviours (Chivers & Smith, 1998; Smith, 1999), such as movement away from the source of the cue (fleeing), a decrease in movement (freezing), and an increase in shoal cohesiveness (Chivers & Smith, 1998; Smith, 1999). Foraging responses also include a variety of behaviours, such as movement towards the source of the cue and an increase in searching behaviour (Mathis *et al.*, 1995; Brown *et al.*, 2001a).

In addition to the well-documented benefits to cue receivers (Chivers & Smith 1998; Smith, 1999; Brown, 2003), there are also potential benefits to cue senders. Cue senders may benefit from secondary predator recruitment (Mathis *et al.*, 1995; Chivers *et al.*, 1996). The attraction of a second predator, and the subsequent interaction of the two predators for the same food source give the prey a chance to escape (Chivers *et al.*, 1996). Cue senders that lose their life can also receive indirect fitness benefits through the survival of kin (Brown & Smith, 1994; Brown & Smith, 1997; Brown, Chivers, Ranalucci and Plura, unpublished data).

As well as benefiting conspecifics, alarm cues can be used interspecifically (Chivers *et al.*, 2000; 2002; Mirza & Chivers, 2001b). Within a prey guild, heterospecific chemical and visual cues allow individuals to learn to recognize novel predators (Mathis *et al.*, 1996; Chivers *et al.*, 2002; Mirza & Chivers, 2001b; Mirza *et al.*, 2003). Since prey guild members are typically under the same pressures of predation and searching for foraging opportunities, individuals should use chemical cues interspecifically (Mathis *et al.*, 1996; Golub & Brown, 2003). Alarm cues also provide valuable information to potential predators. Predators use alarm cues to locate potential foraging opportunities (Chivers *et al.*, 1996; Golub & Brown, 2003). By responding with foraging behaviours predators can reduce search time, and maximize potential fitness.

Aquatic vertebrates rely on both chemical and visual cues to identify and avoid potentially risky situations. Visual and chemical cues, however, provide individuals with different information and carry different levels of risk (Brown *et al.*, 2004). Visual cues provide more reliable information on spatial and temporal relationship, but are very risky as the predator and prey must be within close proximity. Aside from telling an individual when a predator took a prey item, and how far away, visual cues may also convey a predator's attack motivation (Murphy & Pitcher, 1997) and provide information to facilitate potential learned predator recognition (Brown & Godin, 1999; Brown *et al.*, 2001b). If it is indeed a viable predator the cue receiver is at a higher risk of predation, making the collection of visual cues risky. The information provided by chemical cues is spatially and temporally less reliable, but is less risky, as prey and predator do not have to be in close proximity. Previous studies have demonstrated that individuals can determine a predator's recent diet based on chemical cues (Chivers & Mirza, 2001) and can adjust their visual inspection behaviour accordingly (Brown *et al.*, 2001b). Prey can also use chemical cues to distinguish between satiated and non-satiated predators (Jachner, 1996). Therefore, chemical cues can provide reliable information regarding past foraging preferences and predator hunger level (Brown *et al.*, 2001b; Brown & Magnavacca, 2003). In addition, chemical cues function under conditions of high microhabitat structure, turbid conditions or low light conditions, situations where visual cues would be reduced or totally absent (Kats & Dill, 1998).

The limitation of visual cues by biotic and abiotic structures can cause individuals to rely on chemical cues as the primary source of information (Brown & Cowan, 2000; Brown *et al.*, 2001a). Reliance on chemical cues in the absence of visual cues should increase a prey individual's perceived risk, changing the threat-sensitive value of the alarm cue (Brown *et al.*, 2001a; 2002), and therefore changing an individual's response to that cue. When visual cues are limited, individuals will show a more intense anti-predator response, because perceived risks are greater. When visual cues are readily available perceived risk is lower, and individuals should start to switch to foraging behaviours.

The term cue is used when talking about sensory modalities (visual and chemical cues) as opposed to signal or pheromone because in centrarchids they have been demonstrated to be only reliable indicators of predation. Alarm signals demonstrate active communication between signal sender and receiver. Since these cues are not actively being displayed the term signal is not appropriate. The term pheromone is also inappropriate because pheromones demonstrate a benefit to both signal sender and receiver, and have been evolutionarily selected for. Since this has not been looked at in centrarchids the term cue is most appropriate to describe these sensory modalities.

ONTOGENY

Ontogenetic niches are a succession of life history stages that many species of fishes undergo to reach adulthood (Werner & Gilliam, 1984; Olson *et al.*, 1995; Mittelbach & Persson, 1998). Most notably individuals switch to piscivory during the shift from juvenile to sub-adult (Olson, 1996; Mittelbach & Persson, 1998). Individuals in the sub-adult life history stage are piscivorous, but not sexually mature, therefore they are separated from the adult life history stage (Mittelbach & Persson, 1998). The shift points between life history stages are typically size dependent and species specific (Mittelbach & Persson, 1998).

Centrarchids, which undergo several well-documented ontogenetic stages, undergo a brief larval phase before their juvenile phase, in which they become members of the inshore prey guild (Werner & Hall, 1988; Olson *et al.*, 1995; Olson, 1996). During the larval and juvenile, phases their gape limits food size and individuals are only able to handle small prey (i.e. zooplankton; Olson *et al.*, 1995). As their body size increases, so does mouth gape, and they are able to handle progressively larger food items (Polis *et al.*, 1989; Olson *et al.*, 1995; Olson, 1996). As individuals switch to piscivory they also increase their home range and shift their foraging tactics to better capture their prey (Mittelbach & Persson, 1998). With the shift to piscivory there is also a change in prey guild association. Individuals go from being members of the inshore prey guild, to predators of that prey guild, and should therefore change their use of heterospecific alarm cues accordingly (Brown *et al.*, 2001a). Piscivorous individuals perceive a lower risk from heterospecific alarm cues, and may gain potential foraging opportunities on inshore prey guild members, causing them to shift behaviour from anti-predator to foraging (Brown *et al.*, 2001a)

In largemouth bass (*Micropterus salmoides,* Lacépède), the shift to piscivory occurs around 50 – 55 mm SL (Olson, 1996; Mittelbach & Persson, 1998). At this time they undergo a shift in the use of heterospecific alarm cues that corresponds to the shift to piscivory (Olson, 1996; Brown *et al.*, 2001a). This shift is phenotypically plastic, and the results of previous studies suggest that individuals 40 – 65 mm SL tend to show highly variable response patterns, which are context dependant threat-sensitive trade-offs in their response to heterospecific alarm cues (Brown *et al.*, 2001a; 2002). Pumpkinseed sunfish (*Lepomis gibbosus,* Linnaeus) and green sunfish (*Lepomis cyanellus,* Rafinesque) undergo a similar life history shift (Mittelbach & Persson, 1998), and change in the use of heterospecific alarm cues at 80 – 90 mm SL (Golub & Brown, 2003). Sunfish have a more limited gape than largemouth bass of the same standard length (SL), which delays their shift to piscivory. Sunfish are also opportunistic piscivores, and continue to forage on macroinvertebrates throughout adulthood. Therefore, perceived benefits from foraging responses to heterospecific cues are less than the perceived benefits of anti-predator responses, until they are able to forage on fish.

Golub and Brown (2003) demonstrated that both heterospecific and conspecific alarm cues provide reliable, yet different information to cue receivers. Conspecific cues are a more reliable indicator of predation because they are an indicator of direct predation on a member of the same species. While green sunfish demonstrate a shift in response to heterospecific cues, they show no shift from anti-predator behaviour when exposed to conspecific alarm cues (Golub & Brown, 2003). Obligate piscivores such as yellow perch (*Perca flavescens*, Mitchill; Harvey & Brown, 2004) do not treat conspecific and heterospecific cues differently (Mirza & Chivers, 2001c; Mirza *et al.*, 2003). For a cannibalistic species like largemouth bass, there should be a greater perceived benefit from treating conspecific cues with the same threat-sensitive response as they do with heterospecific alarm cues. Marcus and Brown (2003) recently demonstrated that sunfish, opportunistic piscivores, show threshold responses to conspecific alarm cues, responding with foraging responses to a cue diluted to 25% of the initial testing solution. The concentration of chemical alarm cue detected can inform an individual as to the size of the prey taken, or their spatial relationship (i.e. distance) to the predation event. Lower concentrations of alarm cues could signify that the individual being preyed upon is of a smaller size class, or a considerable distance away, lowering perceived risk. Since chemical cues are limited in their spatial reliability, visual cues would be necessary to determine spatial relationship, and size of the potential predator.

Previous laboratory studies of the behavioural shift in response to heterospecific and conspecific alarm cues have been conducted under open, well-lit conditions. Since centrarchids typically inhabit highly heterogeneous inshore habitats during their juvenile and sub-adult ontogenetic stages, habitat complexity and light availability are two potentially important factors for the behavioural decisions of these individuals. Brown *et al.* (2001a) predicted that biotic and abiotic environmental factors that limit visual cues would make alarm cues a more relied upon source of information. Limiting visual cues would increase risk of predation, and individuals would delay their shift to foraging behaviours.

Environmental complexity has previously been demonstrated to delay the predator avoidance response of individuals to overhead visual cues (Grant & Noakes, 1987). Environmental factors such as habitat have also been demonstrated to affect an individual's probability of survival (Gazdewich & Chivers, 2002), learning (Pollock & Chivers, 2003), phenotypic plasticity (Chips *et al.*, 2004), foraging and mating opportunities (Rowe *et al.*, 1996) as well as territory size and defendability (Breau & Grant, 2002). To this point no one has examined the effects of habitat complexity or light availability on the threat-sensitive trade-off between anti-predator and foraging responses to chemical cues.

Chapter 1 of this thesis will focus on a laboratory study using largemouth bass to explore the behavioural decisions of individuals close to the ontogenetic shift point, from juvenile to sub-adult. Conspecific, confamilial heterospecific, and a synthetic cyprinid alarm cue (heterospecific) will be used to study the effects of habitat complexity and light availability on the threat-sensitive trade-off in response to alarm cues by individuals between the juvenile and sub-adult life history stages.

Chapter 2 will focus on field verification of alarm cue use and the effects of habitat on the threat-sensitive decisions of pumpkinseed sunfish when exposed to conspecific, heterospecific and the synthetic cyprinid (heterospecific) alarm cue. This experiment will use live observations to study the effects of habitat complexity on the response to alarm cues, as well as to study and compare the use of heterospecific versus conspecific alarm cue use by sunfish.

It is the intent of this thesis to examine the effects of environmental constraints (e.g. habitat complexity and light availability) on the behavioural responses to conspecific and heterospecific alarm cues during the shift from juvenile to sub-adult life history stages. During this life history phase individuals exhibit highly flexible responses to alarm cues based on perceived threat. Although previous studies have examined the effects of environmental constraints on alarm cue use, they have not considered how environmental constraints affect the shift between life history stages. Environmental factors have important implications in the behavioural decisions of individuals during the shift from juvenile to sub-adult. By limiting visual cues, environmental factors change the reliability on alarm cues, and affect behavioural decisions.

Chapter 1 – Habitat complexity and the threat-sensitive response to conspecific and heterospecific alarm cues by juvenile largemouth bass (Micropterus salmoides): the effects of structure and light level.[*]

INTRODUCTION

The conflicting demands of foraging, mating, and avoiding predation cause individuals to exhibit threat-sensitive trade-offs in their response to anti-predator cues (Werner & Gilliam, 1984; Lima & Dill, 1990; Pettersson & Brönmark, 1993). Individuals that can reliably assess local predation risk should be able to optimize such trade-offs (Lima & Dill, 1990). However, assessment of local predation risk requires the presence of spatially and temporally reliable information (Brown *et al.*, 2004). A wide variety of taxonomically diverse freshwater fishes (Smith, 1992; Chivers & Smith, 1998) as well as amphibian (Marvin & Hutchison, 1995) and invertebrate species (Hazlett, 1994) rely on damage-released chemical alarm cues to provide reliable information regarding local predation risk.

As stated in the general introduction, damage-released chemical alarm cues are released through mechanical damage to the skin, as would occur during a predation event. Chemical alarm cues provide a valuable source of social information, eliciting anti-predator or foraging behaviour in cue receivers (Brown *et al.*, 2001a). Anti-predator behaviours include area avoidance, reduction in movement, and reduced foraging attempts (Chivers & Smith, 1998). Foraging behaviors include movement towards the cue source, increased overall movement and increased time spent searching for food (Mathis *et al.*, 1995; Brown *et al.*, 2001a).

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[*] In preparation for submission to Ethology.

Centrarchids such as green sunfish (*Lepomis cyanellus,* Rafinesque; Brown & Brennan, 2000) and pumpkinseed sunfish (*Lepomis gibbosus,* Linnaeus; Marcus & Brown, 2003) possess and respond to conspecific chemical alarm cues. Aside from conspecific cues, centrarchids are able to learn and use familiar heterospecific alarm cues in a threat-sensitive manner. Heterospecific cues have been demonstrated to elicit anti-predator responses in juveniles, and foraging responses in piscivorous centrarchids (Brown *et al.*, 2001a; Golub & Brown, 2003). As described in the general introduction, when individuals shift to piscivorous life history stages, they switch from anti-predator to foraging responses to alarm cues. In largemouth bass (*Micropterus salmoides,* Lacépède), the behavioural shift from anti-predator to foraging in response to heterospecific alarm cues corresponds closely with the ontogenetic niche shift (~55 mm SL) from juvenile to sub-adult. This same shift occurs in pumpkinseed sunfish (Chapter 2; Marcus & Brown, 2003) and green sunfish (Golub & Brown, 2003), and corresponds with their juvenile to sub-adult ontogenetic shift (80-90mm SL; Mittelbach & Persson, 1998). Ontogenetic shifts are observed in many freshwater predator species and include changes in habitat, diet, and foraging strategies (Mittelbach & Persson, 1998). When an individual shifts to piscivory, it must also increase its search area to find enough suitable prey, and to change their strategy of finding and capturing this new prey. Switching from inshore prey guild members to predators of the inshore prey guild. As they shift into their predatory role they should no longer use the alarm cues of heterospecifics as a sign of potential predation, but they should start to use the cue as a sign of potential foraging opportunities (Brown *et al.*, 2001a; 2002; Harvey & Brown, 2004).

The shift in the use of heterospecific alarm cues is not fixed, but rather is phenotypically plastic (Brown *et al.*, 2002). Factors such as habitat complexity, light and predation risk should affect the behavioural decisions to shift. Brown *et al.* (2002) demonstrated that juvenile largemouth bass with deeper bodies shifted earlier (40 - 45 mm SL) than individuals with shallower bodies (60 – 65 mm SL). Once an individual exceeds the gape limitations of inshore predators, the relative benefits associated with an anti-predator response are likely reduced (Brown *et al.*, 2002). An individual at a lower risk of predation can now benefit from potential foraging opportunities on prey that meet their own gape limitations (Brown *et al.*, 2002). Those with shallower bodies however, are subject to continued predation risk and benefit from continued anti-predator behaviours (Brown *et al.*, 2002).

Visual and chemical cues convey different levels of risk and information (Brown *et al.*, 2004). While visual cues provide more reliable information on spatial and temporal relationships, the collection of this information requires an individual to be in close proximity to a potential predator. This is a potentially risky situation if it is a viable predator, or if the cue recruits additional predators. Chemical cues do not provide as reliable information on temporal and spatial relationships, however, the collection of this information is less risky. Chemical cues can be detected and used over a larger area than visual cues, and are not as easily limited by environmental complexity and light availability. Brown *et al.* (2001a) predicted that increasing habitat complexity would likely influence the response pattern upon detection of heterospecific alarm cues. In the absence of visual information the perceived risk of a predation event is greater, increasing the benefits of anti-predator responses to alarm cues (Smith, 1999; Brown *et al.*, 2001a).

When visual cues are available, they can provide more reliable information on spatial and temporal relationship to a predator (Kats & Dill, 1998; Brown *et al.*, 2001a). If no predator is detected when visual cues are available, perceived risk is low, and individuals should shift their response to foraging behaviours.

Using habitat complexity and light availability in the laboratory setting as factors to limit visual, but not chemical cues, will allow for observation of individual largemouth bass around the ontogenetic shift point (40 - 65 mm SL), and their behavioural decisions under varying environmental conditions. Individuals in the 40 – 65 mm SL size range show high variability in their response to heterospecific alarm cues (Brown *et al.*, 2001a; 2002). Brown *et al.* (2001a) demonstrated that largemouth bass below this size range show little variability in their behaviours, which is consistently anti-predator, while individuals above consistently show foraging responses with very little variability. Individuals in this range should show threat-sensitive variability in their responses based on the context in which the cue is received (Golub & Brown, 2003).

The limitation of visual cues through increased habitat complexity and decreased light availability will result in individuals making context dependant changes in their behaviour. When visual cues are limited by environmental factors (high habitat complexity and low light availability), perceived risk will increase, and the predator avoidance – foraging trade-off should favour an anti-predator response. When visual cues are less limited (low habitat complexity and high light availability), perceived risk will decrease, and the trade-off should favour a foraging response.

METHODS

Fish Collection

Test and donor fish, largemouth bass and pumpkinseed sunfish, were collected from local populations (Lac Seigneurial, Canal Lachine, and Bassin de La Prairie), and held in 225 L basins with a central standpipe and continuous flow-through of dechlorinated water at 18° C, on a 12:12 light:dark cycle. Fish were fed a combination of commercial flake food and frozen brine shrimp (*Artemia* spp.) *ad libitum* twice daily. Green swordtails (*Xiphophorus helleri*, Heckel) were purchased from a commercial supplier, and held in 110 L glass aquaria at 28° C with a recirculating filter and fed commercial flake food twice daily.

Stimulus Preparation

Natural alarm cues were collected from skin fillets taken from both sides of donor fish, in accordance with Concordia University Animal Care Protocol (AC-2002-BROW). Skin extracts collected from donors (mean ± S.E.) in Experiment 1 were pumpkinseed sunfish (N = 3, SL= 67.0 ± 3.10 mm) and largemouth bass (N = 6, SL= 48.1 ± 1.40 mm). In Experiment 2 pumpkinseed sunfish (N = 4, SL= 54.0 ± 2.30 mm) and largemouth bass (N = 4, SL= 59.5 ± 1.70 mm) skin extracts were collected.

Skin fillets were measured (cm^2), homogenized in 50 ml of chilled glass-distilled water, then filtered using polyester filter floss. Using glass-distilled water, skin extracts were diluted to final concentrations similar to those used by Brown *et al.* (2001a; 2002) and Golub and Brown (2003). In Experiment 1 final concentrations were 31.77 cm^2 in 380 ml, and 34.75 cm^2 in 420 ml for pumpkinseed sunfish and largemouth bass respectively. In Experiment 2 final volumes were 27.03 cm^2 in 325 ml, and 33.36 cm^2 in 400 ml for pumpkinseed sunfish and largemouth bass respectively. All samples were then stored in 20 ml aliquots at –20° C until used. Juveniles exposed to skin extracts at these concentrations have previously been demonstrated to increase anti-predator responses compared to distilled water controls (Brown et al., 2001; Golub & Brown, 2003; Harvey & Brown 2004).

Hypoxanthanine-3-*N*-oxide (H3NO) was used as a synthetic equivalent to cyprinid (heterospecific) alarm cues (Brown *et al.*, 2000). H3NO was diluted to 0.001 mg ml^{-1} of stock solution in glass-distilled water and stored as described above. Hypoxanthanine-3-*N*-oxide concentrations used in this experiment have previously been demonstrated to elicit similar response patterns to cyprinid skin extract prepared in concentrations analogous to those described above (Brown *et al.*, 2000; Leduc *et al.*, 2003).

Experiment 1 – Habitat Complexity

Experimental setup

The setup consisted of a series of 110 L glass aquaria with a single airstone in the upper right-hand corner and gravel substrate. A tube (acrylic airline) for stimulus injection and a tube for food injection were attached to the airstone. A black viewing curtain was hung over the front of the tank, and the remaining sides were covered with brown paper. A grid was drawn on the front and back of the tank dividing it into three equal vertical units and five equal horizontal units. Each tank had a single 40 W light bulb overhead on a 12:12 light:dark photoperiod.

Below the substrate a sheet of plexiglass (27 cm x 87 cm) with twenty holes, 7mm in diameter, were cut equidistant from each other in a 4 x 5 grid pattern. In three of these plexiglass sheets a 30 cm length of green nylon rope was passed through each hole and the top of the rope was frayed to simulate vegetation (Figure 1.1). The plexiglass sheets were buried under the gravel. The other three plexiglass sheets were placed under the gravel without any simulated vegetation.

Experimental Protocol

Individual largemouth bass (N = 52, between 41.4 - 64.9 mm SL) were arbitrarily selected and placed in either open or complex habitat test tanks 24 hours prior to testing. Live observations were conducted and video recorded for subsequent data analysis.

Experimental trials were divided into a 10-minute pre- and 10-minute post-stimulus observation periods. Prior to the start of observations 60 ml of water was removed through the stimulus injection tubing and discarded to clear any stagnant water from the tubing. An additional 60 ml of water was removed and saved for later use. The same was also done with the food injection tube. During pre- and post-stimulus periods horizontal and vertical position were recorded every 15 seconds, and an average was calculated for that observation period. Time spent moving and foraging attempts were recorded for the entire pre- and post-stimulus periods. Following the pre-stimulus observation period 10 ml of experimental stimuli were introduced through the stimulus injection tube, at the same time 10 ml of brine shrimp were introduced through the food injection tube. Experimental stimuli were largemouth bass skin extract, pumpkinseed sunfish skin extract, H3NO or distilled water. Stimulus and food were flushed through the tubing simultaneously with 60 ml of tank water at a rate of approximately 2 ml s^{-1}. Once the 60 ml of tank water was used up the post-stimulus observation period began. Post-stimulus observations were conducted in the same manner as described for pre-stimulus observations. Decreases in all behavioural measures (horizontal position, vertical position, time spent moving and foraging attempts) from the pre- to post-stimulus periods are indicative of an anti-predator response (Brown, 1984; Brown *et al.*, 2001a; 2002). An increase in these behaviours is indicative of a foraging response (Brown *et al.*, 2001a; 2002).

At the conclusion of the post-stimulus observation period, fish were moved to a second experiment tank of the opposite habitat type and allowed to acclimate for 24 hours. Fish were observed for a second 10-minute pre- and post-stimulus period using the same experimental stimulus they were exposed to previously. The order in which experimental stimuli were tested was randomized. At the end of the second experimental treatment fish were weighed, and measured (standard length and depth) before being returned to a second holding tank. No fish were retested after the second set of experimental observations.

Statistical analysis

Change in behavioural measures (post – pre) was analyzed as dependent variables in a repeated measures (open and complex habitats) MANCOVA using standard length as a covariate, and stimulus type as the independent variable. For each experimental stimulus (and the distilled water control), direct comparisons between open and complex treatments were made using paired one-tailed t-tests, corrected for increasing Type I error rates with a modified Bonferonni correction, $\alpha` = \frac{(k-1)\alpha}{c}$ (Keppel, 1991) where k is the number of treatments and c is the number of comparisons.

Since each individual was exposed to the same chemical treatment in both open and complex environments, a difference of response patterns was calculated (Open $_{(\text{Post} - \text{Pre})}$ – Complex $_{(\text{Post} - \text{Pre})}$) between the microhabitat treatments (sensu Leduc *et al.*, 2003). This was used to directly compare the effects of stimulus type, on changes in behavioural measures. The mean overall response difference scores were then compared across the four stimuli treatments using a MANOVA, followed by individual ANOVAs for each behavioural measure. Post-hoc comparisons were made with Fisher's Protected Least Squared Difference tests. If, as predicted, individuals exhibit a less intense anti-predator response and/or a foraging response under open versus complex habitats, there should be a higher overall difference for each of the behavioural measures, relative to the distilled water control.

Experiment 2 – Light Intensity

Experimental setup

A series of tanks were set up similar to Experiment 1. An additional overhead lamp was added to each tank with a 40 W red light bulb. Light cycle of each tank was manipulated using one red 40 W light bulb and one white 40 W light bulb set on automatic timers. The new light cycle consisted of 10 hours dark period, followed by a three-hour low light period (red light only, dawn), 8 hours of high light (red and white lights, mid-day), finally a second three-hour low light period (dusk). The low light condition was approximately 6.4 lux at the tank surface and high light was approximately

72.2 lux at the surface. These light conditions represent early and late light intensities during the dawn photoperiod (Breau, 2003). During testing the overhead room lights were kept off and all 4 sides of the shelves were covered with black plastic curtains. Live and video recorded observations were conducted 30 minutes after first low light photoperiod started or 30 minutes after high light photoperiod started respectively.

Experimental Protocol

Experimental protocol was similar to Experiment 1. Individual largemouth bass (N = 36, between 43.3 – 65.0 mm SL) were observed for 10-minute pre- and 10-minute post-stimulus periods. During observation periods vertical and horizontal location, time spent moving and foraging were recorded as described before. At the conclusion of the pre-stimulus observation period, 10 ml of experimental stimuli (described above), and 10 ml of brine shrimp were administered through their respective tubing as described in Experiment 1. After the post-stimulus observation period, fish were moved to a clean tank and allowed to acclimate for a minimum of 18 hours prior to a second set of visual observations. The second set of visual observations was conducted under the opposite light conditions. Each fish was tested to the same stimuli in high and low light conditions, and the order of stimuli use was randomized similar to Experiment 1. After the conclusion of the second set of observations, fish were removed, weighed and measured (standard length and depth) before being returned to a holding tank. No fish were retested after the second observation period.

Statistical analysis

Data were analyzed as described above for Experiment 1. In this experiment treatments of high light and low light conditions were used, and the differences calculated using High Light $_{(Post-Pre)}$ – Low Light $_{(Post-Pre)}$.

RESULTS

Experiment 1

When an individual's size (standard length) was included as a covariate, no significant difference was seen in the response patterns between open and complex habitat treatments (MANCOVA $F_{(7, 52)} = 0.978$, $P = 0.460$). Since no significant effect was found using standard length as a covariate subsequent analysis were conducted without the covariate.

Individuals show a significant change in behaviour between open and complex habitats when exposed to experimental stimuli compared to distilled water control (MANOVA $F_{(7,42)} = 6.148$, $P < 0.001$), but no significant interaction of habitat and stimulus (MANOVA $F_{(7, 44)} = 1.07$, $P = 0.399$). Individual paired sample t-tests, using an $\alpha = 0.0375$, were used to demonstrate the significant differences in response to experimental stimuli between open and complex habitats. Individuals demonstrated a significant increase in the behaviours recorded in open habitats (foraging response) while showing a decrease of these same behaviours when exposed to the same chemical alarm cue in complex habitats (anti-predator response). This is seen in the significant effects of largemouth bass and pumpkinseed sunfish skin extracts on horizontal area use, vertical area use, time spent moving and foraging attempts (Figure 1.2, Table 1.1). H3NO yielded a significant effect on vertical area use, moving and foraging attempts, but no significant effect on horizontal area use (Figure 1.2, Table 1.1). Individuals showed no significant change in behaviour between open and complex microhabitats when exposed to distilled water (Figure 1.2, Table 1.1). This demonstrates a significant difference in an individual's response between complex and open habitats. Individuals are responding differently to alarm cues in open and complex habitats, but showing no change in behaviour to distilled water cues between habitats.

For the calculated response differences (Open $_{(\text{Post} - \text{Pre})}$ – Complex $_{(\text{Post} - \text{Pre})}$), when standard length is accounted for as a covariate, no significant difference among chemical stimuli treatments is seen ($F_{(4,\,52)} = 1.330$, $P = 0.274$). However, a significant overall effect of stimulus type on response differences was observed (MANOVA, $F_{(4,\,47)} = 4.86$, $P = 0.002$). As well as significant individual effects of stimulus on difference in horizontal area use (ANOVA $F_{(3,48)} = 2.88$, $P = 0.046$, Figure 1.3A), time spent moving (ANOVA $F_{(3,48)} = 2.85$, $P = 0.047$, Figure 1.3C) and foraging attempts (ANOVA $F_{(3,48)} = 3.24$, $P = 0.030$, Figure 1.3D). There was no significant effect of stimulus on vertical area use (ANOVA $F_{(3,48)} = 0.922$, $P = 0.438$, Figure 1.3B). A significant overall effect demonstrates that individuals were showing a significant behavioural change in response to conspecific and heterospecific alarm cues while showing no significant change in response to distilled water controls. Significant changes in area use, time spent moving and foraging attempts demonstrate that in conditions of higher habitat complexity, individuals show a significantly more intense anti-predator response to alarm cues.

Experiment 2

When an individual's size (standard length) was included as a covariate, no significant difference was seen in the response patterns between high and low light treatments ($F_{(7, 40)} = 1.674$, $P = 0.155$). Since no significant effect was found using standard length as a covariate, subsequent analysis were calculated without the covariate.

The results demonstrate that there is a significant effect of light level, individuals show different response to the same chemical cue in different light levels, but show no change to distilled water control (MANOVA $F_{(7,30)} = 2.487$, $P < 0.039$). They are also no significant interaction of light and stimulus (MANOVA $F_{(7, 32)} = 1.486$, $P = 0.208$). Individual paired sample t-tests yielded significant effects of largemouth bass skin extract on horizontal area use and time spent moving, but no effect on vertical area use or foraging attempts (Figure 1.4, Table 1.2). A significant effect of pumpkinseed skin extract was found for vertical area use, time spent moving and foraging attempts, but no significant effects were found for horizontal area use (Figure 1.4, Table 1.2). There was no significant effect on horizontal area use, vertical area use, time spent moving and foraging attempts for H3NO or distilled water (Figure 1.4, Table 1.2)

For in the calculated response differences (High $_{(\text{Post}-\text{Pre})}$ – Low $_{(\text{Post}-\text{Pre})}$), when standard length is accounted for as a covariate, no significant difference among treatments is seen (MANCOVA $F_{(4, 40)} = 1.791$, $P = 0.155$). Similar to Experiment 1 a significant overall effect of response differences observed demonstrates that individuals show a change in behaviour to alarm cues while showing no change to distilled water controls (MANOVA, $F_{(4, 31)} = 4.49$, $P = 0.006$). There were also significant individual effects of stimulus on difference in horizontal area use (ANOVA $F_{(3,32)} = 3.15$, $P = 0.039$,

Figure 1.5A) and movement (ANOVA $F_{(3,32)} = 3.02$, $P = 0.044$, Figure 1.5C). No significant effect of stimulus on vertical area use (ANOVA $F_{(3,32)} = 0.567$, $P = 0.641$, Figure 1.5B) and foraging attempts (ANOVA $F_{(3,32)} = 0.709$, $P = 0.554$, Figure 1.5D) were observed. The significant effect of horizontal area use and time spent moving demonstrate a decrease of these behaviours in low light conditions. Decreases in these behaviours signify a more intense anti-predator response in the low light conditions compared to high light.

DISCUSSION

As predicted, habitat complexity and ambient light levels appear to act as significant environmental constraints, increasing perceived risk and shifting the threat-sensitive trade-off in favour of an anti-predator (versus a foraging) response. Although other environmental cues may be available as indicators (e.g. auditory, mechanical and electrical cues) visual and chemical cues provide the most reliable information on local predation events (Smith, 1992). Limiting the availability of visual cues forces individuals to rely primarily on the information provided by chemical cues, increasing perceived risk (Brown *et al.*, 2001a). However, when visual cues are not constrained, the perceived risk is lower and individuals start to show a foraging response. The behavioural decision to respond with either anti-predator or foraging behaviours is context specific, and individuals are constantly adjusting their response to the perceived level of threat.

Previous studies have demonstrated that the shift from anti-predator to foraging behavioural responses correlate with the ontogenetic shift from juvenile to sub-adult in centrarchids (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003). This study suggests that when perceived risk is low, an individual can make the behavioural shift from anti-predator to foraging behaviours. Since their response pattern is still phenotypically plastic, when perceived risk is high they can still respond with anti-predator behaviours to maximize fitness.

Largemouth bass shift from juvenile to sub-adult in the 40 - 65 mm SL size range (Olson, 1996; Brown *et al.*, 2001a; Brown *et al.*, 2002). Individuals closer to 40 mm SL are expected to show anti-predator behaviours while individuals above 65 mm show foraging behaviours. Those that fall into the range of 40 - 65 mm show high variability in their response, because they are subject to threat-sensitive trade-offs in their behavioural decisions. Individuals below 40 mm SL are constantly at risk from the same potential predation and therefore benefit the most from constantly exhibiting anti-predator responses. Individuals above this size range (65+ mm SL) exceed the inshore predator's gape limitations and therefore are at a lower risk of predation and consistently show foraging responses. Individuals that have recently shifted to the sub-adult stage perceive heterospecific alarm cues to be less risky than do juveniles. When environmental factors limit visual cues, perceived risk associated with the cue increases. The increased threat sensitivity in visually limited situations increased the perceived benefits of anti-predator responses. Individuals that have recently shifted to piscivory (~ 55 mm SL) show foraging behaviours when perceived risk is low, but when perceived risk is greater, exhibit anti-predator behaviours. This demonstrates that individuals are constantly making threat-sensitive behavioural decisions based in part on the perceived benefits of chemical alarm cues. Individuals with phenotypically plastic responses are able to adjust their response to their situation. For example, when visual cues are limited perceived risk increases, so individuals with a flexible phenotype respond with anti-predator behaviours because these behaviours provide the maximum perceived benefits in that situation. When perceived risk is low, an individual with a flexible response pattern shows foraging responses, because they yield the maximum benefits. Individuals with rigid response patterns are subject to loss of benefits when perceived risks and benefits change. Individuals that continually show anti-predator responses miss opportunities to increase fitness when perceived risk is low. Individuals that only show foraging responses are at a greater risk of predation in high-risk situations, because they will be less likely to avoid potential predators.

Experiments 1 and 2 demonstrate that the shift in behavioural response seen in centrarchids (Brown *et al.*, 2001a) is flexible and can vary within an individual with environmental conditions. Experiment 1 demonstrates that physical barriers in the environment that limit visual cues have a significant effect on the behavioural decision, while Experiment 2 demonstrates that light availability has a similar effect. Both habitat and light may limit visual cues, which increase perceived risk causing individuals that have become inshore predators to respond with anti-predator behaviours.

Although no direct comparison was done between the results of experiments 1 and 2, comparison of the changes in behavioural responses after the introduction of chemical stimuli suggests that light availability did not have as strong an effect as habitat complexity on the behavioural response patterns of individuals at these levels tested. Though the results suggest a similar trend in response patterns. The two conclusions that can be made from this comparison are that light is not as strong a variable as habitat in threat-sensitive decisions, or that the light levels selected, though demonstrating a slight effect, was not a large enough difference in light levels. Therefore lower light could be perceived as less risky than highly complex habitats, or that the light levels used in this experiment did not mimic as great a difference as the complex and open habitats. Since natural light levels vary more during the day in nature that those used in this experiment, a follow up experiment using a larger change in light levels would help answer these questions. By conducting the experiment in a similar fashion with a larger change in light between the two treatments the effect of light might become more apparent.

Previous work (Brown *et al.*, 2002) has demonstrated similar effects of phenotypic plasticity on the threat-sensitive trade-off in response to predation risk. Largemouth bass with greater body depth shift their response to heterospecific alarm cues earlier than those with more shallow body phenotypes (Brown *et al.*, 2001a; 2002). This is a threat-sensitive behavioural decision where individuals with deeper bodies perceive the act of inshore predation with less risk than individuals with shallower bodies. Predators are less likely to be able to consume deeper bodied prey because they are more likely to exceed their gape limitations (Brönmark & Miner, 1992). Therefore, deep-bodied prey perceive less risk because they have a better chance of exceeding their predators gape limitations than those with shallower bodies. The shallower bodied individuals perceive a greater risk from the same cue, and respond with anti-predator behaviours until their body depth exceeds the predators gape limitations and the perceived risk decreases.

This study demonstrates that largemouth bass possess alarm cues, and respond to conspecific cues. Contrary to previous studies, obligate piscivores, like yellow perch (Harvey & Brown, 2004) and largemouth bass, are less likely to discern between conspecific and heterospecific cues. Golub and Brown (2003) argued that since green sunfish rely less on piscivory, they tend to place a greater value on conspecific cues. However, Marcus and Brown (2003) demonstrated that pumpkinseed sunfish show a shift to foraging responses when conspecific cues are at concentrations below 25% of those used by Golub and Brown (2003). The behavioural shift in response to a 25% cue solution, versus the response to a 100%, demonstrates that when perceived risk is lower (25%) individuals benefit from responding with foraging behaviours, but when perceived risk is high (100%), anti-predator behaviours yield the maximum perceived benefit, a threat-sensitive response.

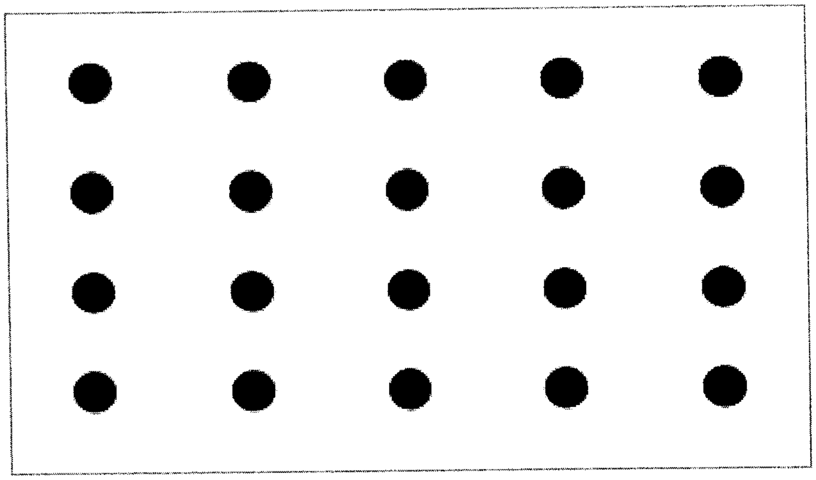
When individuals are exposed to visual cues in the absence of chemical cues, as is typical with avian predators, they reduce their reaction visual distance as biotic/abiotic structure increases (Grant & Noakes, 1987). This might occur because individuals are not receiving chemical cues. By relying on visual cues alone with dense overhead structure decreases the reliability of these cues, so they delay their response until they have identified this as a potential risk. Therefore, highly complex habitats are also limiting visual cues of potential predators, making prey in highly complex habitat harder to locate and catch than prey in open conditions. Increased habitat complexity has also been demonstrated to decrease an individual's ability to learn heterospecific alarm cues (Pollock & Chivers, 2003), and has also been shown to increase an individual's survival (Gazdewich & Chivers, 2002). Habitat complexity has also been demonstrated to affect population phenotypes (Brinsmead & Fox, 2002; Chips *et al.*, 2004). Habitat also can effect an individuals foraging and mating opportunities (Sih, 1988; Rowe *et al.*, 1996), and optimal territory size (Breau & Grant, 2002).

Furthermore, this chapter demonstrates that an individual's response to alarm cues is a threat-sensitive trade-off, and an individual can switch between anti-predator and foraging responses depending on their perceived threat. When visual cues are not limited individuals perceive chemical cues as lower risk than when visual cues are limited. This allows an individual in the range of the juvenile/sub-adult life history shift to respond accordingly to the perceived threat.

Figure 1.1 Schematic diagram of simulated vegetation for the laboratory experiment. Vegetation consisted of 30 cm lengths of green nylon rope passed through the 20 holes cut in a 27 x 87 cm plexiglass sheet. Horizontal area use (HAU) and vertical area use (VAU) are used to measured an individuals position in the tank every 15 seconds during observation periods. Low HAU and VAU scores signify movement away from stimulus injection site, and increasing scores signify a movement towards the source of the cue.

Figure 1.1

A)



B)

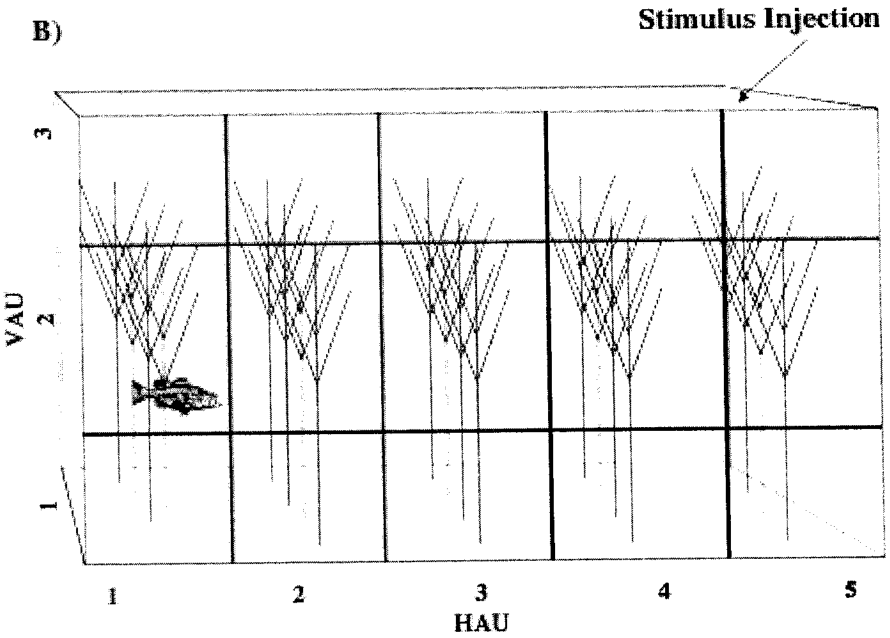


Figure 1.2 Mean (±S.E) change (post – pre) in (A) horizontal area use (out of 5 units), (B) vertical area use (out of 3 units), (C) time spent moving (sec) and (D) foraging attempts (per observation period) between pre- and post-stimulus observation periods. Comparison between complex (gray) and open (white) habitats for largemouth bass (LMB), hypoxanthanine-3-*N*-oxide (H3NO), pumpkinseed sunfish (PSF) and distilled water (DW) stimuli. * denotes a significant difference with a $\alpha = 0.0375$.

Figure 1.2

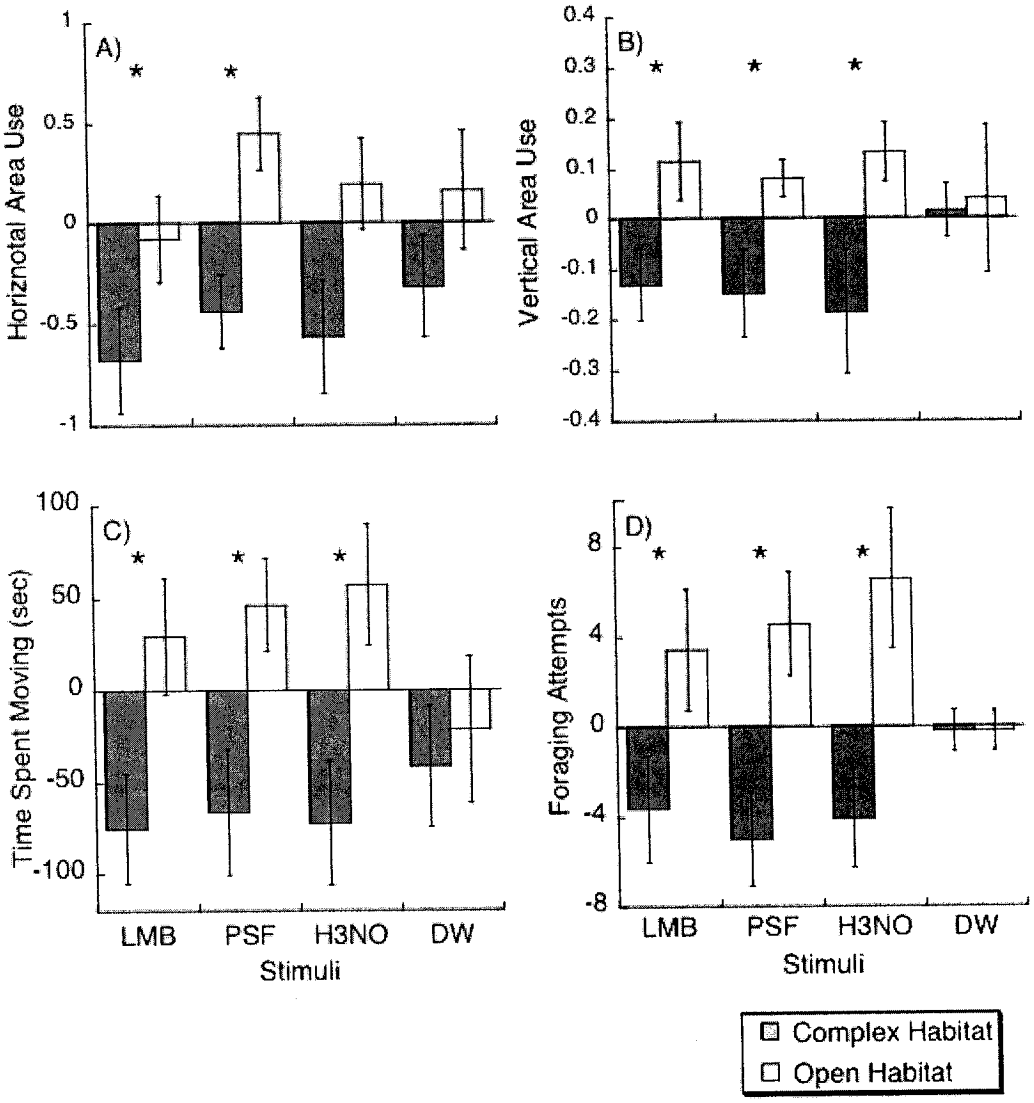

A)
Horiznotal Area Use
1
0.5
0
-0.5
-1
*
*
B)
Vertical Area Use
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
*
*
*
C)
Time Spent Moving (sec)
100
50
0
-50
-100
*
*
*
LMB
PSF
H3NO
DW
Stimuli
D)
Foraging Attempts
8
4
0
-4
-8
*
*
*
LMB
PSF
H3NO
DW
Stimuli
▨ Complex Habitat
☐ Open Habitat

Figure 1.3 Mean (±S.E) response difference (Open $_{(Post-Pre)}$ – Complex $_{(Post-Pre)}$) for largemouth bass between 40 – 65 mm SL. Each individual was exposed to either largemouth bass skin extract (LMB), pumpkinseed skin extract (PSF), hypoxanthanine-3-*N*-oxide (H3NO) or distilled water (control; DW) in both open and complex habitats. Mean response differences were recorded for (A) horizontal area use (out of 5 units), (B) vertical area use (out of 3 units), (C) time spent moving (sec) and (D) foraging attempts (per 10 min observation period).

Figure 1.3

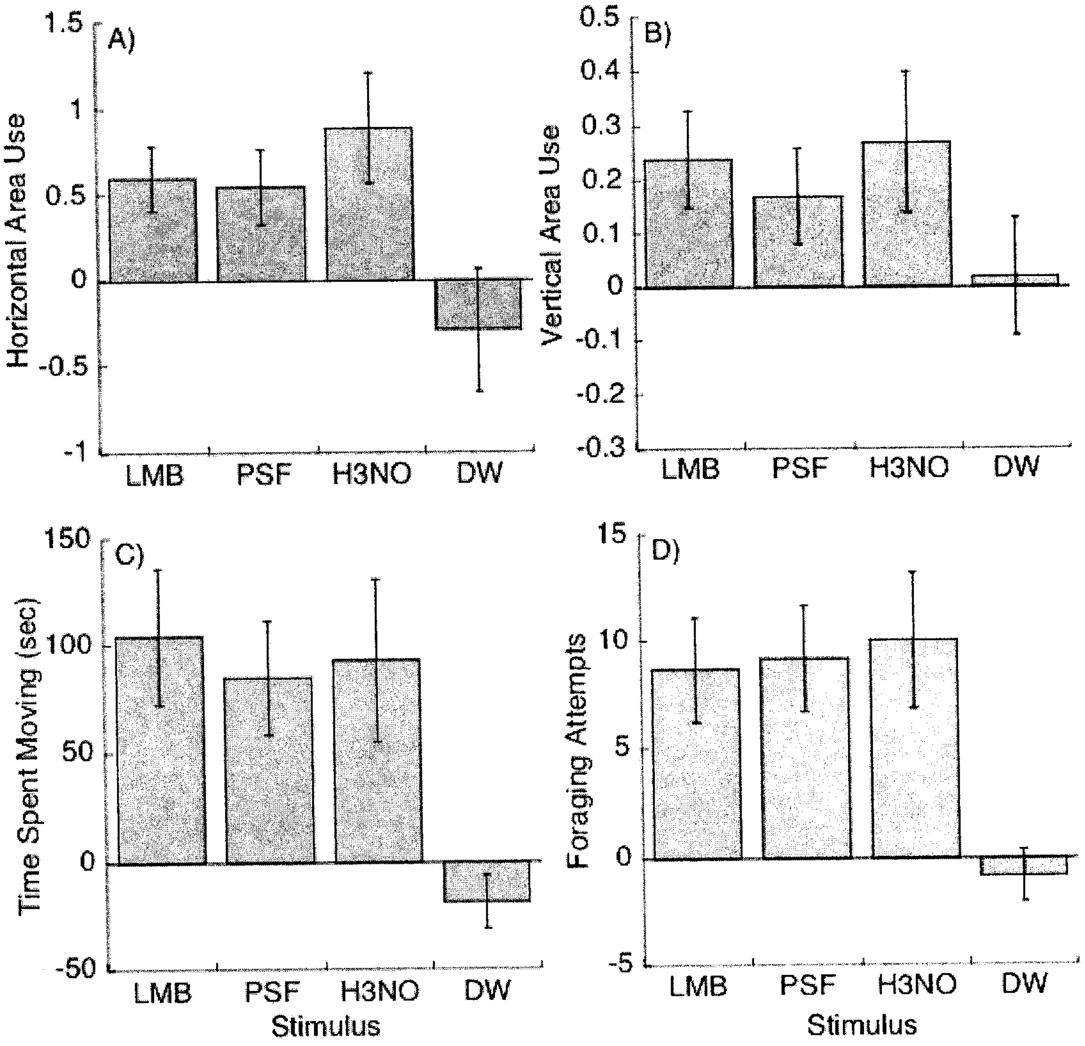

A)
Horizontal Area Use
1.5
1
0.5
0
-0.5
-1
LMB
PSF
H3NO
DW
B)
Vertical Area Use
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
LMB
PSF
H3NO
DW
C)
Time Spent Moving (sec)
150
100
50
0
-50
LMB
PSF
H3NO
DW
Stimulus
D)
Foraging Attempts
15
10
5
0
-5
LMB
PSF
H3NO
DW
Stimulus

Figure 1.4 Mean (±S.E) change (post – pre) in (A) horizontal area use (out of 5 units), (B) vertical area use (out of 3 units), (C) time spent moving (sec) and (D) foraging attempts (per observation period) between pre and post stimulus observation periods. Comparison between low (gray; <10 lux) and high (white; >50 lux) light levels for largemouth bass (LMB), hypoxanthanine-3-*N*-oxide (H3NO), pumpkinseed sunfish (PSF) and distilled water (DW) stimuli. * Denotes a significant difference with $\alpha = 0.0375$

Figure 1.4

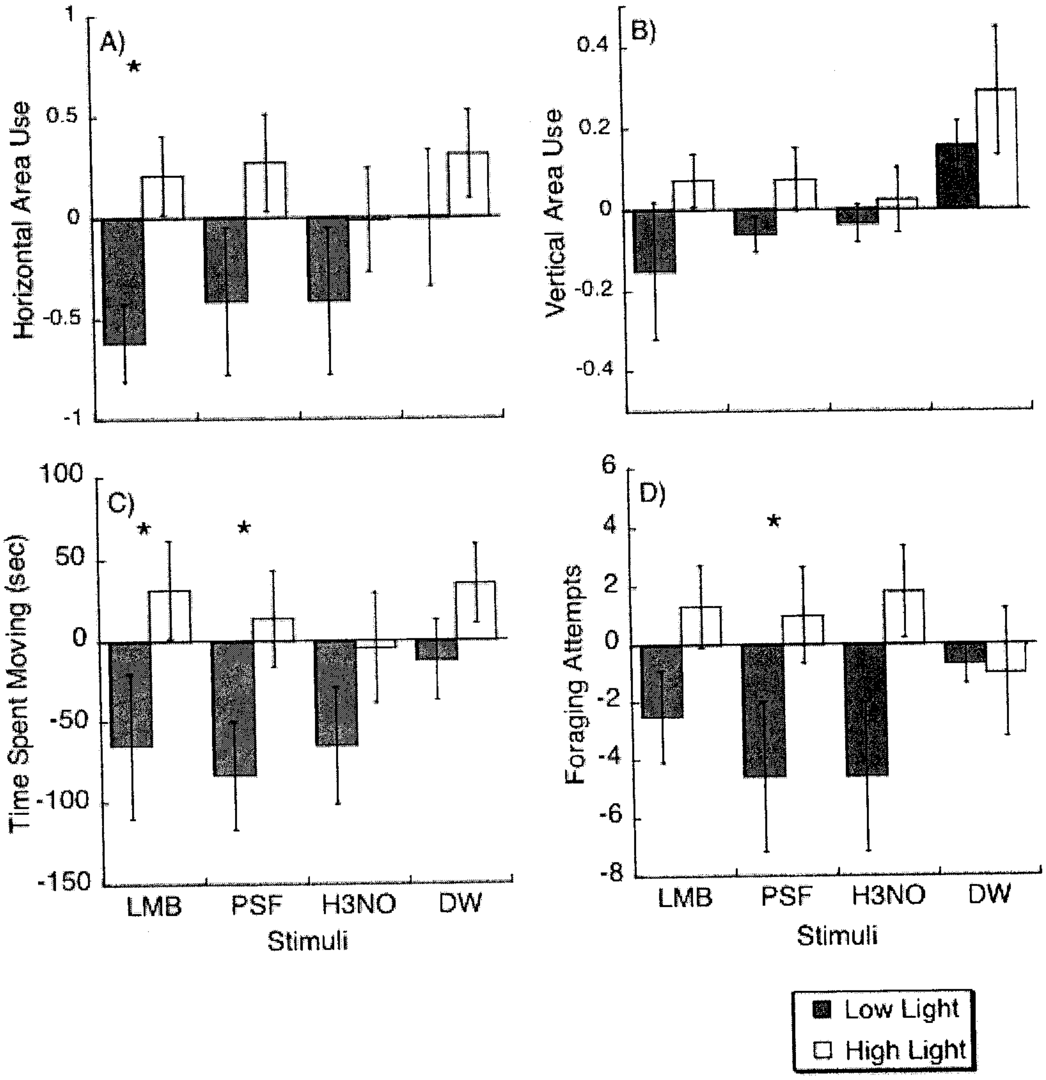

A)
*
Horizontal Area Use
1
0.5
0
-0.5
-1
B)
Vertical Area Use
0.4
0.2
0
-0.2
-0.4
C)
* *
Time Spent Moving (sec)
100
50
0
-50
-100
-150
D)
*
Foraging Attempts
6
4
2
0
-2
-4
-6
-8
LMB PSF H3NO DW
Stimuli
LMB PSF H3NO DW
Stimuli
■ Low Light
□ High Light

Figure 1.5 Mean (±S.E) response difference (High Light $_{(Post-Pre)}$ – Low Light $_{(Post-Pre)}$) for largemouth bass between 40 – 65 mm SL. Each individual was exposed to either largemouth bass skin extract (LMB), pumpkinseed skin extract (PSF), hypoxanthanine-3-*N*-oxide (H3NO) or distilled water (control; DW) in both high and low light conditions. Mean response differences were recorded for (A) horizontal area use (out of 5 units), (B) vertical area use (out of 3 units), (C) time spent moving (sec) and (D) foraging attempts (per 10 min observation period).

Figure 1.5

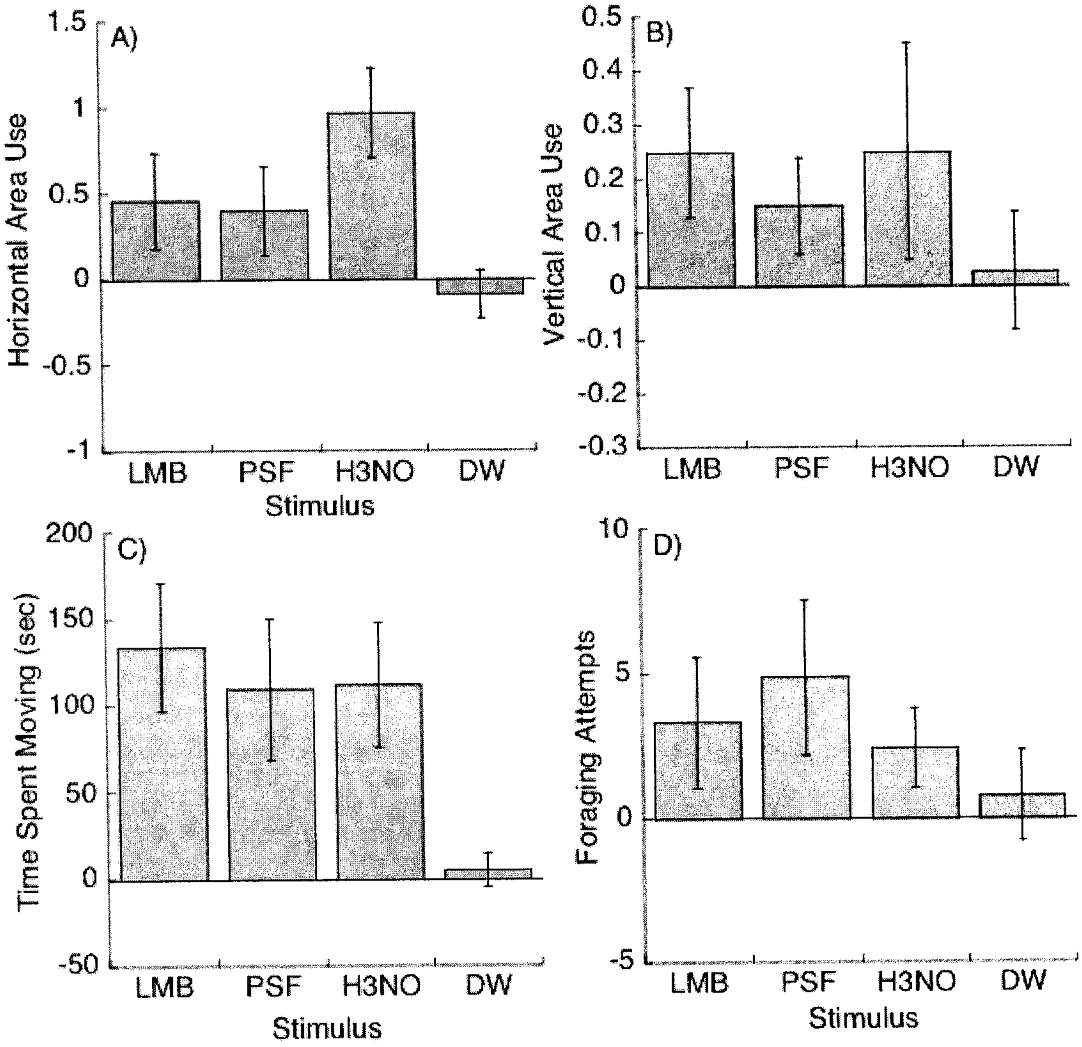

A)
Horizontal Area Use
1.5
1
0.5
0
-0.5
-1
LMB
PSF
H3NO
DW
Stimulus
B)
Vertical Area Use
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
LMB
PSF
H3NO
DW
C)
Time Spent Moving (sec)
200
150
100
50
0
-50
LMB
PSF
H3NO
DW
Stimulus
D)
Foraging Attempts
10
5
0
-5
LMB
PSF
H3NO
DW
Stimulus

Table 1.1 Paired sample t-test values for behavioural measures (horizontal area use, vertical area use, time spent moving, and foraging attempts) in response to largemouth bass, pumpkinseed sunfish, H3NO and distilled water treatments, between complex and open habitat conditions. Significant differences are denoted in **bold** face.

Table 1.1

	Horizontal Area Use		Vertical Area Use		Time Spent Moving		Foraging Attempts	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Largemouth bass	**3.114**	**0.004**	**-2.521**	**0.013**	**-2.821**	**0.007**	**-2.262**	**0.020**
Pumpkinseed Sunfish	**3.498**	**0.002**	**-2.525**	**0.012**	**-2.764**	**0.008**	**-3.624**	**0.001**
H3NO	0.877	0.198	**-2.291**	**0.019**	**-2.618**	**0.010**	**-2.824**	**0.007**
Distilled water	1.62	0.07	-0.216	0.417	-0.364	0.362	0	0.5

Table 1.2 Paired sample t-test values for behavioural measures (horizontal area use, vertical area use, time spent moving, and foraging attempts) in response to largemouth bass, pumpkinseed sunfish, H3NO and distilled water treatments between high and low light conditions. Significant differences are denoted in **bold** face.

Table 1.2

	Horizontal Area Use		Vertical Area Use		Time Spent Moving		Foraging Attempts	
	t	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Largemouth bass	**-3.082**	**0.006**	-1.206	0.129	**-2.586**	**0.014**	-0.755	0.235
Pumpkinseed Sunfish	1.527	0.080	-1.984	0.039	**-2.626**	**0.014**	**-2.342**	**0.022**
H3NO	0.817	0.217	-0.803	0.221	-1.264	0.119	-0.931	0.188
Distilled water	0.662	0.262	-0.962	0.180	-1.475	0.087	0.15	0.442

Chapter 2 - Response to conspecific and heterospecific alarm cues by pumpkinseed sunfish (Lepomis gibbosus): field verification of the ontogenetic shift.[*]

INTRODUCTION

Prey are continually faced with the conflicting demands of foraging and predator avoidance (Werner & Gilliam, 1984; Lima & Dill, 1990; Pettersson & Brönmark, 1993), and often exhibit threat-sensitive trade-offs between the benefits associated with foraging and anti-predator behaviour (Helfman, 1989; Lima & Dill, 1990; Pettersson & Brönmark, 1993; Lima & Bednekoff, 1999). Individuals that can reliably assess local predation risk and/or potential foraging resources should be able to optimize these trade-offs (Lima & Bednekoff, 1999; Golub & Brown, 2003). A wide variety of fishes, including juvenile centrarchids, are known to maximize these trade-offs by undergoing size-specific ontogenetic niche shifts in habitat use, diet, and predation risk (Werner & Gilliam, 1984; Werner & Hall, 1988; Olson, 1996; Mittelbach & Persson, 1998).

It has recently been demonstrated that juvenile centrarchids also undergo a similar ontogenetic shift in their response to damage-released chemical alarm cues (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003). Damage-released chemical alarm cues are found in a wide range of freshwater fishes (Smith, 1992; Chivers & Smith, 1998), including centrarchids (Brown & Brennan, 2000; Golub & Brown, 2003; Marcus & Brown, 2003). Alarm cues are released following mechanical damage to the skin (as would occur during a predation event) and, when detected by nearby conspecifics and sympatric heterospecifics, can elicit dramatic short-term increases in anti-predator behaviour

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(Chivers & Smith, 1998; Smith, 1999). These cues can also lead to an increased probability of survival during predator encounters (Mathis & Smith, 1993; Mirza & Chivers, 2001a). Alarm cues can also provide reliable foraging information to nearby predators (Mathis *et al.*, 1995; Smith, 1999; Brown *et al.*, 2002).

Juvenile largemouth bass (*Micropterus salmoides,* Lacépède) undergo a behavioural shift from anti-predator to foraging response patterns when exposed to chemical alarm cues from a heterospecific prey guild member (finescale dace, *Phoxinus neogaeus,* Cope) at around 55 mm standard length (SL) (Brown *et al.*, 2001a). Green sunfish (*Lepomis cyanellus,* Rafinesque) undergo a similar shift in the use of heterospecific alarm cues at around 90 mm SL, but do not shift in response to conspecific alarm cues (Golub & Brown, 2003). However, these shift points appear to be phenotypically plastic and subject to threat-sensitive trade-offs between foraging and predator avoidance benefits (Brown *et al.*, 2002; Golub & Brown, 2003; Marcus & Brown, 2003).

Brown *et al.* (2001a; 2002) suggested that environmental factors such as the degree of habitat complexity (i.e., biotic and/or abiotic structure) would influence an individual's perceived risk of predation and hence influence the threat-sensitive trade-off between predator avoidance and foraging responses. Highly complex habitats, typically created by dense inshore vegetation, would limit the availability of visual information, and increase the value of chemical information in the assessment of local predation risk (Brown & Magnavacca, 2003; Brown *et al.*, 2004). In the absence of visual information, individuals may perceive a higher risk upon detecting a chemical alarm cue, increasing potential anti-predator benefits (Smith, 1999; Brown *et al.*, 2001a). Thus, under conditions of high habitat complexity, an individual would be predicted to exhibit an anti-predator response more readily, while under lower habitat complexity, the same individual would be predicted to more likely exhibit a foraging response to the same chemical information (Smith, 1999; Brown *et al.*, 2001a; Golub & Brown, 2003).

Previous studies examining the ontogenetic shift in use of chemical alarm cues by juvenile and sub-adult centrarchids have been conducted primarily under laboratory conditions (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003). This study examines the effects of habitat complexity on the juvenile to sub-adult behavioural shift in response to conspecific and heterospecific alarm cues in the field. In addition, this study will examine the use of heterospecific and conspecific alarm cues by juvenile and sub-adult pumpkinseed sunfish (*Lepomis gibbosus,* Linnaeus) under natural conditions. Pumpkinseed sunfish were chosen as our focal species for field observations because they have been shown to use heterospecific and conspecific alarm cues in the lab (Leduc *et al.*, 2003) and tend to occupy highly heterogeneous inshore habitats for most of their life (Scott & Crossman, 1973). Unlike other centrarchids, such as largemouth bass, pumpkinseed sunfish are opportunistic piscivores (Olson *et al.*, 1995; Mittelbach & Persson, 1998). Adults should only be piscivorous when perceived risk is low, making them ideal to test for differences between heterospecific and conspecific alarm cues (Brown *et al.*, 2002).

METHODS

The response to heterospecific and conspecific alarm cues were quantified for three local size classes of pumpkinseed sunfish (small < 40 mm, medium 40 – 80 mm, and large > 80 mm SL). Individuals of less than 40 mm SL were included because they have never been tested to see if they show the same response as larger individuals (40 – 80 mm SL size class). In addition, juvenile sunfish between about 40 and 80 mm are commonly found shoaling together in the littoral zone (personal observations) and hence were considered a single size class.

Focal shoals of each size class of pumpkinseed sunfish were exposed to one of four experimental stimuli: conspecific skin extract (PSF), largemouth bass (LMB), a synthetic cyprinid alarm cue (hypoxanthine-3-*N*-oxide, H3NO, Brown *et al.* 2000) and swordtail skin extract (SWT, *Xiphophorus helleri*, Heckel). Hypoxanthanine-3-*N*-oxide was used because it has been previously shown to elicit a response similar to natural cyprinid alarm cues in pumpkinseed sunfish (Leduc *et al.*, 2003). Swordtails are allopatric from pumpkinseed sunfish, and while they possess an analogous chemical alarm cue (Mirza *et al.*, 2001), it is not recognized by centrarchids (Brown & Brennan, 2000; Golub & Brown, 2003). Thus, swordtails were included as a control for the chemical cues of any injured heterospecific prey.

Field Sites

Field trials were conducted at Lac Seigneurial in Parc du Mont-Saint Bruno (45.55N, 73.32W), near Montreal, Quebec from June to August, 2003. Lac Seigneurial has a diverse inshore population of cyprinids (e.g., redbelly dace, *Phoxinus eos*, Cope; pearl dace, *Semotilus margarita*, Cope), centrarchids (e.g., largemouth bass; rock bass, *Ambloplites rupestris*, Rafinesque), percids (e.g., yellow perch, *Perca flavescens*, Mitchill) and ictalurids (e.g., brown bullhead, *Ictalurus nebulosus*, Lesueur) (personal observations). Trials were conducted at depths of 0.5 - 2 m, up to 20 meters from the shoreline. This inshore region consisted of highly heterogeneous habitat ranging from open patches to densely vegetated regions.

Habitat was ranked as low, intermediate or high complexity. Habitat rank was based on an average of density and height within the focal area (1 m radius of the stimulus injection site). Low complexity habitats consisted of exposed substrate (sand or detritus) with fewer than ten individual plants of varying height. Intermediate complexity habitats were made up of either dense short vegetation (< 30 cm tall) with some intermittent tall vegetation (1 - 10 plants > 30 cm tall), or a moderate density (10 - 20 individual plants) of tall vegetation amongst sand or detritus substrate. High complexity habitats consisted of predominantly tall (> 30 cm) dense vegetation (> 21 individual plants) with short vegetation filling the gaps.

Stimulus Preparation

Donor largemouth bass and pumpkinseed sunfish were collected from locations near Montreal (Lac Seigneurial, Canal Lachine, and Lac de La Prairie). Fish were held in 225 l holding tanks with a continuous flow-through of dechlorinated water at 18° C. Green swordtails were purchased from a commercial supplier and held in a continually filtered 110 l aquarium at ~28° C. All fish were fed twice daily *ad libitum* with commercial flake food and frozen brine shrimp (*Artemia* spp).

Natural skin extracts (mean SL ± SE) were collected from pumpkinseed sunfish (N = 8, SL = 64.4 ± 4.10 mm), largemouth bass (N = 11, 66.6 ± 1.90 mm) and swordtails (N = 16, 43.9 ± 1.50 mm) in accordance with Concordia University Animal Care

Protocol (AC-2002-BROW). Skin fillets of donor fish were measured (cm^2) homogenized in 50 ml of chilled glass distilled water, and filtered using polyester filter floss. Skin extracts were diluted using glass-distilled water to final volumes of (74.89 cm^2 in 895 ml, 70.96 cm^2 in 845 ml and 74.44 cm^2 in 910 ml) for pumpkinseed sunfish, largemouth bass, and swordtails respectively, and frozen in 20 ml samples at –20° C. H3NO was diluted to 0.001 mg ml^{-1} of stock solution in glass-distilled water and stirred for 20 minute. H3NO samples were stored as described above.

Experimental Protocol

This experiment consisted of 10 experimental trials for 4 different stimuli on 3 different habitat types, a total of 120 experimental pre- and post-stimulus observation periods. During each pair of observation periods the number or individuals in 3 different size classes was recorded resulting in a total of 360 experimental observations.

In order to locate focal fish (observation sites), an observer slowly snorkeled towards the shoreline. When a suitable site with fish was found, the stimulus injection tube (see below, Figure 2.1) was positioned and the observer remained motionless for at least ten minutes before the pre-stimulus observation period began. During all observations, the observer remained approximately 2 m from the point of stimulus injection. All stimulus injections were performed by an assistant positioned approximately 2 m behind (i.e., further away from the focal fish), in order to minimize movements by the observer. Focal fish did not show any adverse reaction to the presence of the observer.

For each observation, focal fish were exposed to only one of the four chemical cues. Before placement of the stimulus tube in the focal site the tube was cleared with 120 ml of lake water. Trials consisted of a 5-minute pre-stimulus period followed by a 5-minute post-stimulus injection observation period. At the beginning of the post-stimulus period, 20 ml of stimulus was injected into the tube and slowly flushed through with 120 ml of lake water at a rate of about 2.0 ml s^{-1}. Chemical stimuli were injected though airline tubing affixed to the end of a 2 m graphite pole, such that the stimuli were injected approximately 10 cm above the substrate. Just above the stimulus tube, a 1 m length of bamboo was affixed perpendicular to the pole and five cm increments were marked off. This was used to approximate the perimeter of the observation area, and estimate focal fish length (Figure 2.1).

During both pre- and post-stimulus injection observation periods, the number of individuals of each size class present within one meter of the stimulus injection site were recorded every 15 sec. Areas that contained experimental sites were not revisited for a minimum of two days to reduce the chance of that focal individuals became habituated to observers and stimuli.

Statistical Analysis

Data were analyzed using a three-way ANOVA, using habitat type, size class and stimulus type as independent variables, and the change in number of pumpkinseed sunfish present (post – pre-stimulus period) as the dependent variable. To test for possible effects of fish density before the injection of stimulus, a similar two-way

ANOVA was conducted on the pre-stimulus data to confirm no difference in pumpkinseed density during baseline observations.

RESULTS

During the pre-stimulus observation periods, there was no significant difference in the number of pumpkinseed sunfish present in each microhabitat (ANOVA habitat: $F_{(2, 353)} = 0.87$, $P = 0.42$, size class: $F_{(2, 353)} = 2.51$, $P = 0.08$, habitat x size class: $F_{(4, 353)} = 1.02$, $P = 0.39$), demonstrating that densities (mean ± SE) of small (n = 20.60 ± 2.78), medium (n = 15.97 ± 2.18) and large individuals (n = 12.92 ± 2.31) were similar across all habitats prior to introduction of experimental stimuli. Averages were calculated from the number of fish in each size class observed every 15 seconds during an observation period. Since individual fish were not tracked during observations these averages are unable to account for individual fish being recounted. Following the introduction of the experimental stimuli, individuals of different sizes (small, medium and large) respond significantly differently to alarm cues in different levels of habitats complexity (ANOVA $F_{(4, 326)} = 2.61$, $P = 0.036$), and different size pumpkinseed sunfish also treat conspecific, familiar heterospecific and allopatric control alarm cues significantly differently (ANOVA $F_{(6, 326)} = 7.66$, $P < 0.0001$). There was no significant interaction between stimulus and habitat (ANOVA $F_{(6, 326)} = 1.41$, $P = 0.21$).

Given the significant interaction between stimulus type and focal fish size class, additional ANOVAs were conducted to examine the effects of stimulus and habitat type on each size class independently. For small and medium size classes, a significant effect of stimulus type was found (small: $F_{(3, 109)} = 8.07$, $P < 0.0001$; medium: $F_{(3, 109)} = 4.76$, $P =$

0.0037). There was no significant effect of habitat (small: ($F_{(2, 109)} = 0.005$, $P = 0.99$; medium: $F_{(2, 109)} = 0.51$, $P = 0.59$) or stimulus x habitat interaction (small: $F_{(6, 109)} = 0.18$, $P = 0.98$; medium: $F_{(6, 109)} = 0.39$, $P = 0.88$). In response to largemouth bass and H3NO alarm cues, small and medium individuals exhibited a significant decrease in mean number present (an anti-predator response) in all three-habitat types (low, intermediate, and high complexity; Figure 2.2a, b). However, for large size class individuals, there was a significant effect of stimulus type ($F_{(3, 109)} = 12.81$, $P < 0.0001$), habitat type ($F_{(2, 109)} = 6.78$, $P = 0.0014$) and a significant stimulus x habitat type interaction ($F_{(6, 109)} = 2.18$, $P = 0.04$). Large individuals in this experiment exhibited an increase in number present (foraging response) in low and intermediate habitats, but a decrease in number present in high habitat complexity (Figure 2.2a, b).

When exposed to pumpkinseed sunfish skin extract (conspecific) all three-size classes of individuals (small, medium, and large) showed a decrease number present, regardless of habitat complexity (Figure 2.2c). When exposed to swordtail skin extract no change in number present was observed between pre- and post-stimulus (Figure 2.2d), suggesting that pumpkinseed sunfish do not recognize the allopatric heterospecific skin extract as either a foraging or predation risk cue.

DISCUSSION

These results demonstrate that the threat-sensitive use of conspecific and heterospecific chemical alarm cues as social information sources by juvenile centrarchids is influenced by both body size and microhabitat complexity. Under low and intermediate complexity conditions, smaller pumpkinseed sunfish exhibited increased anti-predator behaviour in response to the chemical alarm cues of heterospecific prey guild members. In contrast, larger pumpkinseed sunfish, exhibited an increase in number present, consistent with a foraging response. However, under conditions of high microhabitat complexity, pumpkinseed sunfish of all size classes exhibited an anti-predator response.

The data also demonstrate that in response to conspecific alarm cues, there was no evidence of an ontogenetic shift. Small, medium and large pumpkinseed sunfish exposed to conspecific alarm cues, regardless of microhabitat complexity, exhibited an anti-predator response. Previous studies have demonstrated similar shifts in the response to the chemical alarm cues of conspecifics and heterospecific prey guild members (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003; Marcus & Brown, 2003). However, these studies were conducted under laboratory conditions.

The current study provides, for the first time, field verification of laboratory results. Recent studies have argued that chemical alarm cues are artifacts of laboratory studies and/or biased field trapping techniques (Magurran *et al.*, 1996; Henderson *et al.*, 1997; Irving & Magurran, 1997; but see Smith, 1997). The strength of the current study lies in the use of direct observations, rather than indirect methods such as trapping studies (Magurran *et al.*, 1996; Smith, 1997). These data clearly demonstrate that under natural conditions, pumpkinseed sunfish rely on damage-released chemical alarm cues as both anti-predator and foraging cues. Recent studies have employed similar direct observation techniques (Brown & Godin, 1999; Brown *et al.*, 2001b) or employed underwater video cameras (Leduc *et al.*, 2004; Wisenden *et al.*, 2004; Wisenden & Barbour, in press) to verify the use of chemical alarm cues under natural conditions in a variety of freshwater prey species.

In order to maximize fitness, inshore juvenile and sub-adult centrarchids should shift from anti-predator to foraging in response to chemical alarm cues in a threat-sensitive fashion (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003). As small, primarily invertebrate feeding juveniles, individuals should gain increased survival benefits associated with an anti-predator response upon detecting a heterospecific chemical alarm cue. However, as they grow, and make a dietary switch to piscivory, potential foraging benefits should increase. In addition, as individuals grow, they may exceed the gape limitations of common inshore predators (Pettersson & Brönmark, 1993; Chipps *et al.*, 2004), hence reducing relative anti-predator benefits. As such, selection should favour individuals capable of making flexible behavioural decisions regarding the form of response to both conspecific and heterospecific alarm cues in a threat-sensitive fashion (Brown *et al.*, 2001a; 2002; Golub & Brown, 2003).

The results of the current study demonstrate that under natural conditions of high microhabitat complexity, large size class pumpkinseed sunfish exhibited an anti-predator response, where similar sized individuals exhibited foraging responses in less complex habitats. Under low and intermediate habitat conditions, individuals can rely on visual cues, in addition to chemical cues, to assess local predation risk. As such, the presence of a chemical alarm cue may be perceived as a lower predation risk by large size class pumpkinseed sunfish than by small class pumpkinseed sunfish (Brown & Magnavacca, 2003; Brown *et al.*, 2004). However, under conditions of high complexity, visual information would be limited and as a result, chemical alarm cues are perceived as a higher risk (Hartman & Abrahams, 2000; Brown & Magnavacca, 2003). Brown *et al.* (2002) similarly demonstrated that perceived predation risk influenced the response patterns of juvenile largemouth bass exposed to heterospecific alarm cues. Additional studies have demonstrated the influence of habitat complexity on the use of chemical alarm cues as risk assessment cues. For example, an individual's ability to learn to recognize heterospecific alarm cues and novel predators is dependent upon habitat complexity (Gazdewich & Chivers, 2002; Pollock & Chivers, 2003).

The observed results confirm the use of both conspecific and heterospecific alarm cues by centrarchids in natural settings as a means of predator detection. Individuals are able to shift their behaviour to heterospecific cues in a threat-sensitive fashion. This optimization of the shift is plastic with environmental conditions, which include habitat complexity. The combination of these inputs yields a dynamic trade-off in the costs and benefits that dictate the response of an individual.

Figure 2.1 Experimental apparatus used for behavioural field observations. Apparatus consisted of a stimulus injection tube affixed to a 2 m pole. At the end, two bamboo rods (1 m each) were affixed perpendicular to each other, marked in 5 cm increments, and used for estimating focal fish size. Below the handle, a plastic plate was attached and used to hold writing tablets. Model is not drawn to scale.

Figure 2.1

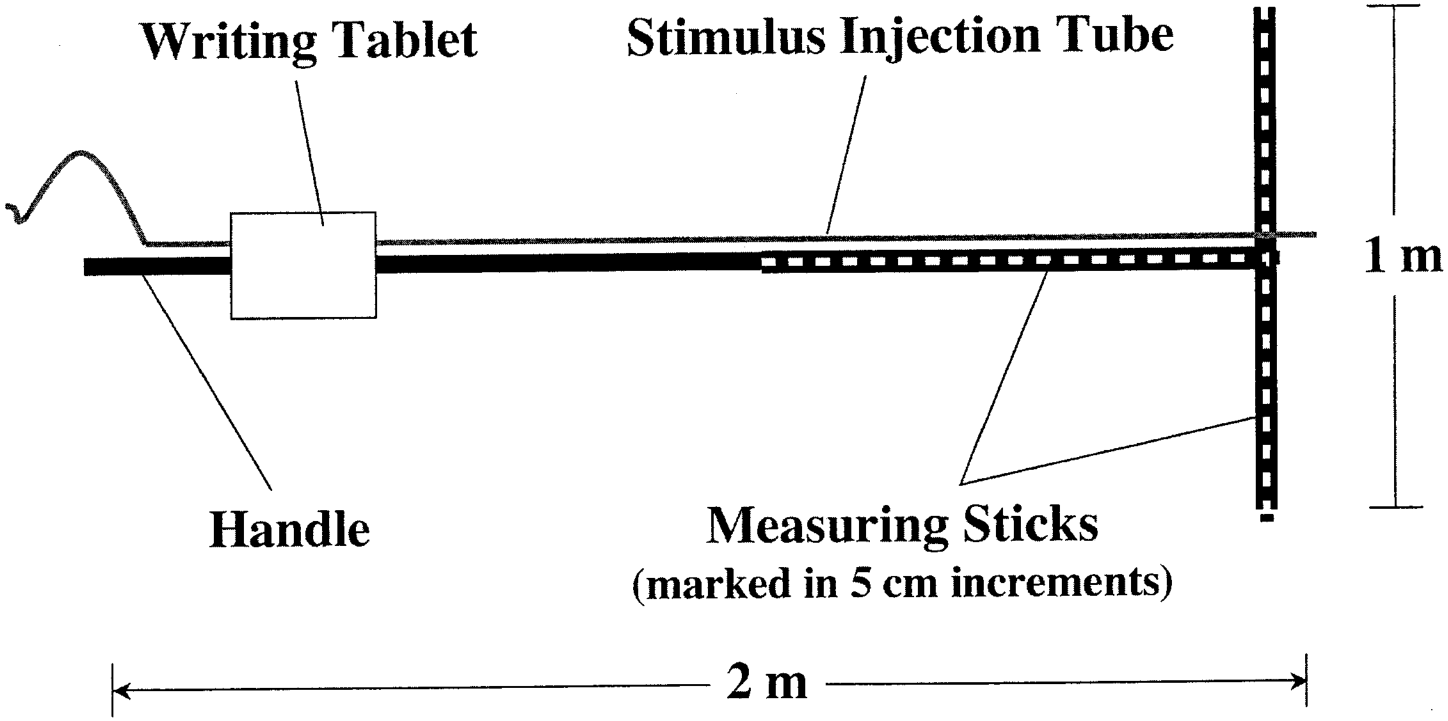
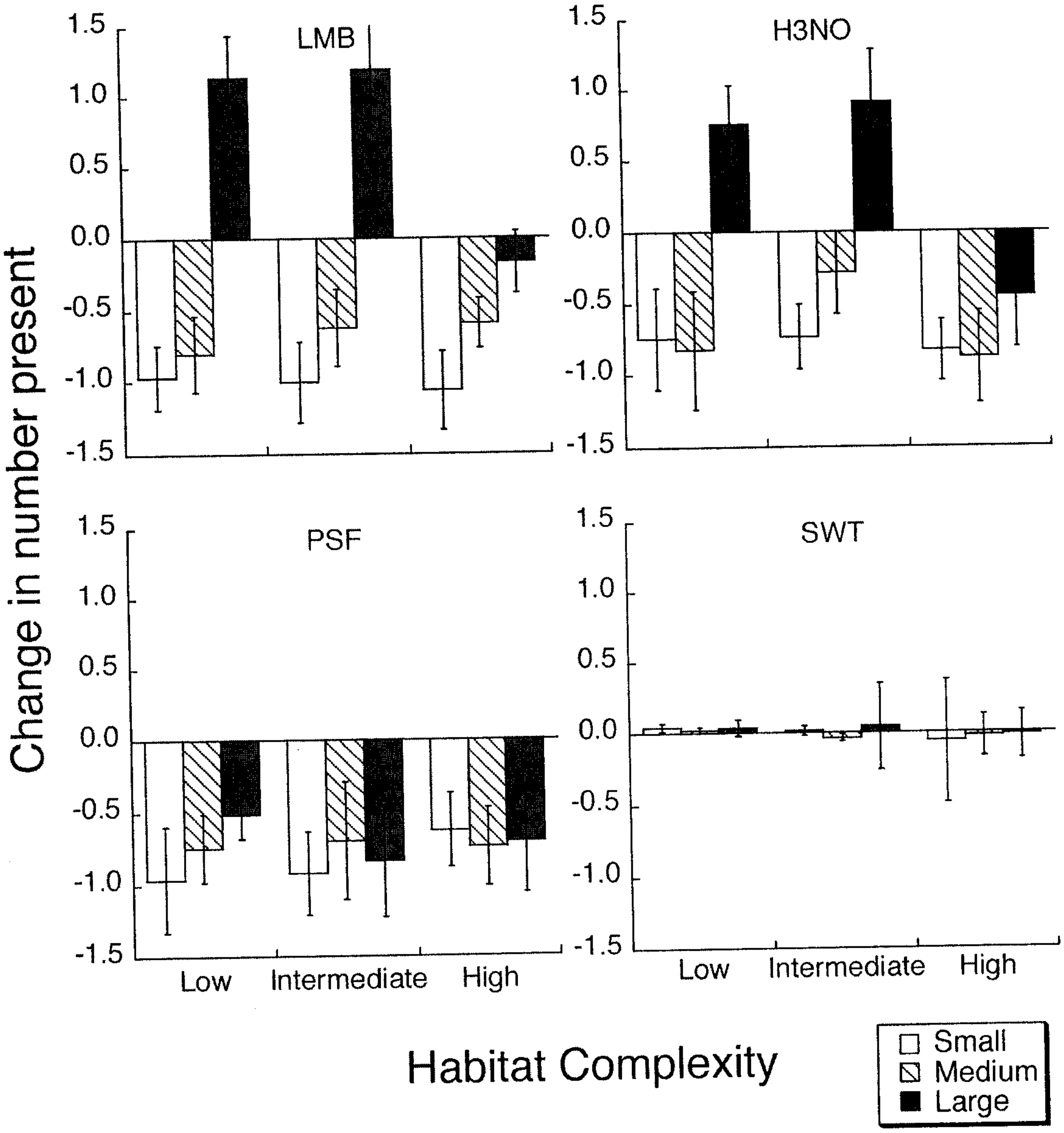

Writing Tablet
Stimulus Injection Tube
1 m
Handle
Measuring Sticks
(marked in 5 cm increments)
2 m

Figure 2.2 Mean (±S.E) change (post – pre) in number of individuals present for low, intermediate and high complexity habitats. Three size classes of individuals; Small (< 40 mm SL, Open bars), Medium (40 – 80 mm SL, Hatched Bars) and Large (> 80 mm SL, Closed Bars) were exposed to: Largemouth bass skin extract (LMB), Hypoxanthanine-3-*N*-oxide (H3NO), Pumpkinseed skin extract (PSF) and Swordtail skin extract (SWT).

Figure 2.2


Change in number present
LMB
H3NO
PSF
SWT
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Low
Intermediate
High
Habitat Complexity
□ Small
▧ Medium
■ Large

General Discussion

The response to damage-released chemical alarm cues appears to be a threat-sensitive trade-off between the benefits associated with anti-predator versus foraging behaviour. When perceived risks are low, the trade-off favours a foraging response. However, when risks are high, the trade-off favours an anti-predator response. As previously predicted (Brown *et al.*, 2001a), these results demonstrate that environmental factors such as habitat complexity and light availability will effect the trade-off of perceived risks and benefits to an individual. The laboratory and field results both demonstrate that under complex habitats (i.e. conditions where visual cues are limited), juvenile centrarchids exhibit a more intense anti-predator response than sub-adult individuals. However, under lower complexity habitats, individuals show behaviour patterns consistent with an increase in foraging behaviour in response to chemical alarm cues.

In Chapter 1, largemouth bass, between the juvenile and sub-adult ontogenetic phases (40 – 65 mm SL), exhibit plastic behavioural responses to conspecific and heterospecific alarm cues. By increasing the level of habitat complexity, or reducing light availability, visual cues become limited and hence perceived risk appears to increase. In test conditions where perceived risk was high (high habitat complexity, or low light availability), individuals exhibited an increase in anti-predator response intensity. In test conditions where visual cues were not limited (open habitat and high light), perceived risk was lower, thus individuals showed less predator avoidance behaviours and switched to foraging behaviours. Since individuals were exposed to the same stimuli in visually limited and non-limited microhabitats, the difference in response demonstrates a constantly adjusting threat-sensitive decision. The data also demonstrated that largemouth bass use conspecific alarm cues in the same threat-sensitive manner as heterospecific cues. Previous studies have demonstrated heterospecific and conspecific alarm cues are used differently by green sunfish (Golub & Brown, 2003). It has been argued that highly cannibalistic species, such as largemouth bass, perceive a greater benefit from conspecific alarm cues than opportunistic piscivorous species, such as pumpkinseed sunfish (Harvey & Brown, 2004). These individuals therefore show a shift in response to conspecific alarm cues much like the shift to heterospecific alarm cues.

Chapter 2 demonstrates that habitat complexity affects the threat-sensitive response to heterospecific alarm cues, in the field. Habitat complexity increases perceived risk, and individuals show increased anti-predator behaviour. It is one of the first field studies to demonstrate that centrarchids use alarm cues in their natural setting, and to demonstrate a threat-sensitive trade-off in their responses, as has been seen in the lab. This is also the first study to demonstrate the effects of habitat on the threat-sensitive response in the field. Smaller individuals (< 40 mm SL) demonstrate anti-predator responses, while larger individuals (> 80 mm SL) demonstrate foraging responses to heterospecific alarm cues. Intermediate size individuals (40-80 mm SL) demonstrated a context-dependant threat-sensitive trade-off in their responses. In low habitat complexity test sites they demonstrate foraging responses (increased number present), while in intermediate and high vegetation sites an increase in anti-predator responses (decreased number present). This study also confirmed the findings of Golub and Brown (2003), demonstrating that individuals perceived a greater risk of predation when exposed to conspecific cues, than heterospecific alarm cues. Recently, Marcus and Brown (2003) demonstrated that pumpkinseed sunfish do exhibit a shift to foraging response when exposed to conspecific cues at very low concentrations, supporting the hypothesis that chemical alarm cues are used in a threat-sensitive fashion.

The use of chemical alarm cues by juvenile centrarchids, such as largemouth bass (*Micropterus salmoides,* Lacépède) and pumpkinseed sunfish (*Lepomis gibbosus,* Linneaus), appears to be context dependant. By exhibiting phenotypically plastic response patterns, individuals are able to maximize benefits in high and low perceived risk situations. When perceived risk is high individuals are able to respond with anti-predator behaviour, to maximize chances of predator avoidance. When perceived risk is low, individuals respond with foraging behaviour because they have a reduced chance of being preyed upon, or increased chance of finding and capturing prey.

The findings of this thesis extrapolate from the predictions of the model proposed by Golub and Brown (2003; Fig 3.1). The model predicts the ontogenetic shift point based on trade-offs in relative benefits of anti-predator and foraging behaviour. Golub and Brown (2003) predicts that as an individual grows the benefits received from responding to inshore prey guild alarm cues with anti-predator behaviour (B_{ap}) decreases as their size increases. It also predicts that the benefits of foraging (B_{for}) upon detection of inshore prey guild alarm cues increase as size increases. For a small juvenile B_{ap} greatly outweighs B_{for}, so the individual responds to alarm cues with anti-predator behaviour. As this individual grows B_{ap} decrease and B_{for} increase. When B_{for} exceeds B_{ap} the model predicts that an individual will switch from anti-predator to foraging behaviour (L1) upon detection of alarm cues because the risk of lost foraging opportunities outweigh the risk of predation.

Based on these initial predictions a second set of predictions was made on the effects of variations in morphology. For example, Brown *et al.* (2002) used young-of-the-year (Y-O-Y) largemouth bass, split them into two groups and fed them different weight diets. This produced largemouth bass with shallower and deeper body morphs. These bass were then individually tested to heterospecific alarm cues. Individuals with shallower bodies were found to delay their shift while individuals with deeper bodies shifted earlier than the average. Individuals with a deeper body would be at a lower risk of predation because they would exceed the gape limitations of their predators earlier (Brönmark & Miner, 1992). Therefore, B_{ap} is reduced and is exceeded by B_{for} earlier, thus the individual should shift earlier (L2). Individuals with shallower bodies are susceptible to predators for longer because of their lower body depth and therefore experience an increased B_{ap}, which exceeds their B_{for} for longer and delays their shift (L3).

The data in Chapters 1 and 2 of this thesis predict that when the behavioural phenotypes are manipulated by limiting visual cues the perceived benefits of anti-predator and foraging behaviour change. When visual cues are limited (i.e. high habitat complexity and low light availability), perceived risk is greater, B_{ap} increases and B_{for} decreases and individuals benefit from continued anti-predator responses, and delaying their ontogenetic shift. When visual cues are readily available the perceived risk is lower and perceived benefits increase, so B_{ap} decreases and B_{for} increases and individuals switch to foraging behaviour sooner.

Recent studies have argued that chemical alarm cues are only used to compensate for lack of visual cues (Hartman & Abrahams, 2000), and that individuals do not rely on alarm cues in natural settings (Magurran *et al.*, 1996; Irving & Magurran, 1997). These studies suggest that in laboratory conditions perceived danger is greater, and therefore individuals show a response to alarm cues. When individuals are in their natural habitat they show no response to alarm cues because perceived danger is low.

Contrary to the findings of Hartman and Abrahams (2000), this thesis directly demonstrates that individuals use chemical cues when visual cues are not limited. Individuals may perceive the presence of chemical cues in situations where visual cues are limited as a greater risk than when visual cues are available; but they use both chemical and visual cues to assess predation risk (Brown & Magnavacca, 2003; Brown *et al.*, 2004). Chapter 2 directly demonstrates, through observations in natural settings, that individuals rely on both chemical and visual cues to assess predation risk. As well, other recent studies have demonstrated the use of alarm cues through live and video observation under natural conditions (Brown & Godin, 1999; Brown *et al.*, 2001b; Wisenden *et al.*, 2004; Wisenden & Barbour, in press). The anti-predator response to chemical cues is equal to that of a model predator (Wisenden *et al.*, 2004).

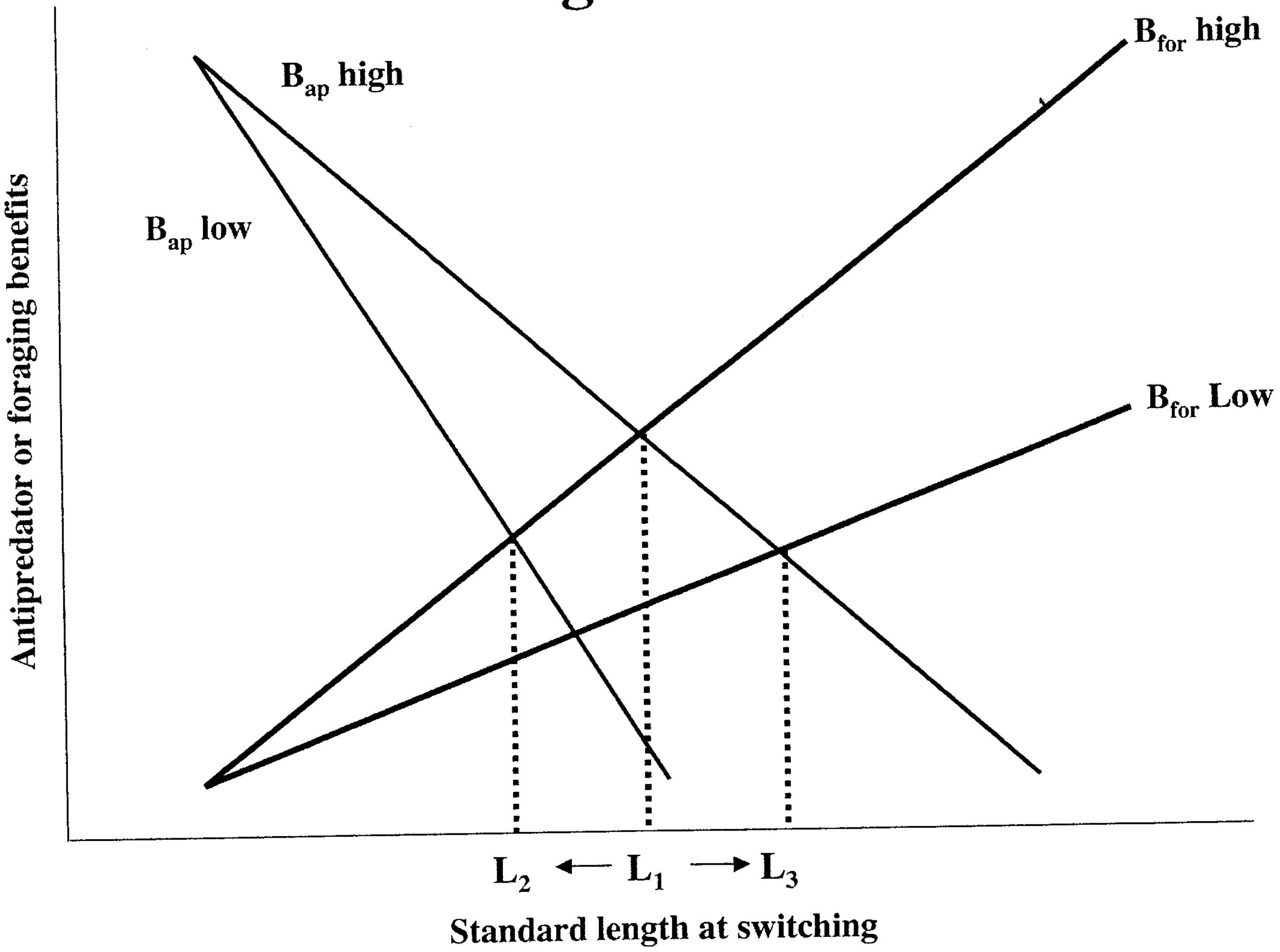
The mechanism influencing the shift from anti-predator to foraging response is unknown. The shift from anti-predator to foraging responses is a behavioural decision, influenced by recent experiences, temporal variations and ambient predation (Brown & Dreier, 2002; Lima & Bednekoff, 1999). Individuals that learn to recognize potential predators and retain the information are able to use this information to avoid viable predators in the future (Kelley & Magurran, 2003; Brown, 2003). Individuals that are also able to recognize non-threatening predators are also at an advantage because they can use this information to switch to foraging behaviours sooner. Individuals in environments under constantly high predation pressure perceive a lower risk from the presence of alarm cues that those in lower risk situations (Lima & Bednekoff, 1999). Therefore, individuals exposed to constantly high predation pressures should shift to foraging responses earlier because constantly responding to these alarm cues results in the loss of foraging opportunities. Individuals in lower risk situations however are not exposed to these predation pressures as often and potentially benefit more from responding with anti-predator behaviour. Also individuals who have greater assets to protect should treat alarm cues as more risky than those with fewer assets to protect (Reinhardt & Healey, 1999). Those individuals that have higher fat stores and are well satiated should favour an anti-predator response because they have a greater asset to loose. Individuals that are hungry and have less fat reserves are at a greater risk of starving to death, and therefore favour a foraging response because the potential risks of predation are outweighed by the benefits of foraging. Another potential mechanism is the potential to exceed the gape limitations of potential predators. By switching to piscivory and being able to take advantage of potential foraging opportunities, individuals are able to grow faster and exceed their predators' gape limitations earlier. By exceeding predators gape limitations individuals reduce their perceived predation risks. Without further experimentation the mechanisms by which individuals shift from anti-predator to foraging behaviours is still unknown.

Other recent studies have demonstrated the effects of habitat complexity and light intensity on survival, learning, and phenotype. Gazdewich and Chivers (2002) demonstrated that chances of survival increase in open habitats versus complex habitats. Individuals learn to recognize the value of heterospecific alarm cues in open habitats over vegetated habitats (Pollock & Chivers, 2003). Individuals can modify and thus adapt their morphology and foraging strategy with habitat complexity (Chips *et al.*, 2004). Habitat complexity can also limit the territory size of an individual, making larger territories with complex structure uneconomic to defend (Breau & Grant, 2002). When exposed to overhead visual cues in the absence of chemical cues, as is typical with avian predators, individuals show a shorter reaction time to potential threats (Grant & Noakes, 1987). Much like the threat-sensitive adjustments in response to chemical cues, individuals can adjust their response to visual cues when habitat affects perceived threat. Other studies have demonstrated that as habitat complexity increases predator avoidance behaviour decreases (Relyea & Werner, 1999). Since these studies used visual cues in situations where they are less reliable (e.g. increased habitat complexity) and in the absence of chemical cues, individuals are unable to assess potential predation risk, and therefore are unable to respond.

Consequently, for individuals to reliably assess predation risk, both chemical and visual cues should be used. When one of these cues is inhibited, behavioural decisions will be made based on the most reliable information available. Typically these decisions will be more cautious because the information provided by chemical and visual cues independently is limited, and therefore less reliable then the two cues together. With less reliable information individuals are likely to perceive situations as more risky then they might actually be and respond with more cautious behaviour.

Figure 3.1 Model predicting relative benefits of anti-predator (B_{ap}) and foraging behaviours (B_{for}) in response to chemical alarm signals and the effects of shifts in perceived benefits of anti-predator and foraging behaviours. As presented by Golub & Brown (2003)

Figure 3.1


B$_{ap}$ high
B$_{ap}$ low
B$_{for}$ high
B$_{for}$ Low
Antipredator or foraging benefits
L$_2$ ← L$_1$ → L$_3$
Standard length at switching

REFERENCE

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