Linking Morphological and Behavioural Defences: Prey Fish Detect the Morphology of Conspecifics in the Odour Signature of their Predators

Douglas P. Chivers, Xiaoxia Zhao & Maud C. O. Ferrari

Department of Biology, University of Saskatchewan, Saskatoon, SK, Canada

Abstract

Predation is a strong selective force acting on both morphology and behaviour of prey animals. While morphological defences (e.g. crypsis, presence of armours or spines or specific body morphologies) and anti-predator behaviours (e.g. change in foraging or reproductive effort, or hiding and fleeing behaviours) have been widely studied separately, few studies have considered the interplay between the two. The question raised in our study is whether antipredator behaviours of a prey fish to predator odours could be influenced by the morphology of prey conspecifics in the diet of the predator. We used goldfish (Carassius auratus) as our test species; goldfish exposed to predation risk significantly increase their body depth to length ratio, which gives them a survival advantage against gape-limited predators. We exposed shallow-bodied and deep-bodied goldfish to the odour of pike (Esox lucius) fed either form of goldfish. Deep-bodied goldfish displayed lower intensity antipredator responses than shallow-bodied ones, consistent with the hypothesis that individuals with morphological defences should exhibit less behavioural modification than those lacking such defences. Moreover, both shallow- and deep-bodied goldfish displayed their strongest antipredator responses when exposed to the odour of pike fed conspecifics of their own morphology, indicating that goldfish are able to differentiate the morphology of conspecifics through predator diet cues. For a given individual, predator threat increases as the prey become more like the individual eaten, revealing a surprising level of sophistication of chemosensory assessment by prey fish.

Introduction

The importance of predation pressure in altering the behaviour and morphology of prey animals has long been recognized (Lima & Dill 1990; Lima 1998). Prey often show dramatic changes in behaviour, such as fleeing or hiding, upon encountering predators. Behavioural responses to predators can also include subtle alterations in foraging rates, foraging postures, level of reproductive effort, etc. (Chivers et al. 1995; Wisenden et al. 1995). Morphological defences include such things as the development of protective spines and armour and the presence of cryptic and aposematic colouration (Edmunds 1974; Appleton & Palmer 1988). Different types of antipredator defences often work at different stages in the predation sequence (Lima & Dill 1990). For example, behavioural defences often work to reduce the
probability of an encounter with a predator by influencing habitat choice of the prey. Alternatively, fleeing and hiding responses often act to reduce the probability of capture during an encounter. These are different than morphological defences, which often act to deter the predator from attacking during an encounter or increase the probability that the prey will survive if it is attacked.

In many cases morphological defences are not fixed but rather are induced upon exposure to predators (Harvell 1990; Clark & Harvell 1992). Examples are particularly common among invertebrate taxa (e.g. Appleton & Palmer 1988; Johansson & Samuelsson 1994; Wiackowski et al. 2003; Laforsch 2004; Hoverman et al. 2005). The pioneering work in vertebrates, completed by Brönmark & Miner (1992) demonstrated that crucian carp (Carassius carassius) increase their depth to length ratio (DLR) in response to predation cues, thus making the prey less vulnerable to gape-limited predators (Nilsson et al. 1995). However, this defence is costly because those individuals with induced morphological defences suffer a hydrodynamic cost and are out-competed by individuals without the induced morphology (Pettersson & Brönmark 1997, 1999; Andersson et al. 2006). The specific cues that induce the change in morphology are prey alarm cues in the predator’s diet (Stabell & Lwin 1997). Chivers et al., (in press) recently documented the same increase in DLR in response to alarm cues by a closely related fish species, the goldfish (Carassius auratus).

Researchers studying antipredator defences often focus on either behaviour or morphology. However, there has been strikingly little consideration of the interplay between the two. In one study, Abrahams (1995) hypothesized that prey species which possess morphological defences should exhibit less behavioural modification in response to predation risk than species lacking such defences. His results showed that brook stickleback (Culaea inconstans) exhibited relatively little behavioural modification in response to the presence of a predator (yellow perch, Perca flavescens), both in terms of their reactive distance to an approaching predator and in avoiding dangerous areas, when compared with fathead minnows (Pimephales promelas). Stickleback are armoured while minnows lack armour. In another study, Hoverman et al. (2005) examined how prey integrate behavioural and morphological defences. They documented that the morphological responses of snails (Helisoma trivolvis) to different types of predators had subsequent effects on the behavioural responses of the snails to predator cues. We suggest that there is a need for more research that integrates both behavioural and morphological defences, particularly as they related to induced morphological defences. Such studies will be particularly fruitful in understanding the dynamic nature of antipredator defences.

A diversity of aquatic organisms assesses their risk of predation using chemosensory information (for review see Chivers & Smith 1998; Kats & Dill 1998; Wisenden & Chivers 2006). The source of the chemicals can arise from the predator (i.e. predator odours) or from cues released from damaged prey (i.e. alarm cues). The information content of predator odours has received increasing attention and demonstrates a surprising level of sophistication. For example, Mathis & Smith (1993a,b) demonstrated that predator-naive fathead minnows could determine the diet of their predators based on the predator’s odour. Minnows showed antipredator responses to cues of pike fed minnows but not pike fed another fish diet (swordtails, Xiphophorus helleri). Such diet-dependent antipredator responses are widespread in predator–prey systems (for review see Chivers & Mirza 2001). In another study, Kusch et al. (2004) showed that fathead minnows could determine the size of a predatory pike from the predator’s odour alone. In that experiment, minnows showed greater intensity responses to the odour of small pike than to the odour of large pike. Small pike likely present more of a risk to minnows than do large pike. Minnows can also determine both the relative density and the relative proximity of predators through predator odour cues (Ferrari et al. 2006). These studies raise the question of what other information prey fishes can acquire from the odour signature of their predators.

In this experiment we investigated the interplay between morphological and behavioural defences of a prey fish and address what other information prey can detect in the odour of their predators. Specifically, we test if goldfish can differentiate the odour of predators that have been fed on conspecifics that have different morphologies (i.e. those with a shallow-bodied morphology vs. those with a deep-bodied morphology). According to the threat-sensitive predator avoidance hypothesis (Hellman 1989; Chivers et al. 2001), prey should match the intensity of their antipredator response to the degree of threat posed by their predators. Three scenarios exist in which the goldfish may accomplish this matching. First, prey may respond most dramatically to cues of predators feeding on conspecifics that match their own morphology. A predator
that is feeding on an individual that is the same size and same shape as the prey could be perceived as the greatest threat. From this we would predict that shallow-bodied goldfish should respond strongest to predators fed shallow-bodied goldfish and deep-bodied goldfish should respond strongest to predators fed deep-bodied goldfish (scenario 1). However, a predator that can consume a deep-bodied goldfish can most certainly consume a shallow-bodied goldfish, but a predator that can consume a shallow-bodied goldfish may not be able to consume a deep-bodied goldfish because of its gape limitation. Consequently, this generates the prediction that both shallow- and deep-bodied goldfish may show stronger responses to cues of predators fed deep-bodied goldfish as they may be perceived as more of a threat (scenario 2). However, are they more of a threat? Fish with deep vs. shallow bodies could rely on different strategies to avoid predation. Fish with a deep-bodied morphology have a reduced swimming speed and are less manoeuvrable (Nilsson et al. 1995). Instead of relying on escape they may rely on their morphology to avoid attack. In situations where predators are efficient enough to capture shallow-bodied prey, which are highly manoeuvrable, they can certainly capture deep-bodied prey. If predators are big enough to consume deep-bodied prey, then predators that can capture shallow-bodied prey may be more of a threat than those that can capture deep-bodied prey. This third scenario predicts that both deep- and shallow-bodied goldfish should respond strongest to cues of predators fed shallow-bodied conspecifics.

**Methods**

To understand what effect a prey’s morphology has on the odour signature of its predator and how other prey respond to this signature, we used a $2 \times 2$ design exposing both shallow-bodied and deep-bodied goldfish to odours of predators fed either shallow-bodied or deep-bodied conspecifics.

Shallow-bodied and deep-bodied goldfish came from a previous experiment examining the induced change in morphology of goldfish (Zhao et al., unpubl. data). Here we provide a brief summary of the experiment. Juvenile goldfish were held for 60 d in 74–1 aquarium at 18°C. They were fed 8% of their body weight per day (5% fed at 10:00 h and 3% at 16:00 h). Each day the fish were exposed to 10 ml of skin extract from conspecifics (experimental treatment), or controls of 10 ml of skin extract from heterospecific swordtails or distilled water. In response to alarm cues from conspecifics the goldfish increased their DLR compared with the control treatments and this higher DLR reduced the vulnerability of the goldfish during encounters with live predators. In the current experiment, fish from the goldfish skin extract treatment represent our deep-bodied fish and goldfish from the swordtail treatment represent our shallow-bodied fish. The body length of the shallow-bodied fish was similar to that of the deep-bodied fish (mean difference in length = 3.9%). However, the two types of fish differed dramatically in their depth (17.2% difference) and DLR (18.0% difference – range of DLR for shallow-bodied goldfish: 0.25–0.34; for deep-bodied goldfish: 0.34–0.42).

Northern pike used in experiments were collected from Pike Lake in central Saskatchewan, kept in a 4000-L pool and fed goldfish (both morphologies – the goldfish were left from previous experiments). Prior to the experiment, pike were fed fathead minnows for 1 mo. We collected cues from pike that were fed either deep- or shallow-bodied goldfish. We used four different pike to prepare the cues: two pike (19.3 and 17.7 cm standard length (SL)) were fed deep-bodied goldfish, and two (19.5 and 17.5 cm SL) were fed shallow-bodied goldfish for nine consecutive days. Thirty minutes after the final feeding, the pike that were fed deep-bodied goldfish were placed into a 74-l aquarium with fresh dechlorinated water. The pike fed shallow-bodied goldfish were likewise placed into a 74-l aquarium with fresh dechlorinated water. After 24 h, pike were removed and the remaining water was used as the stimuli in the experiments. The stimuli were pipetted into aliquots and frozen at −20°C until use. To minimize predator differences in the odour signature, we switched prey types for each of the two sets of northern pike; those fed deep-bodied goldfish were fed shallow-bodied goldfish and vice versa. During each behavioural trial (see below) we introduced 120 ml of the stimulus odour, 60 ml from each of the two sets of stimulus.

We conducted 64 trials, 16 replicate trials in each of four treatments (deep- and shallow-bodied goldfish exposed to cues of pike fed either deep- or shallow-bodied goldfish). Fish were tested individually; the order of the treatments was randomized. The trials were performed in 74-l aquarium (60 x 30 x 40 cm) which were wrapped with black plastic on three sides so that fish in adjacent tanks were not visible to each other. Each tank had two 10-cm horizontal lines on the front of the tank.
which divided the tank into three vertical areas. Each of the test tanks contained a single airstone mounted in the centre of the end wall.

Fish were allowed to acclimate in the tanks for 24 h prior to trials. Each trial was 18 min in length and consisted of an 8-min pre-stimulus period and an 8-min post-stimulus period, with a 2-min stimulus-injection period between the pre- and post-stimulus periods. Prior to the pre-stimulus period, we removed and discarded 60 ml of water through the stimulus injection tube. We then removed and retained an additional 60 ml of water. After the pre-stimulus observation period, we injected 120 ml of either pike fed deep-bodied goldfish cue or pike fed shallow-bodied goldfish cue, and flushed it through with the 60 ml of the previously retained water.

To assess the effect of morphology on the response to predation cues, we calculated the number of line crosses and vertical area scores for each fish for both the 8-min pre-stimulus and the 8-min post-stimulus periods. A line cross was scored when 75% of the fish’s body crossed from one grid square on another. An area use score (1 = lower third of water column, 2 = middle third of water column, and 3 = upper third of water column) was recorded every minute. In response to predation risk cues prey fishes often change their vertical distribution. For example, Zhao & Chivers (2005) and Zhao et al. (2006) demonstrated that goldfish move towards the top of the water in the presence of predation cues. In contrast, studies with brook stickleback (Chivers et al. 1995) and convict cichlids (Brown et al. 2006) have shown movement towards the substrate. In another study, Mirza et al. (2001) showed that male swordtails moved towards the top of the water column whereas females moved towards the substrate in response to predation cues. Prey fishes also change their activity level in response to predation cues. While most studies have documented a decrease in activity in response to predation threat, Zhao (unpubl. data) has shown that goldfish increase activity in response to predation threats. We are confident that this increase in activity represents antipredator behaviour as the fish also altered their vertical distribution and morphology in a manner indicative of an antipredator response (i.e. the goldfish increased their DLR).

For each treatment we calculated the change in line crosses and vertical area occupied from the pre-stimulus baseline. These data were normally distributed with equal variances among treatments. Hence, we performed a $2 \times 2$ ANOVA on each of the two behavioural measures (line crosses and area use) to assess how prey fish morphology (deep-bodied vs. shallow-bodied) and cue type (predator odours made from deep-bodied vs. shallow-bodied goldfish) influenced the responses of goldfish. In case of a significant interaction between the two factors, two series of ANOVAs were performed to assess the effect of fish morphology (deep-bodied vs. shallow-bodied fish) on the responses to each of the cues and to assess the effect of cues (cues from deep-bodied vs. shallow-bodied fish) on the response of fish from each of the two morphologies.

**Results**

Behavioural response to diet cues showed significant interactions between body morphology of the receiver and the morphology source of the diet cue (Line crosses: morph*cue $F_{1,56} = 14.82$, $p < 0.001$; Area use: morph*cue $F_{1,56} = 4.27$, $p = 0.043$, Fig. 1).

Deep-bodied receivers did not respond differently to cues from deep- and shallow-bodied diet cues in terms of change in area use ($t = 1.2$, df = 28, $p = 0.235$). However, they displayed higher antipredator response to diet cues from their own morphs compared with diet cues from shallow-bodied goldfish in

![Fig. 1: Mean (±SE) change in line crosses (top panel) and area use (bottom panel) for shallow- and deep-bodied goldfish exposed to the odour of pike fed either shallow-bodied (empty bars) or deep-bodied (solid bars) conspecifics.](image-url)
terms of change in line crosses \( (t = 3.1, \text{ df} = 28, p = 0.004) \). Similarly, shallow-bodied receivers did not respond differently to diet cues from both morphs in terms of change in area use \( (t = -1.7, \text{ df} = 28, p = 0.104) \). However, shallow-bodied goldfish responded with a higher intensity response to diet cues from their own morph compared with diet cues from deep-bodied goldfish in terms of change in line crosses \( (t = -2.4, \text{ df} = 28, p = 0.021) \).

Shallow-bodied goldfish displayed significantly higher intensity responses to shallow-bodied diet cues than did deep-bodied goldfish both in terms of change in line crosses \( (t = -4.0, \text{ df} = 28, p < 0.001) \) and change in area use \( (t = -3.9, \text{ df} = 28, p = 0.001) \). However, in response to the deep-bodied diet cues, responses of the two receiver morphs did not differ terms of change in line crosses \( (t = 1.2, \text{ df} = 28, p = 0.209) \) or change in area use \( (t = -0.9, \text{ df} = 28, p = 0.381) \).

**Discussion**

The results of our experiment illustrate the dynamics between antipredator behaviour and morphology and greatly extend our understanding of the sophistication of chemosensory risk assessment by prey fishes. In our experiment, deep- and shallow-bodied goldfish have a different history with regards to exposure to alarm cues. In fact, it is this differential exposure to alarm cues that induced the change in morphology of the goldfish. Associated with this change in morphology were differences in the level of antipredator response of deep- and shallow-bodied goldfish to predation cues. Each morph differentiated cues of predators fed goldfish with different morphologies.

Our results provide support for threat-sensitive predator avoidance. The goldfish match the intensity of their antipredator response with the degree of threat posed by the predator. We provide support for the first scenario we outlined in our introduction. Specifically, shallow-bodied goldfish showed their strongest antipredator responses to predators fed shallow-bodied prey and deep-bodied goldfish showed their strongest responses to predators fed deep-bodied goldfish. This means that a predator that is feeding on an individual that is the same size and same shape as the prey is perceived as the greatest threat. A diversity of aquatic animals, including numerous fishes, appears to exhibit threat-sensitive predator avoidance (Helfman 1989; Bishop & Brown 1992; Chivers et al. 2001; Ferrari et al. 2005; Ferrari & Chivers 2006). For example, three-spot damselfish (Stegastes planifrons) exhibit more intense antipredator behaviour to model trumpetfish (Aulostomus maculatus) that are more of a threat (i.e. are larger, closer or in a strike pose, Hellman 1989). In another study, Chivers et al. (2001) demonstrated that slimy sculpins (Cottus cognatus) avoid areas in a creek that contain caged brook char (Salvelinus fontinalis) that are large enough to pose a threat but do not avoid caged trout that are not a threat. In this system the sculpins can determine the threat posed by the predator when visual cues alone were presented. However, when only the predator odour was presented, sculpins responded to the predator regardless of its size. Chemical cues appear to function to warn the sculpins of danger but visual cues are needed in order for the sculpins to assess the risk posed by the predator.

A comparison of the level of antipredator behaviour exhibited by deep-bodied and shallow-bodied fish revealed a striking difference. Deep-bodied goldfish showed a statistically lower intensity response to predator cues than shallow-bodied goldfish. This result is consistent with the hypothesis that individuals possessing morphological defences should exhibit less behavioural modification in response to predation risk than individuals lacking such defences. This hypothesis is a natural extension of Abrahams’ (1995) hypothesis that species possessing morphological defences should exhibit less behavioural modification than species lacking such defences.

The sophistication of chemosensory assessment of risk by prey fishes is amazing. Previous work has shown that prey can differentiate the size of predators, the density and proximity of the predators and the diet of those predators (Mathis & Smith 1993a,b; Kusch et al. 2004; Ferrari et al. 2006). We add to this that prey can differentiate the morphology of the conspecifics eaten by the predator. One fascinating question that arises from this work is what proximate cues are the prey using to differentiate these odour signatures. This is an area that deserves the attention of researchers with expertise in organic chemistry.

Predator-induced changes in morphology are common among a diversity of taxa, including protozoans (Kuhlman & Heckmann 1985; Kusch 1993; Wiackowski et al. 2003), cladocerans (Grant & Bayly 1981; Laforsch 2004), rotifers (Beauchamp 1952a,b; Stemberger & Gilbert 1984), bryozoans (Yoshioka 1982), gastropods (Appleton & Palmer 1988; Hoverman et al. 2005), insects (Hershey & Dodson 1987; Johansson & Samuelsson 1994; Johansson 2002).
and amphibians (McCollum & Van Buskirk 1996; Van Buskirk & Relyea 1998; Relyea 2002, 2004; Schoepppner & Relyea 2005). The question of how such induced changes in morphology affect the subsequent antipredator behaviour of prey remains virtually unexplored. We encourage future research aimed at understanding the interaction between morphology and behaviour in order for us to gain a full appreciation of the dynamic nature of antipredator responses.

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