



# Effect of hunger on the response to, and the production of, chemical alarm cues in a coral reef fish

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A wide variety of fish species release chemical alarm cues upon mechanical damage to their skin by predator attack. We investigated the effects of short-term food deprivation on the response to, and the production of, chemical alarm cues in the coral reef-dwelling goby *Asterropteryx semipunctatus* (family Gobiidae). Gobies deprived of food for 4 days did not react with fright when exposed to conspecific skin extracts. However, when the same individuals had been fed ad libitum, they responded with a typical alarm reaction to the skin extracts, including decreased movement and feeding. In a second experiment, gobies were exposed to skin extracts from conspecifics that had been food deprived and from conspecifics fed ad libitum. Damaged skin from food-deprived individuals did not elicit an alarm response in conspecifics, whereas skin from individuals that had been fed ad libitum yielded a typical alarm response. Histological sections of goby skin showed that food-deprived gobies had fewer sacciform cells in their epidermis than did gobies fed ad libitum. Evidence suggests that there may be a trade-off between foraging and antipredator behaviour, and also between the benefits derived from maintaining chemical alarm cue cells and the cost of producing them in *A. semipunctatus*.

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Many aspects of the decisions made by a prey fish about the timing and frequencies of daily life processes are influenced by the risk of predation (Lima & Dill 1990). Predation risk influences the space used by a prey species and behavioural decisions made with respect to processes such as foraging, maintenance of social organization, courting and reproduction. Many aspects of foraging are adversely altered under the threat of predation since feeding increases the visibility and vulnerability of prey fish to predators. These include where and when a prey fish feeds (Schmitt & Holbrook 1985; Savino & Stein 1989; Biro et al. 2006), its feeding rate (Milinski & Heller 1978; Smith 1989; Whitam & Mathis 2000), what prey items it feeds on (Lima & Valone 1986), how quickly it resumes feeding after interruption (Morgan 1988; Brown

& Smith 1997), and how it handles its food (Newman et al. 1988).

Foraging behaviour may also be affected by other factors such as the body condition or the hunger of prey fish (Lima & Valone 1986; Whitam & Mathis 2000). Where predators are common or resident, prey must assess the risk of predation against the gains obtained through foraging. Food-deprived prey individuals often maximize foraging at the expense of antipredator behaviours or vigilance for predators. Because prey are often more vulnerable to predation while foraging as a result of reduced alertness and increased conspicuousness (Whitam & Mathis 2000), food-deprived fishes may take higher risks when trading foraging for safety.

Many aquatic animals respond with typical antipredator responses to the smell of damaged skin of conspecifics (Smith 1992). Such chemical alarm cues act as a reliable indicator that a potential predator is in the vicinity. When prey are well fed and/or in good physiological condition, they should react with an antipredator response to

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such indirect evidence of danger maximize safety. However, when prey are hungry and/or low in energy reserves, they may decide to delay an antipredator response and keep foraging until they have more direct evidence of predation, such as the sight of the predator.

Studies on a number of freshwater fish species have shown that short periods of food deprivation reduce the antipredator reaction and in some cases even trigger a feeding response in reaction to chemical alarm cues from conspecifics. Smith (1981) showed that food-deprived Iowa darters, *Etheostoma exile*, responded with stereotypic feeding to damaged skin from conspecifics; however, when the same individuals were tested after a period of abundant food they showed a typical fright response. Similar results have been reported for fathead minnows, *Pimephales promelas* (Brown & Smith 1996), reticulate sculpins, *Cottus perplexus* (Chivers et al. 2000), pintado catfish, *Pseudoplatystoma coruscans* (Giacquinto & Volpato 2001), salamanders, *Eurycea multiplicata* (Whitam & Mathis 2000), birds (turnstone, *Arenaria interpres*: Metcalfe & Furness 1984) and molluscs (*Nassarius obsoletus*: Stenzler & Atema 1977; *Pleurobranchaea californica*: Gillette et al. 2000). No studies have investigated the effects of hunger on antipredator behaviours of tropical marine fish species.

Early studies on chemical alarm cues in ostariophysan fishes reported that skin extract from fish with low body condition did not seem to elicit an alarm reaction as effectively as skin from fish in good body condition (von Frisch 1941; Thines & Vandenbussche 1966). These casual observations were later supported by a study by Wisenden & Smith (1997), in which fathead minnows maintained on a low food ration had significantly fewer alarm substance cells in their epidermis than minnows maintained on a high food ration. Wisenden & Smith (1997) concluded that there is a trade-off between the benefits derived from possessing chemical alarm substance cells in the epidermis and the cost of producing them. However, no studies have directly tested the effect food deprivation or hunger of an individual has on the response of conspecifics to its damaged skin.

The coral reef-dwelling goby *Asterropteryx semipunctatus* inhabits areas of coral rubble around coral outcrops and shows a stereotypic alarm response when exposed to extracts of the damaged skin of conspecifics (Smith 1989; Larson & McCormick 2005; McCormick & Larson 2007). Large, clear, vacuolated cells analogous to the chemical alarm substance cells in ostariophysan fishes are found in the epidermis of this species (Smith 1989). *Asterropteryx semipunctatus* therefore serves as a good model species to investigate the trade-offs between foraging and antipredator behaviour in coral reef fish. In addition, it can be used to explore possible trade-offs between the benefits derived from possessing chemical alarm cues and the cost of producing them in coral reef fish species. We addressed three specific questions. (1) Does short-term food deprivation influence the alarm response to conspecific skin extracts in *A. semipunctatus*? (2) Does short-term food deprivation influence the effectiveness of skin extracts from *A. semipunctatus* in eliciting an alarm response in conspecifics? (3) Does short-term

food deprivation influence the number of alarm substance cells in the epidermis?

## METHODS

### Collection and Maintenance of Study Animals

We collected gobies in Pioneer Bay, Orpheus Island, on the central Great Barrier Reef, Australia, using a dilute solution of clove oil in sea water (as an anaesthetic) and a hand net. The gobies were transported to the James Cook University aquarium system in aerated sea water inside dark containers to minimize stress. Once in the aquarium system they were maintained in flowing sea water under a 12:12 h light:dark photoperiod at 27°C. The gobies were fed daily with live brine shrimp, *Artemia franciscana*, and commercial flake food during a 2-week acclimation period. All maintenance and experimental procedures were approved by James Cook University Animal Ethics Committee. After the study, the remaining gobies were released at the site of capture.

### Observation Tanks

All observations were conducted in 33-litre tanks with flowing sea water ( $\bar{X} \pm SD = 0.4$  litres/min  $\pm 0.08$ ). The substratum consisted of a layer of aquarium gravel, 3 cm thick, and an airstone was placed at the back of the tank. Plastic tubing was attached to the airline to enable the injection of extracts into the tank without disturbance. The end of the tubing was attached approximately 1 cm from the airstone, which allowed the extracts to disperse rapidly in the test tank. Broken terracotta tiles were placed in the middle of the tank forming a low, structurally complex shelter to mimic the coral rubble shelters gobies inhabit naturally.

### Stimulus Preparation

The donor fish ( $N = 52$ ) were killed by a quick blow to the head to ensure death. We then prepared the skin extract by putting the fish in a clean disposable plastic petri dish, making 25 superficial vertical cuts on each flank with a razorblade, and then rinsing the fish in 15 ml of sea water. The cuts damaged the skin but caused only minor flesh damage. The stimulus water was then filtered through filter paper (125 mm diameter, qualitative 1) to remove any solid particles such as scales and drawn up into a disposable syringe for injection into the tank through the stimulus tube (15 ml of cue). Extracts had no colour and were not visible to the observer when released. They were used within 20 min of preparation, as in some species extracts may lose potency if frozen or stored for long periods at room temperature (Smith 1989).

### Behaviour of Subjects

We categorized and quantified two behaviours: moves and feeding strikes. *Asterropteryx semipunctatus* is a cryptic,

negatively buoyant, benthic fish that moves episodically and movements are separated by periods when they remain stationary on the substratum. Each time a goby changed position we recorded a 'move'. Moves ranged from 1 to 15 cm. We counted all feeding strikes made by a fish regardless of whether the prey item was caught. The observer stood behind a blind and used mechanical counters to record behaviour of the test subjects.

### Experiment 1: Effect of Receiver Hunger

We examined the effect of hunger on the response of *A. semipunctatus* to conspecific alarm cues by comparing the goby's response in four treatments: (1) gobies deprived of food for 4 days and tested with a conspecific chemical alarm cue (from a fed donor fish); (2) fed gobies tested with a conspecific alarm cue; (3) gobies (from treatment '1') that had been deprived of food for 4 days and refed ad libitum for a further 4 days prior to being tested with a conspecific alarm cue; (4) fed gobies tested with a heterospecific skin preparation (controlling for the response of the goby to any skin preparation). We did not test the response of experimental fish to a saltwater control (controlling for the response of the goby to any injection of cues into the test tank), but previous studies using the same protocol have found that *A. semipunctatus* does not respond to the injection of saltwater alone into the test tank (Larson & McCormick 2005; McCormick & Manassa 2008).

The responses of fed *A. semipunctatus* to conspecific alarm cues and a heterospecific control have been documented before (McCormick & Larson 2007), but are included here for comparison to the food-deprived and refed treatments. We conducted this published study at the same time as the present one, using a random sample of the fish collected from Orpheus Island and the same experimental protocols, legitimating direct comparison. Protocols for all treatments are given in the present study for ease of comparison among treatments.

Groups of three gobies were placed randomly in aquaria ( $\bar{X} \pm SE$ : 16 males =  $35.35 \pm 1.26$  mm; 29 females =  $30.74 \pm 1.04$  mm) to acclimate for 7–14 days. Pilot tests indicated that individuals in groups of two gobies per test tank showed abnormal behaviour, spending most of their time under shelter. For gobies in groups of three, activity levels, as well as the group density, were similar to those observed in the wild. We used fish in only one experimental trial each. After the acclimation period gobies were deprived of food for 4 days before the experiment. They were then tested for a response to skin extracts from conspecifics that had been fed ad libitum since capture. Trials consisted of a 10 min prestimulus and a 10 min poststimulus period. As a standard test protocol, we quantified the behaviour of the three *A. semipunctatus* for 10 min within each tank before we injected the extract into the tank. Immediately prior to experimental observation, 60 ml of tank water were drawn out through the stimulus injection tube and discarded to remove any stagnant water that may have collected in the tubing. Another 60 ml of tank water was drawn through the

tubing and kept for later use. The 15 ml of chemical stimuli were then injected through the tubing followed by 60 ml of tank water to flush the test substance into the tank. We then observed the fish for another 10 min. In trials with vegetable dye, it took a mean  $\pm SE$  of  $39 \pm 2.20$  s after the injection for the dye to disperse throughout the tank.

As a direct comparison to the response of the food-deprived *A. semipunctatus* to conspecific alarm cues, we conducted trials with gobies that had been fed ad libitum since capture. Experimental protocol and cue preparation followed the same methodology as described for the food-deprived gobies. Skin extracts were prepared from five male (mean standard length  $\pm SE = 32.24 \pm 2.12$  mm) and eight female ( $29.64 \pm 2.53$  mm) *A. semipunctatus*. To test for a general antipredator response to a heterospecific, we prepared skin extracts of 13 swordtails, *Xiphophorus helleri* ( $38.78 \pm 1.91$  cm) using the same protocol as for the gobies. We used swordtails because they are phylogenetically distant from *A. semipunctatus*. Although swordtails possess a damage-released alarm cue to which conspecifics respond (Mirza et al. 2001), gobies are known to have no antipredator response to these chemicals (Larson & McCormick 2005; McCormick & Manassa 2008). The swordtails were obtained commercially and maintained in dechlorinated freshwater at approximately 22.5°C. Details of these controls are given in McCormick & Manassa (2008). Thirteen replicates were run for both control treatments.

After the experiment, gobies were fed to satiation for 4 days and then retested for a response to skin extracts from conspecifics fed ad libitum, using the same methodology. Goby skin extract was prepared from 10 female (mean standard length  $\pm SE = 30.18 \pm 1.26$  mm) and five male ( $34.5 \pm 2.41$  mm) *A. semipunctatus*.

### Experiment 2: Effect of Body Condition of Cue Sender

To examine whether hunger of the donor fish affected the potency of the alarm substance, we deprived donor fish of food for 6 days before killing them by a blow to the head and using them for the preparation of the skin extract. Prior to being killed the fish behaved normally and showed no obvious signs of stress. Gobies that had been fed to satiation were then tested for a response to skin extracts from the food-deprived conspecifics. For comparison, we tested a second random sample of gobies for their response to skin extracts from conspecific donors that had a history of being well fed. The responses of gobies to both these treatments were compared to the response of gobies to skin extracts from swordtails. The extract preparation and test protocols were the same as for experiment 1.

Goby skin extract was prepared from five female (mean standard length  $\pm SE = 30.48 \pm 1.28$  mm) and seven male ( $38.13 \pm 1.75$  mm) *A. semipunctatus* that had been deprived of food for 6 days and from six female ( $32.51 \pm 1.15$  mm) and six male ( $34.67 \pm 1.87$  mm) *A. semipunctatus* that had been fed ad libitum.

The influence on fish behaviour of the four treatments in experiment 1 and three treatments in experiment 2 were tested with one-way MANOVA. The dependent variables were the differences between the pre- and post-stimulus counts of feeding strikes and moves for each trial (Pearson correlation between moves and feeding strikes within experimental treatments:  $r < 0.5$ ). Only feeding strikes and moves were sufficiently common to allow statistical comparison. To explore further the nature of significant differences found by MANOVA, we used one-way ANOVAs to examine whether there were significant differences between treatments in one or both variables. Tukey's highly significant difference (HSD) means comparisons tests were used to explore the nature of significant differences found by ANOVA. Residual analysis was used to examine the assumptions of normality and homogeneity of variance.

## Histology

We examined skin samples from gobies histologically to determine whether short-term food deprivation influenced the number of sacciform cells in the epidermis. Cross-sections were taken from between the head and the dorsal fin of two *A. semipunctatus* fed ad libitum and two that had been deprived of food for 7 days, killed by a blow to the head, and fixed in FAAC (4% formaldehyde, 5% acetic acid, 1.3% calcium chloride). Paraffin sections (5  $\mu\text{m}$ ) were then stained with Schiff's reagent and counterstained with haematoxylin (Periodic Acid Schiff-Hematoxylin). For each of the six epidermal sections taken from each fish, we recorded the thickness of the epidermis and number of sacciform cells. The thickness of the epidermis was measured from the basal membrane to the outer surface of the skin. The sacciform cells were counted in a section of the epidermis 9.0 mm long. Averages of the six sections were calculated for the epidermis thickness and number of sacciform cells for each fish. Using a Student's *t* test, we compared the mean epidermal thickness and number of sacciform cells of fish that had been food deprived and fish that had been fed ad libitum.

## RESULTS

### Experiment 1: Effect of Receiver Hunger

We found differences in the responses of food-deprived and fed gobies to conspecific and heterospecific skin extracts (MANOVA: Pillai's trace = 0.656,  $df = 6, 104$ ,  $P < 0.0001$ ; Table 1, Fig. 1). Food-deprived gobies responded to conspecific skin extracts with a significant increase in moves (Fig. 1a), but with no change in number of feeding attempts. This was very similar to the pattern of response by gobies to heterospecific skin extracts. In contrast, fed gobies responded to conspecific skin extracts with fewer moves and feeding strikes (Fig. 1). When gobies that had been refed for 4 days after a 4-day period of food deprivation were retested for a response to conspecific skin extracts, they showed the same reduction in movement and feeding that the continuously fed gobies had shown (Fig. 1).

### Experiment 2: Effect of Body Condition

Gobies responded differently to skin extracts of conspecifics that had either been food deprived for 6 days or continuously fed (MANOVA: Pillai's trace = 0.631,  $df = 4, 70$ ,  $P < 0.0001$ ; Table 1, Fig. 2). Recipients of the extract from fed gobies significantly reduced their movements and tended to reduce their feeding, whereas little change occurred in the behaviour of gobies exposed to extracts from food-deprived gobies (Fig. 2). Gobies exposed to heterospecific skin cues responded with an increase in moves and no change in feeding response (Fig. 2), suggesting an increase in foraging.

## Histology

Gobies that had been fed ad libitum had significantly more sacciform cells in their epidermis (4.12 per 9 mm length) than gobies that had not eaten for 7 days (1.34; Student's *t* test:  $t_1 = 4.62$ ,  $P < 0.05$ ). The mean epidermal thickness was greater for gobies that had been fed ad libitum ( $\bar{X} \pm \text{SE} = 12.96 \pm 1.63 \mu\text{m}$ ) than gobies that had not eaten ( $8.39 \pm 0.72 \mu\text{m}$ ); however, this difference was not statistically significant ( $t_1 = 2.57$ ,  $P = 0.12$ ).

## DISCUSSION

Our results clearly show that there is a trade-off between predator avoidance behaviour and foraging that is strongly influenced by hunger level in *A. semipunctatus* under laboratory conditions. Food-deprived gobies responded to conspecific skin extracts with an increase in number of moves and with no change in number of feeding attempts. This behaviour resembles the response to the introduction of food into the aquarium (personal observation) and may represent an increase in food-searching behaviour (i.e. foraging), rather than a typical alarm response. When the same gobies were retested for a response to conspecific skin extracts after a period of abundant food supply, they responded with decreased movement and feeding, in keeping with the response of gobies with a history of high food intake, which has been described as a typical response to an alarm cue (Smith 1989; Larson & McCormick 2005). Similar results have been reported for freshwater reticulate sculpins (Chivers et al. 2000), which are ecologically similar to *A. semipunctatus*. Food-deprived sculpins did not show antipredator behaviour when exposed to conspecific alarm cues but continued feeding in the water column; in contrast, individuals that had been fed ad libitum moved and foraged less. Although in the wild both the sculpin and goby are unlikely to experience food deprivation of the type experimentally inflicted on the extract donors and receivers, the study does suggest that the production of alarm cues is an energy-demanding process and that foraging risk is dependent upon hunger state. The potential for food deprivation in the field, and the effects of less dramatic nutritional constraints, await further study.

Decreased movement is a common component of crypsis that removes the visual stimulus of movement

**Table 1.** Summary of comparisons of treatment effects on feeding rate and moves of *Asterropteryx semipunctatus* in two experiments

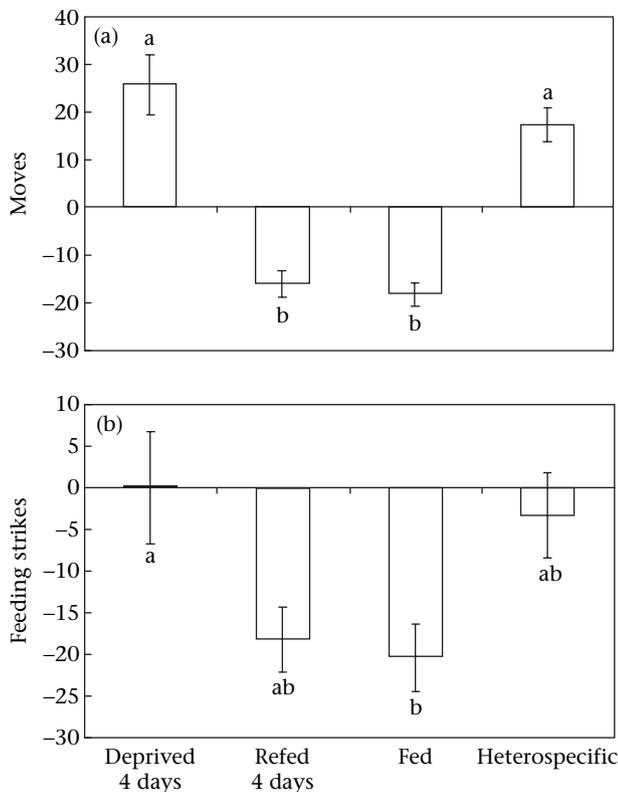
Source of variation		df	Mean square	F	P
Experiment 1					
Feeding	Treatment	3	1501.893	4.058	0.011
	Error	52	379.138		
Moves	Treatment	3	7222.449	32.127	<0.0001
	Error	52	224.808		
Experiment 2					
Feeding	Treatment	2	958.026	2.636	0.085
	Error	36	363.462		
Moves	Treatment	2	4104.876	28.911	<0.0001
	Error	36	141.981		

In experiment 1, gobies were either food deprived or fed ad libitum and their responses to conspecific or heterospecifics skin extracts were recorded. In experiment 2, the responses of gobies to food-deprived or fed conspecifics or heterospecifics were recorded.

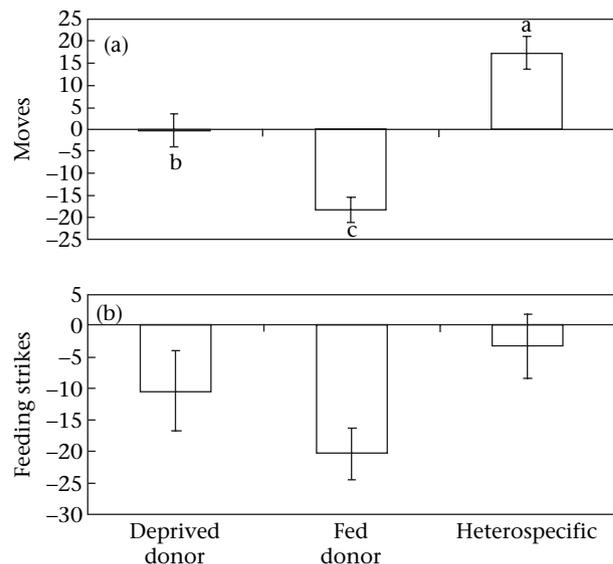
relative to the background (Edmund 1974). Reduced movement in response to conspecific alarm cues increases the survival rate during staged predator encounters in freshwater fathead minnows (Mathis & Smith 1993), but because any movement by the prey fish, whether

associated with mating, defending a nest or foraging, will break crypsis against the background, this antipredator behaviour is associated with a cost of decreased energy intake. Our studies suggest that only gobies that have a good feeding history can afford to lose foraging opportunities to optimize their antipredator behaviour by reducing their activity and foraging intensity.

The increased activity levels and unchanged feeding rates of food-deprived gobies increase their foraging opportunities at the cost of increased visibility to predators. In other words, hungry individuals may take greater risks under predation threat to satisfy their energetic requirements. These results are consistent with findings from similar studies on freshwater fish (Smith 1981; Brown & Smith 1996; Chivers et al. 2000; Giaquinto & Volpato 2001), salamanders (Whitam & Mathis 2000) and birds (Metcalf & Furness 1984).



**Figure 1.** Mean change  $\pm$  SE in (a) moves and (b) feeding strikes of *Asterropteryx semipunctatus* between 10 min observation periods before and after exposure to conspecific skin extracts. Fish were tested after 4 days of food deprivation (Deprived 4 days) and then retested after 4 days of being fed ad libitum (Refed 4 days). Responses are compared to those of gobies fed ad libitum without a food deprivation (Fed), and fed gobies exposed to the skin extract of a heterospecific (*Xiphophorus helleri*). Letters above or below the bars represent Tukey's highly significant difference groupings of means.



**Figure 2.** Comparison of mean change  $\pm$  SE in moves and feeding strikes of *Asterropteryx semipunctatus* between 10-min observation periods before and after exposure to skin extracts from food-deprived conspecifics, conspecifics fed ad libitum, and a heterospecific fish (*Xiphophorus helleri*). Letters above or below the bars represent Tukey's highly significant difference groupings of means.

Although hungry gobies did not show an overt alarm response, they may still have detected the alarm substance. Brown & Smith (1996) showed that food-deprived fathead minnows that were naïve to predators did not show an alarm reaction to a mixture of a conspecific alarm cue and the smell of a predatory pike, *Esox lucius*. However, when minnows were exposed to the pike cue alone after a period of abundant food, they reacted with a typical alarm response. These results suggest that although the minnows did not respond when food deprived they did indeed detect the alarm substance and learned to associate the predator cue with the alarm cue in the absence of an overt alarm response. Similar results have been found for glowlight tetras, *Hemigrammus erythrozonus* (Brown et al. 2004a). Whether this mechanism also occurs in *A. semipunctatus* is unknown.

The alarm substance may have triggered hungry gobies to become more vigilant for a more direct threat of predation, such as a visual cue of a predator. In topographically complex or highly turbid habitats with poor visibility, chemical information can provide valuable forewarning of the presence and actions of a predator (Mirza & Chivers 2001). Visual cues are used to adapt the response to the degree of threat imposed (Hartman & Abrahams 2000; Chivers et al. 2001), often resulting in a graded response (Engstrom-Ost & Lehtiniemi 2004). As chemical cues from injured conspecifics serve only as indirect indication of danger, hungry gobies may require more direct signs of predators before they make a typical alarm response. McCormick & Manassa (2008) reported that *A. semipunctatus* reacted with a similar magnitude of anti-predator response to a strong visual cue of a brown barred rock cod, *Cephalopholis boenak*, and conspecific chemical alarm cue. However, when both cues were presented simultaneously they resulted in an additive response. Hungry gobies may therefore delay an antipredator behaviour and keep foraging until more immediate threats of predation, such as seeing a predator, are detected.

*Asterropteryx semipunctatus* build complex systems of burrows, typically with several exits and entrances, in coral rubble where they also forage and are thus never far from shelter. An increase in vigilance even in the absence of a cryptic antipredator response is likely to improve chances of escape during a predator attack in such an environment.

### Effect of Hunger on Skin Extract Potency

This study showed that skin from food-deprived prey fish was less effective in eliciting an alarm response in conspecifics than skin from fish with a good feeding history. Gobies exposed to skin extracts from hungry conspecifics did not show typical antipredator behaviour but significantly increased the number of moves and did not change the number of feeding attempts. When test fish were retested for a response to skin extracts from fed conspecifics they reacted with a typical alarm response including decreased movement and feeding attempts. Tests on juvenile convict cichlids, *Archocentrus nigrofasciatus*, showed that skin extracts from donors in low body

condition had to be in higher concentrations to elicit antipredator behaviour, and that, once initiated, this antipredator response was less intense than that of fish exposed to extracts from fish in high body condition (Roh et al. 2004; Brown et al. 2004b). The present results suggest that there is a trade-off between the cost of producing the alarm substance and the fitness benefits it provides for prey gobiid fishes. Gobies that have been deprived of food for 7 days divert energy away from maintaining the alarm substance to other needs (Wisenden & Smith 1997).

*Asterropteryx semipunctatus* that had been fed ad libitum had more sacciform cells in their epidermis than individuals that had been deprived of food. This suggests that there is a cost in producing and maintaining these cells and that only gobies with a good feeding history can afford this cost. That skin from food-deprived individuals contained fewer sacciform cells and was less potent in eliciting an alarm response in conspecifics suggests that these cells may be responsible for the production of the alarm substance in *A. semipunctatus*.

The production of chemical alarm cues is thought to have evolved to benefit the cue sender by attracting secondary predators after the prey has been captured; these secondary predators may interfere with the primary predator and facilitate the prey's escape (Chivers et al. 1996). Chemical cues from damaged skin of *A. semipunctatus* attract a coral reef predator, the brown barred rock cod (J. Larson & M. McCormick, unpublished data). Prey individuals that are in good body condition and thus can afford to invest energy in the production of chemical alarm cues may more efficiently attract secondary predators after capture by a primary predator (Chivers et al. 1996; Wisenden & Smith 1997). If the secondary predators increase the probability of the prey escaping, as has been shown in freshwater systems (Chivers et al. 1996), then the cost of the production of the alarm substance is mitigated by a higher survival rate for the prey.

In addition, fish in good body condition may be better at coping with parasites or bacterial infections after injury (Smith 1982; Irving & Magurran 1997; Wisenden & Smith 1997). The club cells that produce the alarm substance in ostariophysan fishes, as well as the sacciform cells thought to produce the alarm substance in acanthopterygian fishes (e.g. gobies and darters), produce mucous lectin. Lectin is a bioactive compound that may be involved in defence against pathogens at the body surface of fishes (Takashima & Hibiya 1995). The alarm substance cells could have evolved initially to protect the fish against bacterial infections after injury and its function as an alarm cue might have been secondarily derived (Smith 1982; Irving & Magurran 1997). Chivers et al. (2007) recently provided support for this hypothesis, with data from a number of species of freshwater Cyprinidae (minnows) and Percidae (darters and perch). They showed that production of alarm cells was not influenced by predation pressure, instead being stimulated by exposure to skin-penetrating pathogens (water moulds) or parasites (larval trematodes), and was correlated with exposure to UV radiation. Moreover, when the immune system was suppressed with cadmium, alarm cell production was inhibited.

Whatever the selection pressures in maintaining these cells are, for gobies that have been deprived of food for 7 days in the present study, the cost of maintaining these cells evidently surpassed the benefits that they provided for the fish.

## Conclusion

It is evident that there were trade-offs between foraging and antipredator behaviour, as well as between the benefits derived from maintaining chemical alarm cue cells and the cost of producing them in *A. semipunctatus*. Prey fish may be able to assess the fitness-related costs and benefits associated with alternative defence tactics. The behavioural decision made during an encounter with an active predator will depend on this assessment. These results have implications for future studies on chemical alarm cues in coral reef fishes. They stress the importance of supplying focal prey fish, to be used both as signal senders and signal receivers, with appropriate amounts of food prior to testing for alarm cue responses. In addition, they pose interesting questions about the costs and benefits involved in producing the alarm substance. Does the production of chemical alarm cues benefit coral reef fishes by attracting secondary predators after prey capture, as it does in ostariophysan fishes? An understanding of the selection pressures involved in maintaining the alarm substance cells would elucidate the factors influencing the energy investment decisions made by coral reef fishes.

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