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The paradox of risk assessment: comparing responses of fathead minnows to capture-released and diet-released alarm cues from two different predators

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Summary. Many aquatic prey are known to use chemical alarm cues to assess their risk of predation. In fishes, such alarm cues can be released either through damage of the epidermis during a predatory attack (capture-released) or through release from the predator feces (diet-released). In our study, we compared the importance of capture- versus diet-released alarm cues in risk assessment by fathead minnows (*Pimephales promelas*) that were naïve to fish predators. We utilized two different fish predators: a specialized piscivore, the northern pike (*Esox lucius*) and a generalist predator, the brook trout (*Salvelinus fontinalis*). Handling time of pike consuming minnows was much shorter than for trout consuming minnows, likely resulting in less epidermal damage to the minnows during attacks by pike. In accordance with this, minnows showed a less intense antipredator response to capture-released cues from pike than capture-released cues from trout. This represents a paradox in risk assessment for the minnows as they respond to the specialized piscivore, the more dangerous predator, with a less intense antipredator response. In contrast, the minnows showed a stronger antipredator response to the specialized piscivore than to the generalist when given diet cues. This work highlights the need for researchers to carefully consider the nature of the information available to prey in risk assessment.

Key words. Chemical alarm cues – predator odour – diet cues – risk assessment – fathead minnow (Pimephales promelas, Family Cyprinidae, Order Cypriniformes, Class Actinopterygii)

Introduction

Due to the unforgiving nature of predation, prey animals are under intense selection to detect and avoid predators (Lima & Dill 1990, Wisenden & Chivers 2005). Because responding to predators is costly, animals displaying adaptive responses, i.e. optimizing the trade-off between antipredator behaviour and foraging or reproduction, should be at a selective advantage (Helfman 1989, Lima & Bednekoff

1999). A prerequisite for effective and adaptive responses against predators is that prey possess accurate information regarding the level of threat posed by the predators. For many aquatic species, chemicals present in the water represent an important source of information regarding foraging, reproduction and predation (Chivers & Smith 1998).

Aquatic animals can gather information regarding predators using chemicals released by the predator, i.e., kairomones or predator odours (Kats & Dill 1998). For example, predator odours have been shown to enable prey fishes like fathead minnows (*Pimephales promelas*) to determine relative size (Kusch *et al*. 2004), proximity and density (Ferrari *et al*. 2006) of predatory northern pike (*Esox lucius*). Many fishes, including fathead minnows, do not have an innate recognition of predators, that is, individuals have to learn to recognize potential predators as a threat (reviewed by Brown 2003).

The second type of chemicals of informative value available to aquatic prey are chemical alarm cues. These chemicals are often released by prey animals when they are attacked or captured by a predator (Chivers & Smith 1998). Such alarm cues have been found in a wide variety of aquatic organisms, both invertebrates (protozoans, flatworms, annelids, arthropods, molluscs) and vertebrates (fishes and amphibians) (reviewed by Wisenden 2003). They often elicit a dramatic increase in antipredator behaviour when detected by conspecifics and some heterospecifics (reviewed by Chivers & Smith 1998). Experimental manipulations of alarm cue concentrations have shown that increased alarm cue concentrations elicit increases in the intensity of antipredator behaviour displayed by some fish (Dupuch *et al.* 2004, Zhao & Chivers 2006), including fathead minnows (Ferrari *et al.* 2005, Ferrari & Chivers 2006). While these chemicals likely did not primarily evolve as true alarm signals (Wisenden & Chivers 2005, Chivers *et al.* unpublished data), prey responding to these chemicals have a selective advantage. Alarm cues increase survival of receivers (Mathis & Smith 1993a; Mirza & Chivers 2001). They also mediate learned predator recognition through the pairing of alarm cues with novel predator odours (Brown 2003).

The exact location of the alarm cues in the body of prey is still unknown for most taxa. However, it has been shown that fish chemical alarm cues are localized in their epidermis (Chivers & Smith 1998). In addition to being released

through mechanical damage of the skin following a predatory attack during which the fish is injured or captured (capture-released), alarm cues have been shown to be released in the feces of their predators (Mathis & Smith 1993b, 1993c, Brown *et al.* 1995) (diet-released). The alarm cues or the breakdown products of the alarm cues are not completely degraded during the digestion process.

While both capture- and diet-released alarm cues have been shown to be important in mediating predator/prey interactions, no studies have attempted to directly compare the efficacy of the two cues in mediating antipredator responses of the prey. Hence, the first goal of our experiment was to compare the responses of fathead minnows to capture- versus diet-released cues of predators in a controlled experiment. The second goal of our experiment was to investigate whether the type of predator (specialist or generalist) would influence the intensity of these responses. Here, we use two different fish predators, the northern pike – a specialized piscivore, and brook trout (*Salvelinus fontinalis*) – a more generalist predator. We hypothesized that the characteristics of specialist piscivores (e.g., a large mouth, pharyngeal suction etc.) would be associated with shorter handling time of prey and would lead to a smaller release of alarm cues during capture. Differential patterns of cue release would likely influence the antipredator responses of prey, as it has been shown that many fish species increased the intensity of their antipredator response when exposed to increased concentrations of conspecific alarm cues (Ferrari *et al.* 2005). We hypothesized that if the specialized piscivore (i.e., the pike) is effective at reducing capture-released cues, then fathead minnows may effectively consider pike as less of a threat than trout when the opposite is true (Bertolo & Magnan 2006, Findlay *et al.* 2000). From the perspective of the prey, this may create a paradox in terms of risk assessment, because fathead minnows assess local predation risk through alarm cue concentration. If specialized predators are more effective at breaking down alarm cues through digestion than are generalist predators, then the prey should likewise consider the pike as less of a threat. This could again create a paradox in terms of risk assessment.

Methods

Fish collection and maintenance

Fathead minnows were collected from a one-ha pond located on the University of Saskatchewan campus in Saskatoon, SK, Canada in January of 2006 using Gee's Improved Minnow Traps. The pond contains fathead minnows and brook stickleback (*Culaea inconstans*) but no predatory fishes. Minnows from this pond do not show innate recognition of fish predators including pike (Chivers & Smith 1994). Immediately following capture minnows were transported to the laboratory and maintained in a 518-L stream tank at approximately 9 °C and kept on a 15:9 h light:dark cycle. The fathead minnows were fed a diet of commercial fish flakes daily.

The swordtails (*Xiphophorus helleri*) used in the experiment came from multi-generational laboratory stock originally purchased commercially from Florida, USA. The swordtails were maintained in a 407-L tank at approximately 23 °C and kept on a 14:10 h light:dark cycle and fed a diet of commercial fish flakes daily.

The pike were collected using seine nets in the spring of 2005 from Pike Lake, SK., an oxbow lake of the South Saskatchewan River. The pike were maintained in 75-L tanks containing approximately

37-L of water at 17°C and kept on a 15:9 h light:dark cycle for at least two weeks prior to the experiment. In order to cleanse their digestive tracts pike were initially denied food for a 10-day period and then fed one convict cichlid (*Archocentrus nigrofasciatus*) (~3.5 cm fork length) each every other day for a total of three feedings. We fed convict cichlids to the predators as cichlids are not Ostariophysans and do not possess alarm cues recognized by fathead minnows (Chivers & Smith 1998, Brown *et al.* 2000). Moreover, cichlids are distantly related to our control species (swordtails). Brook trout (*Salvelinus fontinalis*) were obtained from the Fort Qu'Appelle hatchery in September 2005 and were maintained and cleansed in a fashion similar to the pike. Ten pike and ten trout were used to prepare the stimuli.

The pike were approximately five cm longer than the trout (~20 cm vs. ~15 cm standard length), however, the volumetric displacement of the trout was about twice that of the pike: volumetric displacement (mean ± S.D.) for pike = 74.6 ± 12.8 mL; trout = 157.8 ± 29.2 mL. Given that the two species of predators used in this experiment are morphologically very different, it was difficult to find a morphological characteristic we could use to match the two species. Indeed, a match in predator body length would result in comparing a fusiform slender pike to a deep-bodied trout. Conversely, a match in predator volume would result in comparing a short trout to a very long pike. None of these scenarios were satisfying, thus we decided to compromise, using pike slightly longer but more slender than trout.

Preparation of stimuli

To prepare the capture-released and diet-released cues, we used a total of 10 pike and 10 trout. Half of the pike and half of the trout were randomly assigned to be fed swordtail and the other half were assigned to be fed minnows. A total of 30 swordtails and 30 minnows were used as prey; three prey items were fed to each of ten trout and ten pike. Due to experimental constraints, we did not measure the exact length of each prey fish. Indeed, manipulations of live minnows that are not anaesthetized would elicit skin damage and release of alarm cues. Alarm cells are easily damageable, occurring on the outside of the fish scales. In addition, no anaesthetic could be used to measure the fish. A decrease in activity of the test minnows due to potential contamination with anaesthetic in the stimulus water could confound our results, as a decrease in activity is a typical antipredator response in minnows. Thus, we 'handpicked' 30 swordtails and 30 minnows as similar in size as possible (~5 cm standard length) and randomly assigned them as prey for the two predator types.

The collection of stimuli was done in two phases. The objective of the first phase was to collect the capture-released cues. For each prey type (i.e. swordtails and minnows), five pike and five brook trout were removed from their holding tanks and placed into individual aerated but not filtered 75-L tanks containing approximately 37 L of dechlorinated tap water. The pike and trout were fed one prey (swordtail or minnow) at a time, each receiving three prey fish in total. The handling time for each predation event was measured and recorded. Handling time was defined as the time of first contact made by the pike or trout until oral manipulation of the prey fish ceased and the mouth was closed. After each predator had successfully consumed three prey, they were placed into a 9.4-L tank filled with dechlorinated tap water for a five-minute period to rinse any remnants of the capture-released cues from the body and oral cavity of the predators. Immediately following this 5 min period, the fish were moved to individual aerated but not filtered 75-L tanks (for the collection of diet-released cues) containing approximately 37-L of water at 17°C. Following removal of the predator from the capture-released collection tank, the water was stirred vigorously and 500 mL from each of the 75-L tanks was removed and placed in one of four communal receptacles (one for swordtail cues from pike, one for minnow cues from pike, one for swordtail cues from trout, one for minnow cues from trout). The collected water was then stirred vigorously. From those stocks, 60-mL aliquots of stimulus were removed and frozen at -20°C.

The objective of the second phase was to collect the diet-released cues. Stimulus was collected from each of the individual tanks that the pike and trout were relocated to for three days after collection of the capture-released cues. Diet-released cues were

collected and frozen in a manner identical to that used for the capture-released cues.

Test tanks

Twelve identical 37-L glass aquaria filled with dechlorinated tap water were used as test tanks. The floor of each tank was covered with approximately 3cm of silica sand substrate. A square ceramic shelter object (20 × 20 × 1.3 cm) supported by three glass legs (5 cm long) was placed in the center of each of the 12 tanks. Each tank also contained an airstone near which we attached a two-m long piece of plastic tubing used to introduce the stimuli into the tanks. The injection line allowed the observer to inject test stimuli from approximately 1.5 m from the test aquaria.

Experimental protocol

Our experiment consisted of exposing single minnows to one of eight treatments (capture- and diet-released cues from pike and trout feeding on swordtails or minnows). We tested a total of 131 minnows, 15-18 minnows in each of the eight treatments. Immediately prior to the start of each trial, 60 ml of water was drawn through the stimulus tube and discarded to ensure the tube was clean before each trial. Another 60 ml of water was then drawn, retained, and later used to flush the stimulus into the tank during the trial. Each trial consisted of an 8-minute pre-stimulus period followed by injection of 60 ml of stimulus and an 8-minute post-stimulus period. We recorded the time spent under shelter (the most reliable behavioural measure for testing antipredator responses in individual fathead minnows, Chivers & Smith 1994) in both the pre- and post-stimulus periods, using the difference between the two as our dependent variable. The order of treatments was randomized and fish were assigned to test tanks in a random fashion. The observer was blind to the treatment at the time of testing.

Statistical Analysis

Data for handling time of pike and trout consuming swordtails or minnows were normally distributed but the variances were not homogenous among treatments. Hence, we performed a two-way Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal & Rohlf 2003), which is a multiway ANOVA design for ranked data (the lowest value had the lowest rank). The two-way non-parametric ANOVA allowed us to test for effects of predator (pike vs. trout) and prey type (swordtails vs. minnows) on handling time. We chose this test instead of non-parametric Mann-Whitney U tests as it allowed us to test for possible interactions between factors.

Data for shelter use were normally distributed but did not have equal variances among treatments. We first performed a two-way non-parametric ANOVA (Scheirer-Ray-Hare extension of the Kruskal-Wallis test) on the swordtail data to investigate the effect of predator (pike vs. trout) and release mechanism (capture vs. diet release) on the response of minnows to swordtail cues. Swordtails are used as a control in this experiment as they do not possess alarm cues recognized by fathead minnows (Chivers & Smith 1998). Thus, we would expect no effect of either predator or release mechanism on the response of minnows to swordtail cues. We then performed a two-way non-parametric ANOVA on the minnow data to investigate the effect of predator and release mechanism on the response of minnows to minnow cues. In case of a significant interaction, subsequent Mann-Whitney U tests were performed to further investigate the nature of the interaction. We adjusted the alpha-value to 0.025 following a Bonferonni correction using an alpha value of 0.1. This alpha level was chosen to account for the highly conservative nature of the Bonferonni correction.

Results

The results of the handling time revealed a significant effect of predator (pike vs. trout: H = 117.65, p < 0.001) and prey
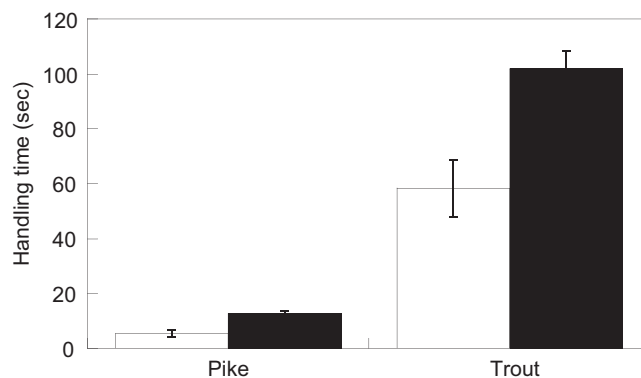
Fig. 1   Mean (± S.E.) of the total handling time (in sec) of the two predators (pike and trout) preying on swordtails (empty bars) or fathead minnows (solid bars)

type (swordtails vs. minnows: H = 22.8, p < 0.001) but no interaction between the two factors (H = 0.0, p = 0.831). Trout handled the prey much longer than pike, and it took longer for both predators to handle the minnows than the swordtails (Figure 1).

The results of the two-way non-parametric ANOVA on swordtail data revealed no effect of either predator (pike vs. trout: H = 1.3, p = 0.255) or release mechanism (capture vs. diet release: H = 0.8, p = 0.362), as well as no interaction between the two factors (H = 1.4, p = 0.236) on the response of minnows to swordtail cues (Figure 2).

The results of the two-way non-parametric ANOVA on minnow data revealed a significant interaction between predator and release mechanism on the response of minnows to minnow cues (H = 16.7, p < 0.001, Figure 2). The subsequent Mann-Whitney U tests revealed that minnows responded with a greater intensity to diet-released cues than capture-released cues coming from pike (U = 60.0, p = 0.017) but reversely, responded with a greater intensity to capture-released cues than diet-released cues from trout (U = 18.0, p < 0.001). When comparing between predators, the Mann-Whitney U tests revealed that minnows responded with a greater intensity to capture-released cues from trout than pike (U = 21.0, p < 0.001) but reversely responded with a greater intensity to diet-released cues from pike than trout (U = 69.0, p = 0.016).

Discussion

The results of our handling time observations showed that a specialist piscivores, the northern pike, handled prey fishes at least six times faster than a generalist predator, the brook trout. This can easily be explained by anatomical characteristics of specialist vs. generalist predators. Brook trout use their tongue-bite apparatus to manipulate different types of prey (e.g. crickets, fish or worms) via raking and open-mouth chewing behaviours (Sanford 2001). On the other hand, pike catch prey fishes through a suction feeding mechanism, in which a strong, inward-directed water current is created via rapid expansion of the buccal cavity. These characteristics likely allow them to catch large prey more efficiently.
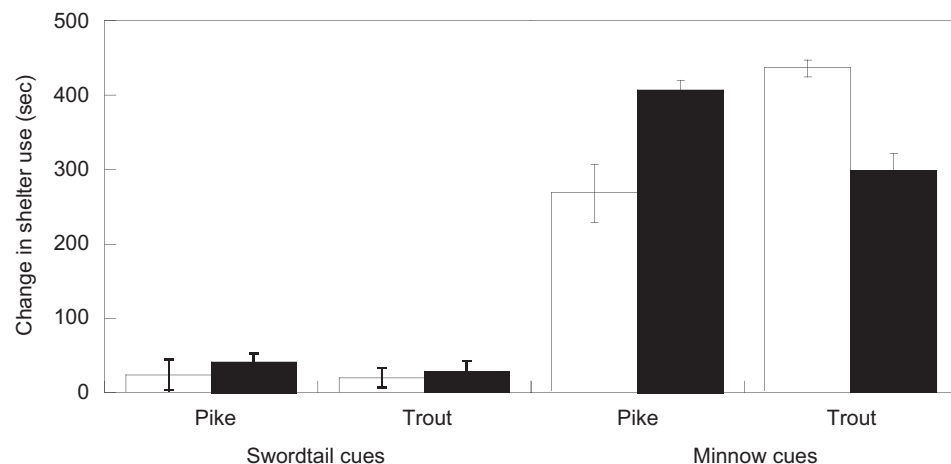
Fig. 2 Mean (± S.E.) change in shelter use (from the pre-stimulus period) of fathead minnows responding to either capture-released cues (empty bars) or diet-released cues (solid bars) from either pike or trout preying on either swordtails or minnows.

The dramatic difference in handling time between the two predators makes it likely that the pike released less alarm cues when capturing minnows than did the trout. This is evident when examining the intensity of response of minnows to capture-released cues from pike and trout; minnows showed a higher intensity response to capture-released cues from trout than from pike. From the perspective of the prey this means that they mistakenly show a stronger response to the predator that is actually less of a threat. Such results could have far reaching implications in predator/prey interactions. For example, minnows are known to learn to recognize predators when they detect alarm cues associated with the odour of a novel predator (Chivers & Smith 1994). Ferrari *et al.* (2005) extended these findings showing that minnows learn to recognize predators in a threat-sensitive manner; minnows learn to recognize brook trout as a high level of threat if the trout odour is associated with a high concentration of alarm cues and as a low level threat if the trout odour is associated with a low concentration of alarm cues. The fact that trout release more alarm cues than pike during capture would mean that minnows should learn that the trout represent a bigger threat than the pike, when the opposite is likely true (Bertolo & Magnan 2006, Findlay *et al.* 2000). Thus, this represents a paradox of risk assessment by prey fishes. While alarm cue concentration may not always predict the exact level of risk the prey is exposed to, prey animals still gain information about the potential threat and must subsequently tune their response to specific predators through experience (Ferrari *et al.* 2006).

The results of our observations also showed that, for both predators, the handling time for fathead minnows was longer than for swordtails. This is likely a result of morphological differences between the two prey species or alternatively could result from a differential ability for prey to escape as a result of different predation pressure on the two species.

Our results confirmed that fathead minnows respond to conspecific alarm cues associated with pike and trout predation, but not to swordtail cues associated with pike and trout predation. This confirms that the minnows lack innate recog-

nition of both predators (Chivers & Smith 1994). They only respond when the predators are fed minnows, indicating recognition of the alarm cue and not recognition of the predator per se. Minnows displayed stronger responses to diet-released cues than capture-released cues for pike. However, when trout was the predator, the minnows showed higher responses to capture-released cues than diet-released cues. When compared between predators, minnows displayed higher intensity of antipredator response to capture-released cues from trout than pike, but reversely responded with a higher intensity of response to diet-released cues from pike than trout. These responses reflect that the intensity of antipredator response of minnows is directly linked to the concentration of alarm cues released during attacks and after digestion (Ferrari *et al.* 2005, Ferrari & Chivers 2006).

We may expect that specialized piscivores like pike may be under intense selection to mask their odours or be better able to break down the alarm cues of their prey during digestion. However, this does not seem to be the case in our experiment. A comparison of the responses to diet-release cues between predators showed that pike released more diet cues recognized by minnows than did trout. A proximate explanation for the response patterns of the minnows to capture- and diet-released cues from the two predators could be that the amount of cues released in the predator's diet is reversely proportional to the amount of cues released during capture. Thus, since pike release less alarm cues than trout during capture, it means that more alarm cues will be released by the pike in its diet, assuming both predators have the same ability to breakdown the cues in their digestive tracts. However, the shorter digestive tract and high gut evacuation rate of specialized predators like pike may also have reduced the ability of the specialist to degrade the cues. Behavioural adaptations of the predators might compensate for the lack of ability to breakdown the alarm cues. Pike are known to minimize the ability of minnows to detect alarm cues released in their feces by defecating away from their foraging area (Brown *et al.* 1995). Pike do this when they are fed minnows but not other fishes that lack alarm cues (Brown *et al.* 1996). Whether other specialized piscivores also have

this ability is unknown. Further work should attempt to address the mechanisms by which piscivores could minimize the cues available to the prey for risk assessment.

Acknowledgements

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