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REPORT

Predation risk assessment by olfactory and visual cues in a coral reef fish

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Abstract Assessment of predation risk is vital for the success of an individual. Primary cues for the assessment include visual and olfactory stimuli, but the relative importance of these sources of information for risk assessment has seldom been assessed for marine fishes. This study examined the importance of visual and chemical cues in assessing risk for the star goby, *Asterropteryx semipunctatus*. Visual and chemical cue intensities were used that were indicative of a high threat situation. The behavioural response elicited by both the visual cues of a predator (the rock cod, *Cephalopholis boenak*) and the chemical alarm cues from conspecifics were similar in magnitude, with responses including a decrease in feeding strikes and moves. A bobbing behaviour was exhibited when the predator was visible and not when only exposed to the chemical alarm cue. When visual and chemical cues were presented together they yielded a stronger antipredator response than when gobies were exposed solely to conspecific alarm cues. This suggests additivity of risk assessment information at the levels of threat used, however, the goby's response is also likely to depend on the environmental and social context of the predator–prey encounter. This study highlights the importance of chemical cues in the assessment of predation risk for a coral reef fish.

Keywords Behaviour · Chemical alarm cue · Coral reef fish · Olfaction · Predation risk · Visual cue

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Introduction

A trade-off exists between predator avoidance behaviours and regular fitness-associated behaviours, such as foraging, maintenance of social organization and reproduction (Sih 1980). Selection should favour those individuals who are most efficient at assessing risk and hence are able to direct the most energy to reproduction (Helfman 1989; Lima and Bednekoff 1999; Mirza and Chivers 2001a; Brown 2003; Golub and Brown 2003). Aquatic vertebrates can use a variety of information sources to assess predation risk, with the most commonly used cues being visual (Helfman 1989; Murphy and Pitcher 1997; Smith and Belk 2001) and chemical stimuli (Chivers and Smith 1998; Wisenden 2000; Brown 2003; Brown and Chivers 2006).

Studies on freshwater Ostariophysan fishes have underscored the importance of chemical cues in predator–prey interactions (Chivers and Smith 1998; Brown and Chivers 2006), in particular the pivotal role that species-specific alarm cues play in risk assessment. For many species, once mechanical skin damage occurs during a predator encounter, a chemical cue from the prey is involuntarily released that is a reliable indicator of a predator threat to conspecifics in the vicinity of the predator strike. Upon detection of the chemical by conspecifics a fright reaction is elicited, which enhances survival of those forewarned conspecifics (Chivers et al. 1996; Mirza and Chivers 2000) and at times heterospecifics (Wisenden et al. 1995; Chivers et al. 2002; Pollock and Chivers 2004). The prevalence of a well-developed response to chemical cues in many aquatic organisms has been attributed to the high turbidity and structural complexity of the areas inhabited (Mirza and Chivers 2001b) or as a response to the activity of nocturnally active predators (Chivers et al. 1996).

In contrast, visual cues are likely to be important for risk assessment by prey that inhabit areas of high visibility and low structural complexity, which facilitate the timely evasion of a predator strike. Research on the visual and olfactory contributions to risk assessment in freshwater amphibia suggests that chemical cues may play a more important role than visual or other stimuli (e.g., Stauffer and Semlitsch 1993; Kiesecker et al. 1996; Mathis and Vincent 2000), in part attributable to the low visual acuity of the study organisms (Mathis and Vincent 2000). Recent research on freshwater fishes has revealed a more complex situation, with chemical alarm cues indicating the presence of a predator, and visual cues being used to accurately modify the response to the degree of threat imposed (Hartman and Abrahams 2000; Chivers et al. 2001). Moreover, some fish respond to both sensory cues with a graded response (e.g., Dupuch et al. 2004; Engstrom-Ost and Lehtiniemi 2004; Zhao and Chivers 2005; Ferrari et al. 2006; Zhao et al. 2006), with chemical cues warning prey of a potential threat and visual cues being used to assess the level of risk posed (Chivers et al. 2001).

The majority of studies on roles of chemical and visual cues in risk assessment have been conducted on freshwater species (Smith and Lawrence 1992; Mirza and Chivers 2001a), with a paucity of research on marine organisms. Despite the obvious benefits of using chemical alarm cues in marine systems, only two families have been shown to possess them: Gobiidae (*Asterropteryx semipunctatus* and *Brachygobius sabanus*) and Ariidae (*Arius felis*) (Smith and Smith 1989; Smith et al. 1991; Smith and Lawrence 1992; Smith 2000). This lack of representation is probably more a feature of the low number of studies undertaken than their diminished importance in marine environments. While it is known that some marine species will respond to chemical and visual cues of predators with a fright response, there is limited understanding of the independent contributions of both visual and chemical cues, together with their interaction, to predator risk assessment.

Coral reefs represent an environment where water clarity is typically high, making visual cues a dominant sensory input into behavioural decisions. Indeed, it has been suggested that high water clarity and the typically well-developed visual system of the inhabitants (Losey et al. 2003) has lead to the development of the spectacular colouration of many fish species (Helfman et al. 1997), and the development of complex forms of visual communication (Marshall et al. 2003). Possibly due to the obvious efficacy of using visual cues for predator risk assessment in a low turbidity environment, little work has been conducted on the role of other information sources, such as olfactory stimuli. Whilst coral reef environments are known for their water clarity, they are also topographically complex, with a strong relationship between species diversity and structural complexity (McCormick 1994). Research on the use of chemical cues in freshwater systems strongly suggests that chemical cues may also play an important role in behavioural decisions associated with risk assessment. The relative importance of visual and olfactory cues in influencing the magnitude of antipredator response is currently poorly understood for marine systems.

The present study investigated if the magnitude and nature of an antipredator response in the tropical marine goby, *A. semipunctatus*, differed depending on whether visual or chemical cues were used to assess risk, and whether the cues interacted. A previous study in Hawaii by Smith (1989) indicated that the species responds to conspecific chemical alarm cues and sighting a model (lizardfish predator, though uncontrolled for) pulled from the sand by a string with a fright response. The first aim of the present study was to assess whether *A. semipunctatus* from a location on the Great Barrier Reef also responded to conspecific chemical alarm cues and visual cues of a live predator. This study uses the rock cod, *Cephalopholis boenak*, as the predatory stimulus since it is known to be a common predator in the habitat occupied by *A. semipunctatus* (Beukers-Stewart and Jones 2004). The second aim was to compare the magnitude of the response by *A. semipunctatus* to isolated visual and chemical cues to the response elicited when both sources of risk assessment information were present.

Materials and methods

Study species

Asterropteryx semipunctatus is a coral reef dwelling goby that forms dense aggregations in shallow areas of coral rubble (Randall et al. 1990). This species is a negatively buoyant small benthic fish, which moves episodically interspersed with periods when they remain stationary on the bottom. The species exhibits a behaviour known as bobbing, which has been suggested to be a visual alarm signal to other fishes in the vicinity, or a signal to predators that their approach has been detected (Sweatman 1984; Smith 1997).

Smith (1989) examined the response of *A. semipunctatus* from Hawaii to conspecific alarm cues and found they responded with reduced movement, feeding and increased bobbing. He also examined their response to a model lizardfish, which was pulled from the sand by a monofilament line. Unfortunately, this disturbance protocol was not controlled, so it is unclear whether it was a specific response to the visual stimulus of the model predator, or simply a startle response to general visual disruption. This made any comparison of the magnitude of response between visual and chemical cues difficult to interpret in the context of the goby's ecology.

The rock cod, *C. boenak*, was used as the predator in the present study since it is common on coral reefs and preys on small reef fishes and invertebrates (Beukers-Stewart and Jones 2004). This predator ranges over the habitats occupied by *A. semipunctatus*, where it is known to eat gobies (Beukers-Stewart and Jones 2004).

Fish collection and maintenance

Asterropteryx semipunctatus and *C. boenak* were collected in Pioneer Bay at Orpheus Island (146° 20′E, 18° 35′S), Great Barrier Reef, Australia on SCUBA using clove oil and hand nets in February 2006. Fish were transported in aerated seawater inside a dark container to the James Cook University Marine and Aquaculture Research Facility Unit where they were maintained in aerated 38 l tanks with flowing saltwater at a mean minimum temperature of 27.8°C and a maximum of 28.6°C (12L:12D photoperiod). Fish were acclimated for a period of 3 weeks, with *A. semipunctatus* fed commercial marine fish flakes and live brine shrimp (*Artemia franciscana*) and *C. boenak* fed frozen brine shrimp (*A. franciscana*) daily. Swordtails, *Xiphophorus helleri*, were used as controls for the addition of skin extract as they are phylogenetically distinct from the goby. Furthermore, although swordtails are known to possess a damage-released alarm 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). Swordtails were obtained commercially and maintained in dechlorinated fresh water at approximately 22.5°C.

Observation tanks

Experiments and behavioural observations were undertaken in 15 l tanks with an air stone at the back corner of each tank. An additional piece of plastic tubing, for stimulus 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 following the injection for the dye to disperse through the tank. Aquaria had a 2 cm deep substratum of aquarium gravel and a shelter consisting of three identical plastic pipes (4.3 cm long, 3 cm wide), which were used 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.

Stimulus preparation

Skin extract was prepared from 45 *A. semipunctatus* (mean standard length (SL) 38.45 mm ± 1.05 SE) and 15 *X. helleri* (as a fish odour control; mean SL 42.43 mm ± 1.65 SE). Specimens were euthanized by a quick blow to the head, and then placed in a clean disposable petri dish, with 25 superficial cuts to the skin (minor flesh damage) made on each flank using a clean razor blade. Specimens were then rinsed in 15 ml of saltwater, previously obtained from each test tank. In order to remove any solid matter each 15 ml of stimulus water was filtered through filter paper (125 mm Ø, qualitative 1) prior to being drawn up into a syringe. Skin extracts were prepared within 20 min of injection as previous studies have suggested that potency is decreased if extracts are stored or frozen (Smith 1989).

Experimental protocol

The experimental protocol is described as three separate experiments because a series of different control protocols were required to validly assess the occurrence and magnitude of the responses to chemical or visual cues. It was only once the response to the chemical and visual cues had been determined in experiments 1 and 2, respectively, that they could be compared to the goby's response to the combined effect of visual and chemical cues (experiment 3). Experiments were conducted at the same time with similar protocol (below), on random subsets of *A. semipunctatus* collected at the same time and location, making comparisons across experiments valid.

Prior to experimentation each set of gobies (mean SL 38.45 mm ± 1.05 SE) were acclimated in the observation tanks for a period of 5 days, with each tank receiving 30 ml of live *A. franciscana* daily (approximately 1 individual per ml of tank water). In each trial the behaviour of focal gobies was quantified (as detailed below) from behind a blind for 10 min before (pre-stimulus period) and 10 min after (post-stimulus period) the addition of a water-borne stimulus (control or treatment). Prior to behavioural observations 60 ml of water was drawn up the stimulus injection tube and discarded to remove any stagnant water from the tube. A further 75 ml was collected and kept in experiments 1 and 3 (comprising 15 ml for rinsing the chemical cue from the donor, or as a saltwater control cue, and 60 ml to flush the cue into the tank).

The procedure undertaken after the pre-stimulus period differs in each experiment and is detailed below.

Experiment 1: response to conspecific skin extracts

The behaviour of the focal gobies to three stimuli was quantified: extracts from damaged skin of conspecifics; extracts from skin of *X. helleri* (swordtail); and an equal volume of saltwater. Both the swordtail extracts and the saltwater stimulus serve as controls, with the swordtail extract controlling for behavioural changes that may result from exposure to an extract of any injured fish and the saltwater controlling for changes resulting from any stimulus injected into the tank via the stimulus tube.

Immediately following the pre-stimulus period 15 ml of the stimulus was injected into the tank followed by 60 ml of previously obtained tank water, to ensure all the stimulus was flushed into the tank. The post-stimulus period was then conducted. Fifteen replicates were undertaken for each stimulus, with the exception of the chemical alarm cue treatment where 20 replicates were conducted.

Experiment 2: response to visual cue of a predator

The behaviour of the focal gobies to three stimuli was quantified: the visual cue of a predator, *C. boenak* (128.0 mm SL ± 2.75 SE); the visual cue of a non-predatory individual (the planktivorous damselfish *Acanthochromis polyacanthus*; 48.8 mm SL ± 5.25); and the visual cue of an empty tank. The last two experimental treatments were controls, with the exposure to a tank containing a non-predator controlling for behavioural changes resulting from the sight of any fish, and the exposure to an empty tank controlling for changes resulting from the experimental procedure.

Two adjacent aquaria were used for this experimental series, one containing the acclimated *A. semipunctatus* and the other containing one of the three treatment stimuli (i.e., predator, non-predator, and nothing). After the pre-stimulus observations a plastic opaque barrier separating the focal and stimulus aquaria (external to both tanks) was slowly removed to reveal the second tank containing the stimuli and 10 min post-stimulus behavioural observations were conducted. Fifteen replicates trials were undertaken for each stimulus.

Experiment 3: response to the co-occurrence of conspecific skin extract and visual cue of a predator

The behavioural response of the focal gobies to the simultaneous presentation of a conspecific skin extract and the visual cue of a predator was examined in this experiment. Prior to the post-stimulus period 15 ml of conspecific skin extract was injected into the tank followed by 60 ml of previously collected tank water. The plastic opaque barrier between treatment and stimulus tanks was also slowly removed to reveal a predator individual (*C. boenak*) in the adjacent tank (setup and procedure being similar to experiment 2). This was followed by a 10-min behavioural observation period. Fifteen replicate trials were undertaken for the treatment. The magnitude of the response of the focal gobies to the combined chemical and visual cues was then compared to the magnitude of response to chemical and visual information in isolation (i.e., the key results of experiments 1 and 2).

Quantification of behaviour

The behavioural responses to all experimental stimuli were quantified by recording the frequency of three behaviours commonly used by *A. semipunctatus*: the number of feeding strikes; the number of moves; and the number of bobs. This species is known to move frequently with pauses between moves. Each change in position after a stationary period was recorded as a move. The number of feeding strikes was recorded regardless of success. A bob consisted of a fish raising its anterior then slowly descending whilst gulping water. Bobbing could occur regardless of whether the individual was stationary or not.

A typical antipredator response in this species included a decrease in feeding strikes and moves and an increase in the number of bobs. *A. semipunctatus* was predicted to display the same alarm behaviour to conspecific skin extracts and the visual sighting of a predator. The co-occurrence of chemical and visual cues may amplify the magnitude of antipredator behaviours described above. The controls in each experiment were not expected to show any changes between pre and post-stimulus periods in the variables measured.

Statistical analyses

The difference in the total counts of fish behaviour between the 10-min pre-stimulus and the 10-min post-stimulus periods were compared among treatments with one-way MANOVA. Only feeding strikes and moves were sufficiently common to allow statistical comparison. To further explore the nature of significant differences found by MANOVA, one-way ANOVAs were used to examine whether there were significant differences between treatments in one or both variables. When ANOVA found significant differences these were examined using Tukey's HSD means comparison tests. Residual analysis was used to examine the assumptions of normality and homogeneity of variance. Since two ANOVA's were completed on potentially interrelated variables in each dataset a Bonferroni correction was employed (modified alpha level of 0.025; Keppel 1982).

Results

Experiment 1: response of gobies to conspecific skin extracts

There was a significant change in behaviour between pre- and post-stimulus periods for the gobies exposed to conspecific skin extracts compared to the two control treatments (MANOVA, $p < 0.0001$; Table 1a; Fig. 1). During the pre-stimulus period individuals fed on live brine shrimp, swimming around the majority of the tank area. Upon

Table 1 Summary of comparisons of treatment effects on (a) overall behaviour and (b) separately for feeding rate and moves of *Asterropteryx semipunctatus* in three experiments (Exp)

a. MANOVA	Source of variation	Effect *df*	Error *df*	Pillai's trace	*p*
Exp 1	Treatment	4	94	0.459	<0.0001
Exp 2	Treatment	4	84	0.478	<0.0001
Exp 3	Treatment	4	94	0.226	0.0225

b. ANOVA	Source of variation	*df*	MS	*F*	*p*
Exp 1. Feeding	Treatment	2	6,650.442	6.237	0.0039
	Error	47	1066.205		
Exp 1. Moves	Treatment	2	13,048.715	8.712	0.0006
	Error	47	1,497.671		
Exp 2. Feeding	Treatment	2	14,239.356	11.897	<0.0001
	Error	42	1,196.879		
Exp 2. Moves	Treatment	2	4,749.867	5.542	0.0073
	Error	42	857.054		
Exp 3. Feeding	Treatment	2	8,071.148	4.400	0.0177
	Error	47	1,834.312		
Exp 3. Moves	Treatment	2	3,246.641	1.280	0.2874
	Error	47	2,535.558		

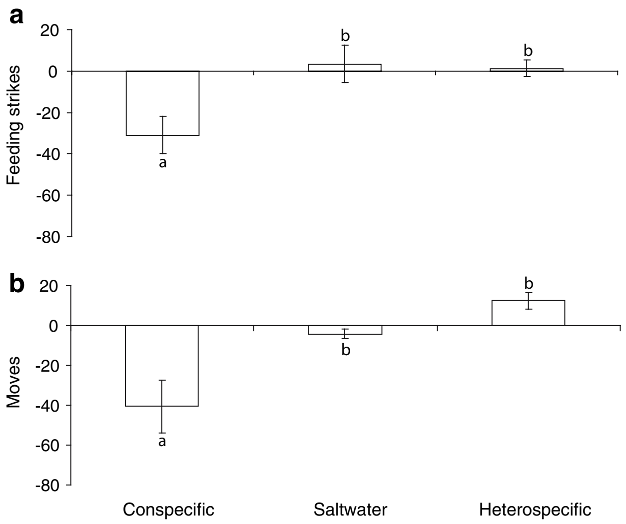

Fig. 1 Mean change (± SE) in **a** feeding strikes and **b** moves by *Asterropteryx semipunctatus* between 10-min pre and post-stimulus periods, for fish exposed to: conspecific skin extract; saltwater; or swordtail skin extract (heterospecific). Letters above or below the bars represent Tukey's HSD groupings of means

introduction of the stimuli, gobies exposed to conspecific skin extracts exhibited a significant decrease in the number of feeding strikes (ANOVA, $p = 0.0039$; Table 1b; Fig 1a) and number of moves (ANOVA, $p = 0.0006$; Table 1b; Fig 1b) compared to fish in the two control treatments, which showed no response to the swordtail extract or salt water stimuli. No bobs were recorded during the pre- or post-stimulus periods by fish in either of the two controls or the chemical alarm treatment.

Experiment 2: response of gobies to the visual cue of a predator

There was a significant change in behaviour between pre- and post-stimulus periods for the gobies visually exposed to a predator compared to fish within the two control treatments (MANOVA, $p = 0.0001$; Table 1a; Fig. 2). Similarly to experiment 1, during the pre-stimulus period gobies fed on live brine shrimp, swimming around the majority of the tank area. Upon exposure to the stimuli, gobies visually exposed to *C. boenak* exhibited a significant decrease in the number of feeding strikes (ANOVA, $p < 0.0001$; Table 1b; Fig 2a) and number of moves (ANOVA, $p = 0.0073$; Table 1b; Fig 2b) compared to fish in the two control treatments, who showed no response from the exposure to a non-predator (*A. polyacanthus*) or an empty tank (disturbance control). Bobs were absent from the behavioural repertoires of gobies in both the controls but occurred in 12 of the 15 predator treatment replicates, with an obvious increase seen between pre- and post-stimulus periods.

Experiment 3: response of gobies to the coupling of conspecific skin extract and visual cue of predator

The behaviour of *A. semipunctatus* in the combined visual and chemical stimulus treatment differed from their response when exposed to the stimuli in isolation (i.e., key treatments of Exp 1 and 2; MANOVA, $p = 0.0225$, excluding bobs; Table 1a). Gobies showed a decrease in feeding and moves, but an increase in bobs in response to the combined stimuli of chemical alarm cues and the visual cue of a

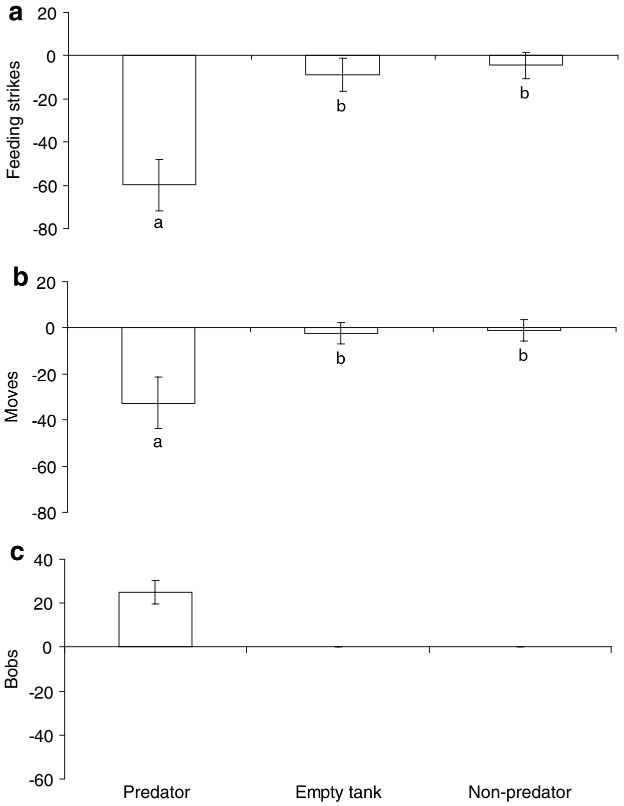

Fig. 2 Mean change (± SE) in **a** feeding strikes, **b** moves and **c** bobs by *Asterropteryx semipunctatus* between 10-min pre and post-stimulus periods, for fish exposed to: visual cue of a known predator; an empty tank; visual cue of a non-predatory heterospecific. Letters above or below the bars represent Tukey's HSD groupings of means

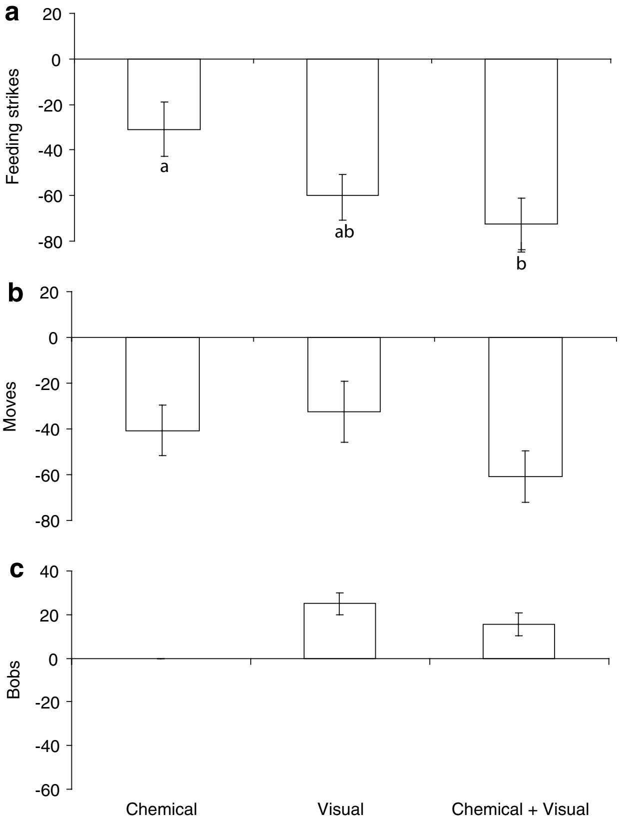

Fig. 3 Mean change (± SE) in **a** feeding strikes, **b** moves and **c** bobs by *Asterropteryx semipunctatus* between 10-min pre and post-stimulus periods, with the stimuli being: the conspecific skin extract (chemical alarm cue); the visual cue of a predator; and the co-occurrence of chemical and visual cues. Letters above or below the bars represent Tukey's HSD groupings of means

predator (Fig. 3). When compared against the visual and chemical alarm cue treatments on their own there was a significant difference in feeding strikes between fish in the isolated chemical alarm treatment and the combined visual and chemical treatment, but there was no difference between fish from the visual predator cue in isolation and the combined cue treatment (Fig. 3a). While fish in all three treatments showed a decrease in moves after all stimuli, there were no statistical differences in the magnitude of the decrease among treatments (Table 1b). Gobies did not bob in response to conspecific skin extracts, and bobs were only recorded in response to visual stimuli, or when visual and chemical alarm cues were combined (Fig. 3c).

Discussion

This study highlights the potential for olfactory cues to be an important source of information on which coral reef fish can make decisions on the risk of predation. At the levels of the stimuli delivered in the present study the antipredator response of the goby, *A. semipunctatus*, to chemical alarm cues from a conspecific was the same magnitude as that provoked by the visual cue of a predator. When visual and olfactory cues were present together they elicited a stronger antipredator response than when fish were exposed solely to conspecific alarm cues. The response of the gobies to the predator cues depended on the behavioural variable measured. Bobs were only elicited when the goby had access to visual cues. This study is the first step in determining the relative importance of visual and olfactory information in risk assessment. Research on freshwater fishes strongly suggests a graded response to chemical cues (e.g., Brown et al. 2001; Ferrari et al. 2006; Zhao et al. 2006), and the potential for a graded response to sensory cues that indicate predation risk has yet to be assessed for marine fishes.

The response of the goby to conspecific skin extracts and visual cues of a natural predator involved a decrease in the number of moves and the number of feeding strikes, which has been interpreted by other studies on *A. semipuntatus* as an antipredator response (Smith 1989; Larson and McCormick 2005; McCormick and Larson 2007). No antipredator response was observed upon exposure to skin extracts of a phylogenetically distant fish, suggesting that the response to conspecific skin extracts was a response to a chemical alarm cue. This supports Smith's (1989) conclusion that *A. semipunctatus* from Hawaii possess an alarm pheromone system similar to that found in ostariophysans and darters.

The present study also demonstrated that there was no change in behaviour as a result of visual exposure to a non-predatory heterospecific, while gobies reduced their feeding and moves in response to seeing a known predator. This suggests that *A. semipunctatus* recognized the threat of the predator in the adjacent aquaria. The gobies also responded to the visual cue of a predator by undertaking a bobbing behaviour. Bobbing only occurred in response to a visual predatory cue or when visual and chemical alarm cues were presented together; they did not bob in response to chemical alarm cues alone. This contrasts with the study of Smith (1989), which found that *A. semipunctatus* bobbed in response to chemical alarm cues. These contrasting results may represent a location difference in a learned response to chemical alarm cues.

The bobbing behaviour found in this study parallels earlier research on this species (Smith 1989; Larson and McCormick 2005) and is known to occur as a defensive response in two other species of gobies, *Gnatholepis anjerensis* and *Coryphopterus nichlosii* (Smith and Lawrence 1992). Smith (1989) suggested that the motion of bobbing widened the gobies area of view, enhancing the visual assessment of the predator. Gulping of water occurs in conjunction with bobbing and this may also increase the acquisition of chemical information leading to a more informed decision on risk. Smith and Smith (1989) suggested that bobbing may function both as a signal to predators that they have been seen and thereby reduce the probability of the predator striking at an aware prey, and also to alert conspecifics to an active predator.

It is not surprising that chemical and visual stimuli elicit similar levels of anti-predator behaviour in the gobies due to the complex nature of the coral reef habitat in which they live. Topographic complexity aids ambush and cryptic predators by obscuring visual cues, and this means that prey within such environments should use a variety of other cues to assess predation risk. Chemical cues can provide information on density, health or ontogenetic stage, and reproductive state of fish in the vicinity of a receptive individual (Brown et al. 2002; Olivotto et al. 2002; Shohet and Watt 2004) and will influence their use of space and behavioural decisions (Tremaine et al. 2006). When the olfactory cues are chemical alarm cues released through a predator strike, then cues may provide additional information on the identity of the predator (Kats and Dill 1998; Brown 2003), whether it fed on a conspecific or possibly a guild member (Brown et al. 1995), the ontogentic stage of the prey item (Golub and Brown 2003), together with some information on timing and location of predator activity. Selection should strongly favour prey that are able to correctly use such chemical cues to inform their decisions under the threat of predation (Mathis and Smith 1993; Mirza and Chivers 2001a).

In environments such as a coral reef, where prey may be exposed to multiple predators with different attack modes, prey will seldom have comprehensive information on which to base a risk assessment decision. To minimise erroneous decisions, prey are expected to use multiple sensory cues to best assess types of predators and the nature of the risk posed (Amo et al. 2006). Chemical cues may remain well after a predator has left the vicinity and a reliance on chemical information may lead to an overestimate of risk (Turner and Montgomery 2003). Visual identification of the predator and its attributes (e.g., size, speed and area of interest) may provide information on the predator's level of motivation and threat (e.g., Helfman 1989; Smith and Belk 2001). Helfman (1989) suggested that the information from multiple sensory cues should contribute in an additive way to yield a graded response in the level of risk-sensitive behaviour (the "threat-sensitivity hypothesis"). Like many other studies, the present study only used two levels of each sensory cue (low threat versus high threat), so determining the shape of the response of *A. semipunctatus* to varying degrees of visual and chemical stimulation was not possible. While data in the present study is sufficiently robust to show that *A. semipunctatus* responds in a similar intensity when given strong visual and olfactory cues of a predator, it is insufficient to strongly characterize whether the cues are additive. Without characterizing the behavioural response to varying intensities of cues it is impossible to determine the extent to which, and conditions under which, the sensory cues have truly additive or multiplicative effects. Clearly, different sensory cues provide different types of information and the more complete the information received by prey on the predation risk the more intense the antipredator behaviour.

Studies on freshwater fishes show that the response elicited through a co-occurrence of visual and olfactory cues will depend on the context of the predator–prey interaction. Brown and Magnavacca (2003) found that glowlight tetras (*Hemigrammus erythrozonus*) primarily used chemical cues in the form of prey alarm cues in the diet of the predator as the source of information on which to assess predation risk. Visual cues were used when chemical information was unavailable or ambiguous. Fathead minnows (*Pimephales promelas*) were also seen to elicit a stronger response to conspecific chemical alarm cues when visual cues were limited (Hartman and Abrahams 2000). In contrast, Smith and Belk (2001) suggested that mosquitofish (*Gambusia affinis*) relied on visual cues during high-risk behaviours, such as predator inspection, whereas general avoidance behaviour was determined by additive responses from visual and chemical cues. Smith and Belk (2001) also noted that there are situations where additivity in response to predator cues may not be expected; for instance, when predator cues overlap in information, responding twice as strongly to two different cues providing the same information may be uneconomical. Further study is required to determine whether this is the situation in the present study.

There are many attributes of a predator that may influence the nature and magnitude of a prey's response. These include: the motivation or hunger of the predator (Licht 1989); its mobility (Dugatkin and Godin 1992); when it last attacked (Murphy and Pitcher 1997); and whether the last victim released chemical alarm cues relevant to the next prey (Chivers and Smith 1998). The balance of information from visual, olfactory and other senses on which behavioural decisions are based is likely to differ with the context of the predator–prey encounter, but also will change over the course of the encounter sequence (Lima and Dill 1990). The present study indicates that chemical cues can be equally as important in the marine environment as they have been shown to be in freshwater systems. How the utility of chemical cues is modified by the context of the predator–prey encounter awaits further study.

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