



Risk assessment via predator diet cues in a coral reef goby

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ABSTRACT

Recent studies conducted in freshwater systems have shown that predator faeces can provide important chemosensory information to prey individuals. To date, no studies on the ability of prey to assess risk through predator diet cues have been undertaken in coral reef ecosystems. Therefore, the present study investigated whether the conspecific chemical cues of a coral reef goby (*Asterropteryx semipunctatus*) elicited an anti-predator response once they had passed through the gut of a common piscivore (the rock cod, *Cephalopholis boenak*). Predators were fed one of two different diets: *A. semipunctatus* or *Xiphophorus helleri* for three consecutive days. Acclimated *A. semipunctatus* were then exposed to: faecal cues containing digested *A. semipunctatus* or *X. helleri*; conspecific chemical cues; *X. helleri* chemical cues; and seawater. A significant reduction in moves and feeding strikes was observed in response to the faeces containing conspecifics, compared to those containing *X. helleri*. This change in behaviour was of a similar magnitude to that exhibited towards the conspecific chemical cue, suggesting that a key component of the cue may not completely degrade during the digestion process. This is the first study to document behavioural responses to predator faeces in a coral reef fish, highlighting the effectiveness of diet cues as a method of risk assessment.

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1. Introduction

An individual's ability to assess, identify and react to a predator directly influences their probability of survival (Holmes and McCormick, 2010a; Lima and Bednekoff, 1999). Spatial and temporal variations in predator abundance require prey to continuously regulate their use of habitat, foraging requirements and reproductive effort, with predator avoidance (Kelley and Magurran, 2003; Schoepner and Relyea, 2009a). Therefore, individuals able to minimize the trade-off between predator avoidance behaviours and other fitness-associated behaviours (foraging, mating) through accurate risk assessment are likely to have a higher chance of survival and the highest inclusive fitness.

Prey identify potential predators through a variety of senses including: visual, chemical, auditory and tactile (Kats and Dill, 1998; Schoepner and Relyea, 2009a). Chemosensory assessment of predation risk is suggested to be of significant advantage for aquatic vertebrates with numerous studies demonstrating their effectiveness (e.g. Brown, 2003; Brown and Chivers, 2006; Chivers and Smith, 1998; McCormick and Manassa, 2007; Wisenden, 2000). Research has shown that the chemical cues released by injured or masticated prey stimulate a fright reaction in nearby conspecifics (Chivers and Smith, 1998). These cues represent a reliable indication of an

immediate threat, with a direct response known to increase prey survival and overall fitness (Mirza and Chivers, 2001a,b). Predator identities can also be rapidly learnt by associating the smell of a predator with those of the prey chemical cue (Ferrari et al., 2007). However, identification of predators in this way comes at a potential cost; prey must first be in the vicinity of an actively foraging predator targeting that particular prey species before the initial association can be made.

One way prey are able to reduce the danger associated with identifying predators is through the use of predator diet cues (Chivers and Mirza, 2001). As predator diet cues require the presence of faecal matter before an association can occur, it is likely that this mechanism is used concurrently with other chemosensory methods to ensure that prey accurately assess risk whilst identifying potential predators within the local area (Mirza and Chivers, 2003). This mechanism of olfactory risk assessment has been shown in a number of freshwater fishes (Brown et al., 1995a; Gelowitz et al., 1993; Kats and Dill, 1998; Mathis and Smith, 1993a; Mirza and Chivers, 2003), molluscs (Griffiths and Richardson, 2006; Smee and Weissburg, 2006), arthropods (McBean et al., 2005; Mortensen and Richardson, 2008), echinoderms (Hagen et al., 2002) and cnidarians (Howe and Harris, 1978). Prey capable of recognising and responding to chemical cues in the faeces of predators may further reduce their risk of predation by learning to avoid predator prone areas (Brown, 2003; Schoepner and Relyea, 2009a,b).

To date, no studies have investigated the use of this anti-predator mechanism in marine systems (reviewed in Ferrari et al., 2010).

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Seawater contains dissolved gases, nutrient molecules, organic substances and salts (Duxbury et al., 2002), making it more chemically complex and biologically active than freshwater. It has been shown that the physical, chemical and biological properties of a prey's environment have a significant influence on the response of individuals to chemical cues (Ferrari et al., 2010), suggesting that an individual's response may differ significantly between freshwater and seawater environments. As coral reefs are among the most biodiverse ecosystems on earth, prey fishes are exposed to a diverse and unpredictable array of predators, with periods of extreme predation risk often occurring at critical life history stages (Almany and Webster, 2006). As such, it is suggested that a mechanism of predator recognition which decreases the threat of capture and/or death during these transitions would be advantageous.

The present study investigates whether the conspecific chemical cues of a coral reef fish, *Asterropteryx semipunctatus*, elicit a response once they have passed through the gut of a common predator (*Cephalopholis boenak*). Specifically, we aimed to: (1) investigate the behavioural response of *A. semipunctatus* to the faeces of *C. boenak* fed a diet conspecifics (*A. semipunctatus*) or heterospecifics (*Xiphophorus helleri*), and (2) whether the potency of the conspecific chemical cue is retained through the process of digestion.

2. Materials and methods

2.1. Study species

A. semipunctatus (Gobiidae) is a coral reef dwelling goby inhabiting shallow areas of coral rubble (Smith, 1989). Previous studies examining the anti-predator behaviour of this species have shown that it responds to conspecific chemical cues with a reduction in movement and feeding, compared to extract from skin of a phylogenetically distant heterospecific or a seawater control (Larson and McCormick, 2005; McCormick and Larson, 2007; McCormick and Manassa, 2007; Smith, 1989). This reduction in activity has previously been interpreted as an anti-predator response.

The predatory species used was the rock cod *C. boenak* (Serranidae), a common predator on coral reefs that preys on small reef fishes and invertebrates (Beukers-Stewart and Jones, 2004).

2.2. Fish collection and pre-experimental maintenance

A. semipunctatus and *C. boenak* were collected using hand nets from Pioneer Bay at Orpheus Island (146° 20'E, 18° 35'S), Great Barrier Reef Australia. Fish were maintained in 38 l tanks at James Cook University Marine and Aquaculture Research Facility Unit and allowed to acclimate for three weeks prior to experimental use. *A. semipunctatus* were fed commercial marine fish flakes and live brine shrimp nauplii (*Artemia franciscana*) daily. *C. boenak* were fed frozen brine shrimp (*A. franciscana*) during the acclimation period and swordtails (*Xiphophorus helleri*) prior to the experiment.

X. helleri were used as a control diet for *C. boenak* throughout the experiment as they are phylogenetically distant from *A. semipunctatus*. Furthermore, although *X. helleri* are known to possess a damage-released cue to which conspecifics respond (Mirza et al., 2001), *A. semipunctatus* are known to have no anti-predator response to these chemicals (Larson and McCormick, 2005). *X. helleri* were obtained commercially and maintained in dechlorinated fresh water at approximately 22.5 °C.

2.3. Observation tanks

Observation tanks were set up as per McCormick and Manassa (2007), with experiments and behavioural observations undertaken in 15 l tanks with an air stone at the back right corner of each. An additional piece of plastic tubing, for cue injection, was attached to the

airline with the end fixed approximately 1 cm above the air stone. Trials with dye showed that it took 12 s for the dye to disperse through the tank. Three identical plastic pipes (4.3 cm long, 3 cm wide) were placed horizontally at the back left corner of each observation tank on a 2 cm deep substratum of aquarium gravel. These pipes were used as a shelter to mimic the natural habitat of gobies. Each tank was surrounded on three sides by black plastic to avoid test fish observing adjacent tanks.

Previous tests have shown that groups of less than three gobies demonstrated abnormal behaviour (Larson and McCormick, 2005; McCormick and Larson, 2007); therefore, three randomly selected individuals were used in each replicate trial, with no individuals used more than once.

2.4. Treatment preparation

The five treatments used in this experiment were: (1) conspecific (*A. semipunctatus*) faecal cues; (2) heterospecific (*X. helleri*) faecal cues; (3) conspecific chemical cues; (4) heterospecific chemical cues; and (5) seawater.

To prepare the faecal cues, six individual *C. boenak* were starved for 24 h then fed one of two different diets for three consecutive days: either two *A. semipunctatus* of equal size per day or one female *X. helleri* per day (ensures similar biomass of fish). One hour after the final feeding, each predator was removed from their holding tank and placed individually into a clean 15 l tank (with continuous air-flow and no water flow). Defecation occurred between 8 and 14 h after the final feeding. At 14 h a siphon was placed directly over the faeces, to collect all faecal matter and a further 300 ml of tank water. The sample was then shaken vigorously until the majority of solid matter had dissolved, filtered and then separated into five 60 ml aliquots. The faecal cues were used no longer than 100 min after preparation.

Chemical cues were prepared according to the protocol from McCormick and Manassa (2007), with a total of 15 individual *A. semipunctatus* (mean standard length (SL) 38.45 mm ± 1.05 SE) and 15 individual *X. helleri* (mean standard length (SL) 42.43 mm ± 1.65 SE) used. Specimens were euthanized by a quick blow to the head, with 25 superficial (minor flesh damage) cuts made to the skin with a clean razor blade. Specimens were then rinsed in 15 ml of seawater, previously obtained from each observation tank. Following this the 15 ml of chemical cue was filtered prior to use, with the cues used no longer than 20 min after preparation.

2.5. Experimental protocol

Prior to experimentation gobies were acclimated in the observation tanks for a period of five days, with each tank receiving 30 ml of live *Artemia franciscana* daily (approximately 1 individual per ml of tank water). In each trial the behaviour of focal gobies was quantified for 10 min before and 10 min after the addition of each treatment ($n = 3$ individuals per trial, 5 treatments, 15 trials per treatment).

Before the observation period was undertaken 60 ml of tank water was extracted through the injection tube and discarded to remove any stagnant water. A further 135 ml (chemical cues and seawater trials) or 120 ml (faecal cues) was collected and retained. Immediately before cue injection, 30 ml of live *A. franciscana* followed by 60 ml of previously collected tank water (to flush the tube) was injected into the tank. The behaviour of the gobies was then recorded for 10 min. Following the initial observation period 15 ml (chemical cues and seawater trials) or 60 ml (faecal cues) of treatment cue was injected into the tank, followed by 60 ml of tank water to ensure the cue was flushed through. A final 10 min behavioural observation period was then conducted.

2.6. Quantification of behaviour

A typical anti-predator response in this species included a decrease in moves and feeding strikes (McCormick and Manassa, 2007). Therefore, the behavioural responses to all experimental stimuli were quantified by recording the number of moves and the number of feeding strikes. Each change in position after a stationary period was recorded as a move, with the number of feeding strikes recorded regardless of success. The controls in each experiment were not expected to show any changes between initial and final observation periods for the variables measured.

2.7. Statistical analysis

The difference in the total counts of moves and feeding strikes between the initial and final 10 min observation periods was compared among the five treatments with a one-factor MANOVA. To further explore the nature of the significant differences found by MANOVA, ANOVAs were undertaken on both variables (moves and/or feeding strikes) followed by a series of planned comparisons. Residual analysis was used to examine the assumptions of normality and homogeneity of variance, which were satisfied.

3. Results

There was a significant change in behaviour between the initial and final observation periods among the five treatments (MANOVA, Pillai's Trace = 0.540, $F_{8,140} = 6.480$, $p < 0.001$; Fig. 1) ($n = 3$ individuals per trial, 5 treatments, 15 trials per treatment).

There was a significant difference in moves among stimuli ($F_{4,70} = 7.771$, $p < 0.001$; Fig. 1a) and a significant difference in feeding strikes ($F_{4,70} = 6.415$, $p < 0.001$; Fig. 1b). Planned comparisons revealed there was a reduction in movement and feeding in response to the conspecific faecal cues and the conspecific chemical cues compared to the three remaining stimuli (moves: $F_{1,70} = 28.146$, $p < 0.001$; feeding strikes: $F_{1,70} = 24.082$, $p < 0.001$; Fig. 1). Planned comparisons also revealed that there were no differences in behaviour between fish exposed to the heterospecific faecal cues and

those exposed to heterospecific chemical cues or seawater (moves: $F_{1,70} = 0.085$, $p = 0.772$; feeding strikes: $F_{1,70} = 1.527$, $p = 0.221$; Fig. 1).

4. Discussion

This is the first study to document behavioural responses to predator faeces in a coral reef fish. The results clearly demonstrate that *A. semipunctatus* are capable of responding to the faeces of a predator which has recently consumed conspecifics. The magnitude of response to conspecific faecal cues was similar to that observed for conspecific chemical cues, suggesting that a key component of the chemical cue may not completely degrade during the digestion process. Predator diet cues ensure that the faeces of a predator will always be coupled with both the predator scent and those of recently consumed individuals. Therefore, when used concurrently with other risk assessment mechanisms, predator diet cues extend the window of opportunity in which prey have to identify potential predators.

The response of *A. semipunctatus* to conspecific faecal cues resembles a fright reaction (decrease in activity levels), which is not only common among gobies but is also seen in many marine fish species (Ferrari et al., 2010; Holmes and McCormick, 2010a; McCormick and Manassa, 2007). Studies on freshwater Ostariophysan fishes demonstrate that areas containing the faeces of a predator fed conspecifics were actively avoided (Brown et al., 1995b), with detection significantly decreasing activity levels (Mathis and Smith, 1993b). A further study demonstrated that after exposure to predator diet cues, anti-predator behaviour in conditioned individuals occurred almost immediately compared to those that had not experienced predator faeces (Mirza and Chivers, 2003). This study highlights the advantages of chemosensory risk assessment by demonstrating that predator detection through diet cues elicits anti-predator responses which are known to enhance survival during subsequent encounters (Mirza and Chivers, 2003).

The presence of recently consumed conspecifics within the diet of predators allows prey to instantly recognise a potential threat, without the need for visual confirmation. As such, predator diet cues are

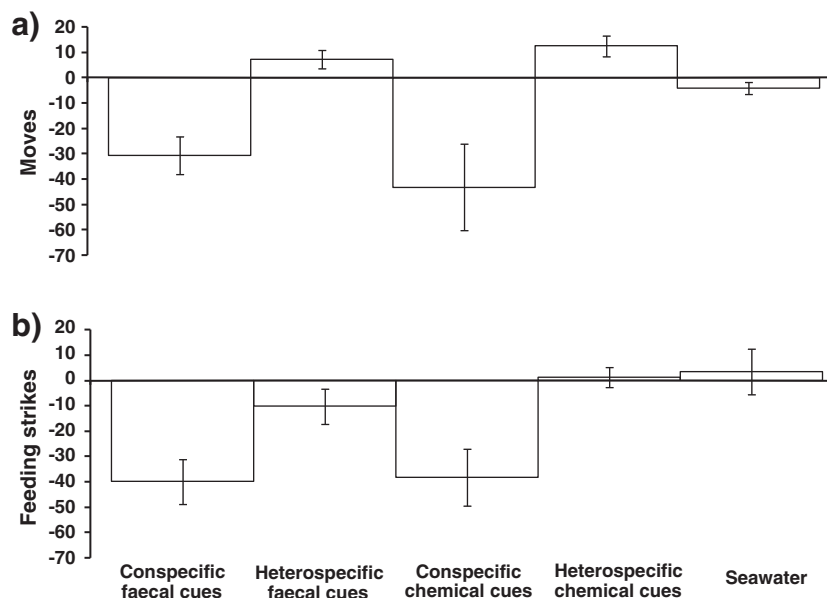


Fig. 1. Mean change (\pm SE) in (a) moves and (b) feeding strikes by *Asterropteryx semipunctatus* between initial and final 10 min observation periods, for fish exposed to: faecal cues of *Cephalopholis boenak* fed a diet of conspecifics or a diet of heterospecifics, conspecific chemical cues, heterospecific chemical cues or seawater ($n = 3$ individuals per trial, 5 treatments, 15 trials per treatment).

likely to be effective in coral reef environments where the diversity and composition of predators differ with ontogeny (Holmes and McCormick, 2010b). Following a pelagic developmental stage, the majority of coral reef fish settle with little to no knowledge of predators within the local area. Over the first 2 days after settlement, mortality due to predation averages 56% (Almany and Webster, 2006), therefore a method of rapidly identifying predators is essential for survival. Labelling predators as risky through diet cues guarantees that prey do not waste time or energy responding to irrelevant species (Mirza and Chivers, 2003), thereby increasing the likelihood that anti-predator responses are proportional to the perceived risk.

In the natural environment prey individuals may be exposed to several predators at any one time, highlighting the need for mechanisms that facilitate the rapid identification of predators and reinforcement of relevant stimuli. A recent study by Mitchell et al. (2011) demonstrated the ability of *Pomacentrus moluccensis* to learn the identity of multiple previously unknown predators simultaneously. However, any previously unknown odours (predator or non-predator) coupled with a known chemical cue may be recognised as dangerous, resulting in responses towards non-relevant threats. Chemical cues from an injured prey individual may immediately warn conspecifics of a potential threat, then following consumption act to label a predator through diet cues, allowing for reinforcement of relevant stimuli (Ferrari and Chivers, 2006; Mathis and Smith, 1993a). On coral reefs, reliance on predator diet cues may be particularly important at the end of the larval phase during settlement, when individuals are most naive to predators. Following settlement, fine tuning of predator identities is likely to occur from interaction with knowledgeable conspecifics and heterospecifics (e.g. social learning; reviewed in Brown and Laland, 2003), further eliminating a response to irrelevant cues.

While chemical cues can be temporally and spatially unreliable, cue concentration is often correlated with cue relevancy (Ferrari et al., 2010). A number of studies on a diverse range of taxa have found that prey match their response intensity to cue concentration (e.g. larval mosquitoes (*Culex restuans* and *Ochlerotatus triseriatus*), roach (*Rutilus rutilus*), fathead minnows (*Pimephales promelas*), goldfish (*Carassius auratus*), damselfish (*Pomacentrus amboinensis*), larval American toads (*Bufo americanus*) and larval woodfrogs (*Rana sylvatica*)). Other studies demonstrate an all-or-nothing response (e.g. northern clearwater crayfish (*Orconectes propinquus*), convict cichlids (*Archocentrus nigrofasciatus*), rainbow trout (*Oncorhynchus mykiss*), and pumpkinseed sunfish (*Lepomis gibbosus*)). As predator diet cues require prey chemical cues to pass through the digestive system of a predator, one would assume that the concentration of the original chemical cue would be lower when defecated. This study suggests that the response of *A. semipunctatus* to conspecific faecal cues was similar in magnitude to conspecific chemical cues. However, this does not necessarily mean that the chemical composition of the cue is unmodified, but rather a key component of the cue may not have completely degraded during the digestion process. This finding emphasises the potential importance of dietary information in identifying locally active predators.

Predator diet cues are temporally unreliable as the time between a predation event and detection of chemical cues within a predators faeces may be several hours. This delay could result in the failure of prey to learn a potential predator prior to attack (Ferrari et al., 2010). In this study the time between prey consumption and evacuation from the gut was 8 to 14 h (*C. boenak*). However, in the natural environment it is possible that following digestion, cues may leak out of the predatory individual prior to defecation, acting as a warning signal. Although a temporal delay may exist, consistency in diet choice may ensure the effectiveness of this method of predator cataloguing. A study by Bryan and Larkin (1972) showed three species of trout (brook trout (*Salvelinus fontinalis*), cutthroat trout (*Salmo clarki*) and rainbow trout (*Salmo gairdneri*)), had marked

consistency in their diet preference for time periods spanning days to 6 months. A study by Beukers-Stewart and Jones (2004) found similar consistency for two species of tropical marine predators (*Cephalopholis cyanostigma* and *C. boenak*). These studies, and the idea that predator diet cues are used simultaneously with other risk assessment methods, suggest that the temporal limitations may have minimal effect on overall prey survival.

The use of predator diet cues as an additional predator identification method has profound implications for predator–prey interactions in marine systems as they minimise the threat of capture during the identification process. It is likely that diet cues may be a universal mechanism used by coral reef fishes to gain knowledge on potential predators, as the majority of species exhibit similar life-histories and associated selection pressures. This study is the first to document the use of predator diet cues as a predator labelling mechanism in a coral reef fish and further highlights the importance that chemical cues play in predator–prey dynamics in marine systems.

Author contributions

Conceived and designed the experiments: RPM MIM. Performed the experiments: RPM. Analyzed the data: RPM. Contributed reagents/materials/analysis tools: MIM. Author of the paper: RPM MIM.

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