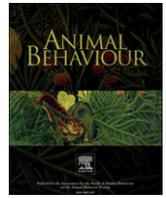




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Response across a gradient: behavioural reactions of newly settled fish to predation cues

Thomas H. Holmes^{a,b,*}, Mark I. McCormick^{a,1}

^aARC Centre of Excellence for Coral Reef Studies, and School of Marine and Tropical Biology, James Cook University

^bMarine Science Program, Science Division, Department of Environment and Conservation

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How individuals respond to predation threats will have a large influence on which individuals survive within a population. The magnitude and timing of these responses will be particularly important during periods of high predation susceptibility, such as that experienced by coral reef fishes immediately following settlement to the reef environment. Although reef fish are known to adopt certain antipredator behaviours when exposed to chemical alarm scents during this early period, the role of visual cues, and how the response varies with different levels of predation threat (both olfactory and visual), remains unknown. This study examined the behavioural response of a newly settled coral reef fish (*Pomacentrus amboinensis*) to different levels of an olfactory and visual predation threat. The concentration of a conspecific chemical alarm cue and the visual proximity to a potential predator (*Pseudochromis fuscus*) were manipulated in separate aquarium experiments. Behavioural responses were found to be threat sensitive in nature, with higher-level threat cues eliciting a more intense response. Although significant changes were observed, responses to visual cues were more inconsistent, while responses to extremely low chemical cue concentrations were marginal, indicating a possible threshold lower limit. These findings demonstrate the ability of newly settled fish to assess the level of predation risk using both visual and chemical cues, and respond appropriately.

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The manner and intensity with which an individual responds to a perceived predation threat can greatly influence the probability of survival (Rhoades & Blumstein 2007). The effects on survival can be both direct, through behaviourally induced changes in vulnerability to the threat (Sih 1986; Spieler & Linsenmair 1999; Blumstein et al. 2002; Hunter et al. 2007), and indirect, through changes in body condition arising from associated behavioural trade-offs (e.g. decrease in foraging time; Amo et al. 2007; Sunardi et al. 2007; Cresswell 2008; Heithaus et al. 2008). If individuals react too strongly to a weak or irrelevant threat, the cost may be an unnecessary loss of overall fitness. On the other hand, if individuals react too weakly, or not at all, to a strong threat, the cost may well be death. It is therefore advantageous to detect the relevance and strength of a predation threat before choosing an appropriate response (Helfman 1989; Godin 1997).

A number of methods can be used in the detection and recognition of predation threats. These include visual (Amo et al. 2006;

Collier et al. 2008; Lohrey et al. 2009; McPhee et al. 2009), acoustic (Durant 2000; Blumstein et al. 2008), olfactory (Amo et al. 2006; Gonzalo et al. 2008; Roth et al. 2008) and seismic means (Warkentin et al. 2007; Lohrey et al. 2009). In aquatic organisms, olfactory, or chemical, cues are a key source of threat information (Sih 1986; Mathis & Vincent 2000; Chivers et al. 2001a, b; McCormick & Manassa 2008; Kim et al. 2009). Olfactory cues have the advantage over other threat cues because they are available early in the predator–prey interaction sequence, often well before an initial encounter with a predator has occurred. However, their utility later in the predation process (i.e. once prey have been visually engaged by the predator) is arguable, owing largely to the relatively slow rate at which these cues travel in an aqueous environment. Once the predator has started the capture sequence, visual cues may play a larger role in the threat detection process, particularly during interactions with noncryptic predators (Brown & Magnavacca 2003).

With respect to olfactory and visual cues, the level of threat posed will be a function of whether the cue is a recognized predation threat, and its relative strength (assessed as cue concentration or source distance, respectively; Mathis & Vincent 2000). Although the identification of relevant cues is often innate (Semlitsch & Gavasso 1992; Kiesecker & Blaustein 1997; Hawkins et al. 2007), or can be acquired through experience (Chivers et al.

* Correspondence: T. H. Holmes, Marine Science Program, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

E-mail address: thomas.holmes@dec.wa.gov.au (T.H. Holmes).

¹ M. I. McCormick is at the ARC Centre of Excellence for Coral Reef Studies, and School of Marine and Tropical Biology, James Cook University, Townsville, QLD 4811, Australia.

1995; Woody & Mathis 1998; Larson & McCormick 2005), how individuals respond to different levels of these may vary. In some circumstances, the intensity of the response may be proportionate to the level of the threat detected ('threat-sensitive' response; Mathis & Vincent 2000; Chivers et al. 2001a, b; Mathot et al. 2009). Alternatively, individuals may choose to respond to a threat only when it is above a certain threshold level ('threshold' response; Mirza & Chivers 2003; Roh et al. 2004; Brown et al. 2006). In this case, the lower levels are not deemed threatening enough to warrant any investment in a response (Brown et al. 2004). However, although some focus has been given to the response of prey to differing predation threat levels in freshwater systems, to date this has received little attention in the marine environment (see Helfman 1989 for an exception).

In coral reef fishes, the period immediately following settlement to the reef environment is characterized by extremely high levels of mortality (Doherty et al. 2004; Almany & Webster 2006). The intense predation pressures present during this stage mean that individuals must quickly learn to respond appropriately to authentic predation threats or face death. We already know that the innate recognition of olfactory alarm cues released by damaged conspecifics plays at least some role in this process (Holmes & McCormick 2010b). However, the function of visual cues in threat recognition during this early period is largely unknown. Given the generally high clarity of water on coral reefs, and the degree of development of visual acuity in settlement stage fish (at least over short distances; Losey et al. 2003; Siebeck et al. 2008), it is reasonable to suggest that it may play a role at some point in the predation process. It is only through understanding how individuals identify and respond to potential predation threats that we can fully comprehend the mechanisms of survival during this potentially critical life stage.

We examined the behavioural response of the newly settled coral reef damselfish, *Pomacentrus amboinensis*, to varying levels of olfactory and visual predation threat cues. Previous work indicates that this species is able to recognize conspecific chemical alarm cues during this early life stage, and responds accordingly by decreasing its feeding rate (Holmes & McCormick 2010b). However, it is unknown how fish respond to potential visual alarm cues (i.e. presence of a potential predator within the prey's visual field) or whether they possess an innate recognition of predator identity in this unfamiliar reef system. We used controlled aquarium experiments to manipulate the exposure of reef-naïve individuals to both a range of concentrations of conspecific chemical alarm cue and potential visual predation cues over a range of distances. Specifically, we aimed to: (1) identify the behavioural response of a naïve newly settled individual to a potential visual predation cue, and whether this differs in response to predatory and nonpredatory individuals; and (2) identify how behavioural responses to both visual cues and conspecific chemical alarm cues change across a concentration/distance gradient.

METHODS

Study Site and Species

This study was conducted at Lizard Island (14°40'S, 145°28'E), northern Great Barrier Reef (GBR), Australia during November and December of 2006 and 2007. The laboratories and flow-through salt water aquarium system at Lizard Island Research Station were used to conduct all experiments, while fish were collected from surrounding waters.

We used *P. amboinensis* as the prey species for all experimental trials. This species is common within coral reef fish communities within the Indo-Pacific, particularly in the central GBR. They settle

to a wide variety of habitats on the northern GBR, but are found in highest densities associated with small reef patches at the base of shallow reefs. The pelagic larval phase lasts between 15 and 23 days and settles at 10.3–15.1 mm standard length (SL; Kerrigan 1996) with its juvenile body plan largely complete (McCormick et al. 2002). Once settled, *P. amboinensis* is site attached, making it an ideal species for experimental manipulation. They recruit in substantial numbers at Lizard Island around the new moon during the austral months (October–January), and are easily collected at the time of settlement with light traps (Milicich & Doherty 1994).

During the visual stimulus trials, the brown dottyback, *Pseudochromis fuscus*, was used as the predator species, while the blue-green damselfish, *Chromis viridis*, was used as the control 'nonpredator' species. *Pseudochromis fuscus* is a small (up to 72.4 mm SL), cryptic, solitary, pursuit predator commonly found on small coral bommies or along reef edges. It is common on shallow reefs throughout the West Pacific and Indian Oceans and is known to feed heavily on newly settled reef fishes in these habitats (Martin 1994; personal observations). *Chromis viridis* is a small reef-associated planktivore commonly found in large aggregations around live coral heads in the West Pacific and Indian Oceans. Although frequently found in similar habitat and in close proximity to many newly settled fish species, they are not known to prey upon them.

Fish Collection, Housing and Release

Settlement stage *P. amboinensis* were collected using light traps moored overnight close to the reef crest, and transported back to the Lizard Island Research Station at dawn in 20-litre buckets of aerated sea water. Fish were collected as close to dawn as possible so as to avoid mortality within the traps. All fish were maintained in 25-litre flow-through aquaria systems for about 24 h, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Aquaria were maintained under a 12:12 h light:dark regime. Fish were collected in batches and used in experiments within about 48 h of capture to avoid biases associated with ontogenetic development. Growth during the handling period was minimal (T. H. Holmes & M. I. McCormick, unpublished data) and mortality during transfer and handling within aquaria was negligible. Fish were released immediately following trials on surrounding shallow lagoonal reef, close to the point of capture and the characteristic habitat of this species. All released fish were observed to be in good condition at the time of release and were shielded from potential predators until within their characteristic habitat to prevent mortality.

Predatory and nonpredatory 'control' fish (*P. fuscus* and *C. viridis*) were collected from surrounding reefs using hand nets and the fish anaesthetic clove oil, and immediately transported back to the Lizard Island Research Station in 20-litre buckets of aerated sea water. *Pseudochromis fuscus* were transported and housed separately owing to aggressive territorial behaviour. All fish were maintained in 25-litre flow-through aquaria systems for about 24 h before being used in trials, to allow for recovery from the stress of capture. Fish were not fed during this period, with the length of time in captivity not deemed long enough to decrease condition significantly. All fish were transported back to the reef in 20-litre buckets of aerated sea water and released immediately following trials at the point of capture so as to facilitate re-establishment in their natural habitat.

Flow-through aquaria systems were fed directly from surrounding lagoonal waters so that water temperatures in aquaria mirrored that found in the natural environment (about 26–29 °C at this time of year). All research was conducted under permits from the Great Barrier Reef Marine Park Authority and James Cook University Animal Ethics Committee.

Experimental Aquaria

Visual stimulus experiments (experiments 1 and 2) were conducted in 24-litre glass aquaria (350 × 255 mm and 280 mm high), with flow-through sea water ('prey aquaria'). Two sides of each aquarium were covered in black plastic to reduce external stimuli. A third side was placed against a similar-sized second glass aquarium ('predator aquarium') maintained on a separate flow-through sea water line. The glass sides of both aquaria were transparent, allowing vision between the two tanks. However, a removable opaque partition could be placed between the two tanks to prevent vision. The fourth side of the prey aquarium was left open to allow for behavioural observations. Two artificial branching coral were placed against one end of the tank for shelter. The predator aquarium was divided into three chambers (of approximately the same size), such that chambers were a minimum of 0, 100 and 200 mm away from the edge of the prey tank, respectively. Given that the artificial branching coral was positioned against the opposite side of the prey aquarium and that it was approximately 55 mm in width, this meant that chambers were 200 (minimum), 300 (medium) and 400 mm (maximum) from the edge of the prey shelter. Chambers were separated by clear Perspex, allowing vision between the 'prey aquarium' and all predator chambers.

The chemical stimulus experiment (experiment 3) was conducted in transparent 12-litre (320 × 230 mm and 200 mm high) aquaria with flow-through sea water. Three sides were covered in black plastic to reduce external stimuli, with the fourth being left open to allow behavioural observations. Two artificial branching coral were placed against one end of the tank for shelter, and a single airstone was placed at the other end. A 1.5 m length of plastic tubing was attached to the airline to allow for the injection of extracts into the aquaria without disturbance. The end of the tubing was attached just above the airstone, enabling rapid dispersal of the extracts throughout the aquaria.

All aquaria were maintained under an approximately 12:12 h light:dark regime, with a constant flow of sea water until the commencement of trials. Lighting was partially provided by natural lighting through windows in the aquarium complex, but boosted to natural daytime levels using fluorescent lighting mounted 1.5 m directly above the aquaria. Observations were conducted from behind a black plastic barrier to avoid human disturbance.

Behavioural Observations

We conducted 10 min observation periods both before and after exposing the fish to the test stimulus. During each period, we quantified seven behaviours: number of feeding strikes, number of 'bobs', time spent in shelter, number of movements into/out of shelter, amount of horizontal movement outside of shelter, distance from shelter, and vertical position in water column. The number of feeding strikes was recorded as the total number of strikes (successful or unsuccessful) made by an individual during each 10 min observation period. Similarly, the number of 'bobs' was recorded as the total number of defined upward or downward bobbing movements by an individual within the period. Although its function is unknown in this species, juvenile *P. amboinensis* possess a distinctive bobbing behaviour, consisting of short, sharp vertical movements in the water column. It is possible the behaviour serves a purpose in either predator vigilance or avoidance.

Every 15 s, we recorded the fish's horizontal distance from shelter and vertical position in the water column. If the fish was within the confines of the branched shelter, its horizontal position was recorded as 0. From these data, approximate measures of time spent within shelter, average distance from shelter and average vertical position within the water column were obtained. To obtain

rough estimates of space use, in the form of movement in and out of shelter and horizontal movement outside of shelter, we divided the experimental aquaria into a series of horizontal zones. The first zone was set at the outside edge of the branching coral shelter, and the zones continued at 50 mm intervals along the horizontal axis, away from the shelter. The number of times a test fish moved both in and out of shelter and between horizontal zones outside of the shelter was recorded during each observational period.

Experiment 1: Response to Visual Stimulus

Before fish were added to the experimental system, the removable partition was placed between the predator and prey aquaria. Individual *P. amboinensis* were then placed into the prey aquaria, while either a single adult *P. fuscus* (known predator) or an adult *C. viridis* (nonpredator) was placed into the closest chamber (0 mm distance from prey aquaria) of the predator aquaria. Shelter was provided at the back end of the aquaria so that predatory fish could take refuge. Fish were left to acclimate for 12 h before the commencement of trials. Flowing sea water and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding.

The behaviour of the single *P. amboinensis* was first recorded for the 10 min prestimulus period. At the completion of this time, the removable partition between the predator and prey aquaria was removed and the prey was exposed to the visual cue of the single *P. fuscus*, the single *C. viridis* or an empty chamber control. The behaviour of the prey was then recorded for the 10 min post-stimulus period.

Fifteen individuals were tested for each of the three visual stimulus treatments, with all fish being tested for one cue only to maintain independence between trials.

Experiment 2: Changing Visual Stimulus Distance

As in experiment 1, the removable partition was placed between the predator and prey aquaria before any fish were added to the experimental system. A single *P. amboinensis* was once again placed into the prey aquaria, while a single adult *P. fuscus* was placed into one of the three predator aquaria chambers. Fish were left to acclimate for 12 h before the commencement of trials. Flowing sea water and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding. The experimental protocol followed that of experiment 1, except that prey fish were exposed to the visual *P. fuscus* cue at distances of 200, 300 or 400 mm from the prey shelter. An empty chamber control was also included in the analysis.

Fifteen individuals were tested for each of the four visual stimulus treatments, with all fish being tested for one cue only to maintain independence between trials.

Experiment 3: Changing Chemical Alarm Concentration

Skin extracts were prepared using settlement stage *P. amboinensis*, collected in light traps. The donor fish were killed individually through cold shock by placing them in a small clip-seal bag along with a small amount of sea water, and completely immersing in ice slurry for about 10 min. Owing to the lower tolerances of juvenile fish of this species, mortality (considered in this case to be a lack of opercula movement) generally occurred within 2 min. However, fish were immersed for the full 10 min to ensure

complete brain death. Cold shock was used as an alternative to concussion and immersion in anaesthesia owing to the fish's small size and to reduce the possibility of chemical contamination of the skin extract sample (i.e. residue anaesthetic solution altering the effectiveness of the chemical cue). An effective and clean blow to the head was near impossible owing to the fish's size, and a spike to the brain would cause issues with the release of internal fluids into the alarm cue. The chemical alarm cue is found in the skin cells and, as such, we wanted to limit the damage to the fish to being superficial. In a real-life context, this allows the release of the alarm cue, even if a fish is only superficially injured by a predatory attack. A clean scalpel blade was used to make seven superficial vertical incisions along each flank. Incisions damaged the skin but caused minimal flesh damage. Killed fish were rinsed in 15 ml of sea water, which was filtered through filter paper (125 mm diameter, qualitative 1) to remove any solid particles, and drawn into a disposable 60 ml syringe for injection into the experimental aquaria. Extracts were prepared in three concentrations, according to the number of equal-sized killed fish (12–14 mm SL) rinsed in the 15 ml of sea water: low (one fish), medium (two fish) and high concentration (four fish). All extracts were used within 20 min of preparation, as they have been shown to lose potency if frozen or stored for long periods (Smith 1989).

Individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 12 h. Flowing sea water and constant aeration were provided during this period, and fish were fed twice daily with newly hatched *Artemia* sp. Immediately prior to the trial period, the water flow was turned off and 10 ml of extra *Artemia* sp. was added to the aquaria to stimulate feeding. We first drew 60 ml of salt water out of the stimuli injection tubing using a disposable syringe, and discarded it to remove any stagnant water from within the line. An additional 60 ml of salt water was then drawn out and kept within the disposable syringe.

The behaviour of the single fish within each aquarium was first recorded for the 10 min prestimulus period. After 10 min, we injected 15 ml of the experimental stimulus (low concentration, medium concentration, high concentration or salt water control) into the tank via the injection tubing, immediately followed by the 60 ml of salt water previously removed from the aquarium. The behaviour of the fish was then recorded for the 10 min poststimulus period. Food colour trials showed that it took approximately 58 ± 4.2 s for the stimulus to disperse evenly throughout the aquarium.

Fifteen individuals were tested for each of the four chemical stimulus treatments, with all fish being tested for one cue only to maintain independence between trials.

No fish died during the experimental procedure of any of the three experiments.

Analysis

Changes in the seven prey behaviours were calculated between pre- and poststimulus observation periods. We compared these values between treatments within each experiment using a multivariate analysis of variance (MANOVA). Canonical discriminant analyses (CDAs) were performed for each experiment to determine how fishes differed between treatments with respect to changes in the seven behavioural traits. Trends in the behavioural variables are represented as vectors, which are plotted on the first two canonical axes, together with treatment centroids and their 95% confidence clouds (Seber 1984). The assumption of multivariate normality was examined before the analysis. To explore further the differences between treatments, we used one-way ANOVAs to identify significant differences within individual behaviours of interest. Bonferroni corrections were used to correct for multiple ANOVAs on

potentially interrelated variables within a single data set. Any differences were further examined using Tukey's honestly significant difference, HSD, means comparison tests. Data met the assumptions of normality and heterogeneity of variances, which were examined using residual analysis.

RESULTS

Experiment 1: Visual Stimulus

There was a significant difference in the overall change in behaviour between the three visual cue treatments (Pillai's Trace_{14,74} = 0.72, $P = 0.001$). The CDA shows this separation among treatments clearly, with most of the variation seemingly caused by differences in a combination of the number of bobs, the mean distance from shelter, mean height in the water column, the number of horizontal movements between zones, and the number of feeding strikes (Fig. 1). These five behavioural characters were further examined using ANOVAs, resulting in a revised alpha level of 0.01. Individuals bobbed more in response to the predator stimulus than to the nonpredator and control stimuli ($F_{2,42} = 6.456$, $P = 0.004$; Fig. 2b), while fish exposed to both the predator and nonpredator cues decreased their mean distance from shelter significantly more than those responding to the control stimulus ($F_{2,42} = 5.953$, $P = 0.005$; Fig. 2c). There was no significant difference between treatments with regard to the other three behaviours (mean height in water column: $F_{2,42} = 3.185$, $P = 0.052$; horizontal movements between zones: $F_{2,42} = 2.983$, $P = 0.062$; feeding strikes: $F_{2,42} = 2.21$, $P = 0.122$; Fig. 2).

Experiment 2: Changing Visual Stimulus Distance

There was a significant difference in the overall behavioural change between the four visual stimulus treatments (Pillai's

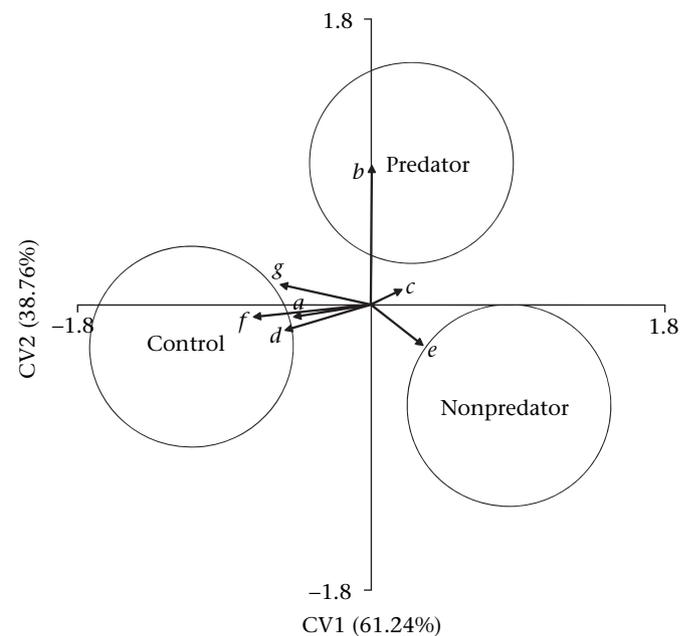


Figure 1. The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to visual predation cues. A canonical discriminant analysis compares the behavioural changes between pre- and poststimulus periods for the three visual cue treatments tested in experiment 1: predator stimulus, nonpredator stimulus and control (empty tank). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (a); bobs (b); movement in/out shelter (c); horizontal movement between zones (d); time in shelter (e); distance from shelter (f); height in water column (g). The circles represent 95% confidence intervals.

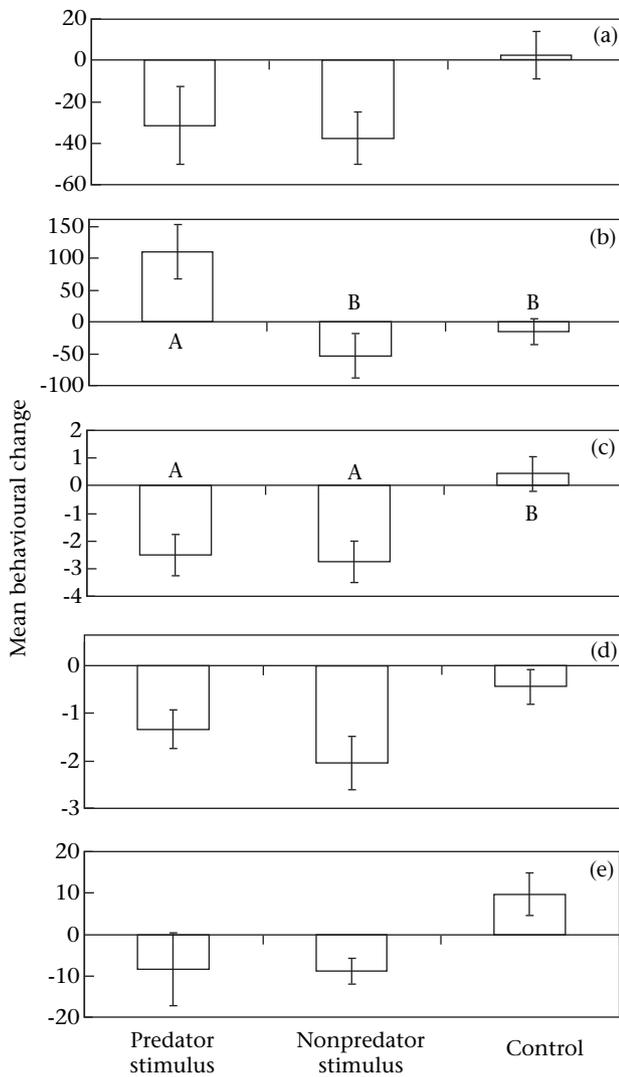


Figure 2. Mean change \pm SD in (a) feeding strikes, (b) bobs, (c) distance from shelter, (d) height in water column and (e) horizontal movement between zones, between pre- and poststimulus periods by newly metamorphosed *Pomacentrus amboinensis* for the three visual cue treatments tested in experiment 1: predator stimulus, nonpredator stimulus and control (empty tank). Post hoc tests are presented only for those behaviours where we found a significant difference between treatments. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

$\text{Trace}_{21,156} = 0.657$, $P = 0.006$). The CDA shows distinct separation between the three visual distance stimuli (minimum, medium and maximum; Fig. 3). However, the behavioural changes in response to the control stimulus are relatively similar to those observed in the maximum stimulus distance trials. Differentiation between groups appears to be largely a result of variation in the number of feeding strikes, the number of bobs, the mean distance from shelter and the number of horizontal movements between zones. These four behavioural characteristics were further examined using ANOVAs, resulting in a revised alpha level of 0.0125. This showed a significant difference in both the change in number of feeding strikes ($F_{3,56} = 4.289$, $P = 0.009$) and mean distance from shelter between treatments ($F_{3,56} = 4.100$, $P = 0.011$). The largest behavioural changes were observed in the minimum distance treatment, with a large decrease in both the number of feeding strikes and distance from shelter in response to the visual cue being recorded (Fig. 4). The magnitude of the change in feeding strikes decreased as the distance from the

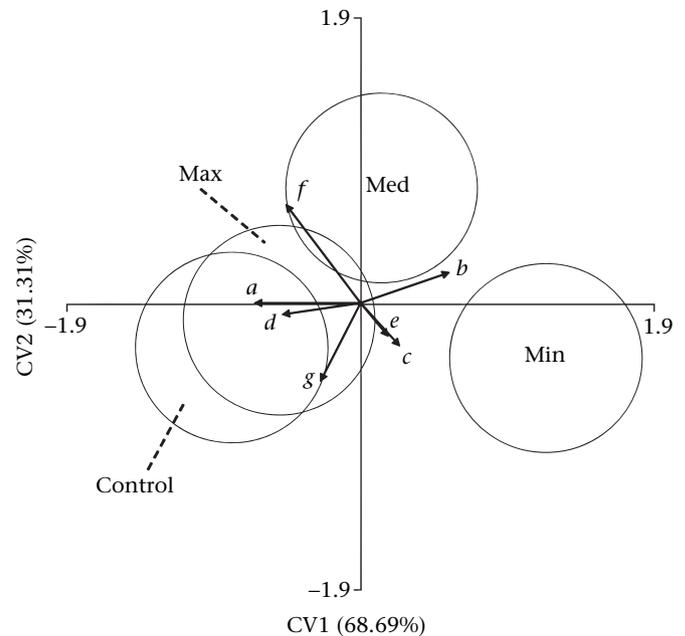


Figure 3. The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to visual predation cues over different distances. A canonical discriminant analysis compares the behavioural changes between pre- and poststimulus periods for the four visual cue treatments tested in experiment 2: minimum distance (Min), medium distance (Med), maximum distance (Max) and control (empty tank). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (a); bobs (b); movement in/out shelter (c); horizontal movement between zones (d); time in shelter (e); distance from shelter (f); height in water column (g). The circles represent 95% confidence intervals.

predator stimulus increased (Fig. 4a). In contrast, mean distance from shelter decreased significantly only for the minimum distance treatment (Fig. 4b). There was no statistical difference between treatments with regard to the change in either the number of bobs ($F_{3,56} = 3.110$, $P = 0.034$) or the number of horizontal movements between zones ($F_{3,56} = 2.579$, $P = 0.063$).

Experiment 3: Changing Chemical Alarm Concentration

There was no significant difference in the overall behavioural change between the four chemical stimulus treatments (Pillai's $\text{Trace}_{21,150} = 0.395$, $P = 0.372$). The CDA clearly shows this overlap in behaviour between the treatments (Fig. 5). Despite this lack of difference, an examination of the CDA showed one vector (i.e. change in the number of feeding strikes) to be responsible for a disproportionate amount of the overall variation. An ANOVA showed there to be a significant difference in the change in the number of feeding strikes between treatments ($F_{3,54} = 4.217$, $P = 0.009$). Those individuals exposed to the high concentration treatment had the largest decrease in the number of feeding strikes, with those exposed to the medium concentration treatment showing a slightly smaller decrease (Fig. 6). Individuals in both the low concentration and control treatments showed little change in response to the chemical cue.

DISCUSSION

Both olfaction and vision are thought to play important roles in threat detection in a wide range of systems, and have previously been demonstrated to operate in tandem in both freshwater (Mathis & Vincent 2000; Brown & Magnavacca 2003) and marine environments (McCormick & Manassa 2008). Their utility over other sensory

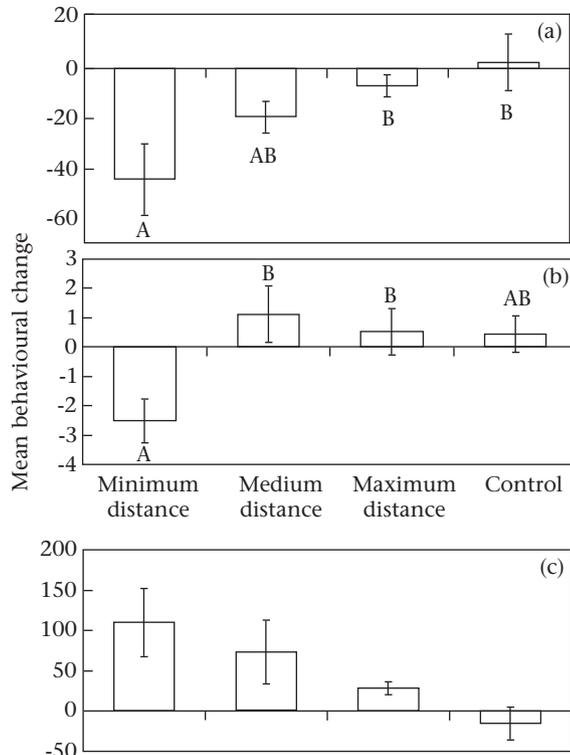


Figure 4. Mean change \pm SD in (a) feeding strikes, (b) distance from shelter and (c) bobs, between pre- and poststimulus periods by newly metamorphosed *Pomacentrus amboinensis* for the four visual cue treatments tested in experiment 2: minimum distance, medium distance, maximum distance and control (empty tank). Post hoc tests are presented only for those behaviours where we found a significant difference between treatments. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

systems in aquatic environments is largely a result of the aqueous medium in which they function, allowing, in particular, for the effective transmission of chemical cues over relatively large areas (Wisenden 2000). However, how each of these sensory cues may be used by animals transitioning to previously unknown habitats is largely unknown. Our results show that the reef fish, *P. amboinensis* was able to detect and respond to both visual and chemical predation cues during their settlement transition. For a subset of the behaviours recorded, the intensity of the behavioural responses to both sensory cues varied, with a closer or more concentrated cue eliciting a more intense response. The nature of the behavioural responses differed slightly between the two sensory cues, while individuals were not able to distinguish between predatory and nonpredatory visual cues while still naïve to the reef environment. This indicates that the mechanisms underlying predator avoidance and the learning of predator identity immediately following settlement (see Holmes & McCormick 2010b) may be a complex interaction between multiple sensory cues.

The nature of antipredator responses can differ greatly between systems, species and even life history stages (Ferrari et al. 2009). However, generally these responses are characterized by one or more of a decrease in foraging activity, a decrease in overall movement and an increase in shelter use (Stauffer & Semlitsch 1993; Chivers & Smith 1998; Griffiths et al. 1998). The behavioural response to conspecific chemical alarm cues across all concentrations in this study was characterized by a decrease in feeding rate only (although differing in intensity between treatments). Although this response is perhaps not as diverse as those found in other studies of adult marine fishes (see Smith & Smith

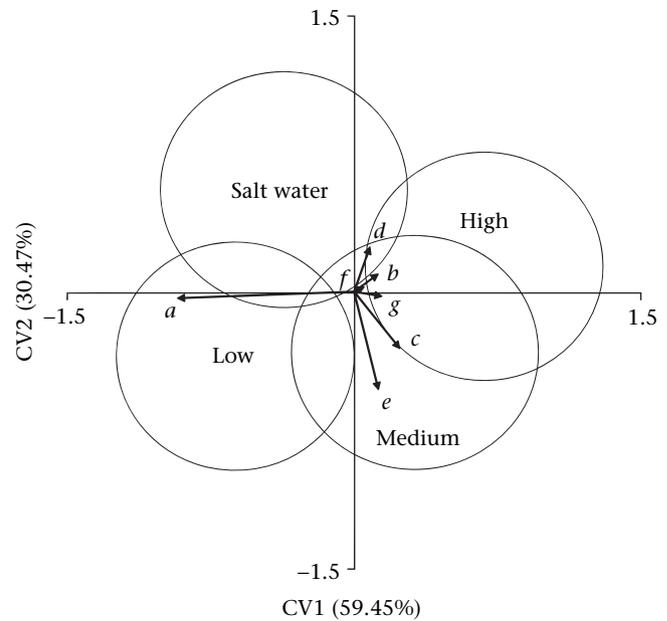


Figure 5. The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to different concentrations of conspecific chemical alarm cue. A canonical discriminant analysis compares the behavioural changes between pre- and poststimulus periods for the four chemical cue treatments tested in experiment 3: high concentration, medium concentration, low concentration and salt water control. Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (a); bobs (b); movement in/out shelter (c); horizontal movement between zones (d); time in shelter (e); distance from shelter (f); height in water column (g). The circles represent 95% confidence intervals.

1989; Larson & McCormick 2005; McCormick & Manassa 2008), it is consistent with the only other study examining the response to conspecific alarm cues during the early postsettlement period (Holmes & McCormick 2010b). This consistency in the nature of behavioural change both between experiments and between concentration levels in this study highlights the important role that this innate cue appears to play in the identification of relevant predation threats during this early transitional period.

Unlike the response to chemical cues, the response to visual cues involved multiple facets of behaviour and was somewhat inconsistent in effect between the two experiments. The response was most consistent with regard to the mean distance from shelter (decreased with exposure to visual cues), while significant changes

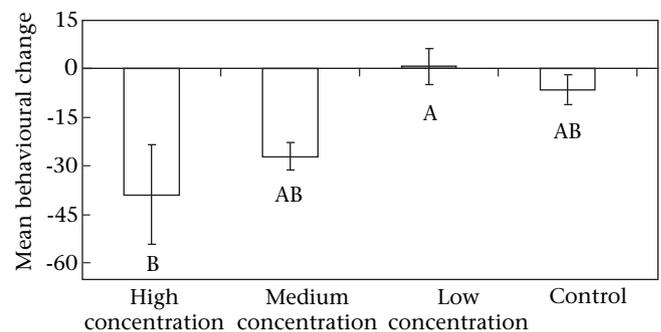


Figure 6. Mean change \pm SD in feeding strikes between pre- and poststimulus periods by newly metamorphosed *Pomacentrus amboinensis* for the four chemical cue treatments tested in experiment 3: high concentration, medium concentration, low concentration and salt water control. Post hoc tests are presented only for those behaviours where we found a significant difference between treatments. Alphabetic notations (A, B) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

in the number of bobs (increased with exposure to visual cues) and feeding rate (decreased with exposure to visual cues) were also observed in experiments 1 and 2, respectively. This inconsistency with regard to the number of bobs and feeding rate may in part be caused by insufficient replication in experiments, with Figs 2 and 4 suggesting a more consistent result might have been achieved with more replication. Overall, these responses are similar to those found by McCormick & Manassa (2008) in a recent study on the response of the marine goby, *Asteropteryx semipunctatus*, to visual and chemical cues. This diverse, yet variable response indicates two things: that visual cues may elicit a more diverse behavioural response because of the more immediate nature of a visual threat; and that there appears to be a degree of uncertainty as to the identity of relevant predation threats without prior knowledge of the reef environment, based on visual cues alone.

The uncertainty in the visual identification of relevant predators during the early postsettlement period is further highlighted by the inability of naïve individuals to differentiate effectively between predatory and nonpredatory fishes. While differentiation was apparent in the number of bobs, there was no difference in the mean distance from shelter in response to predatory (*P. fuscus*) and nonpredatory (*C. viridis*) cues. Similar patterns of nondifferentiation during transitional early life history stages have also been found by Mathis & Vincent (2000) and Kiesecker et al. (1996) during studies of predator recognition and avoidance in amphibian tadpoles. Given that a number of studies have demonstrated that later-stage aquatic organisms can visually distinguish between relevant and irrelevant threats (Gerlai 1993; Kelley & Magurran 2003; Ferris & Rudolf 2007; McCormick & Manassa 2008), this demonstrates that the visual identity of relevant predators is not innate and suggests that such knowledge must be acquired through experience in the new habitat.

Threat-sensitive responses to predator cues have been well documented among freshwater organisms (Mirza et al. 2006; Ferrari et al. 2007; Kesavaraju et al. 2007; Brown et al. 2009). This study presents a similar pattern of threat-sensitive behavioural responses to both visual and chemical predator cues with regard to feeding rate, for a newly settled reef fish. In this case, the behavioural response decreased in intensity with decreasing chemical cue concentration and increasing distance from the visual cue. However, there is also some evidence to suggest that threshold response levels may exist at the lower levels of these graded responses, with individuals showing no change in the distance from shelter in response to visual cues at either the medium or maximum distances. Additionally, there was little to no change in feeding rate at the lowest chemical alarm concentration, indicating that individuals either cannot detect or choose not to respond to this type of alarm cue at extremely low levels. Such a response mechanism where threat-sensitive responses possess a response threshold at the lower end has previously been described by Foam et al. (2005) in an assessment of the behavioural response of juvenile convict cichlids, *Amatitlania nigrofasciata*, to olfactory predation cues.

This is not the first example of a graded antipredator response in the marine environment. Helfman (1989) previously demonstrated a threat-sensitive response to visual predation cues in the three-spot damselfish, *Stegastes planifrons*, while Legault & Himmelman (1993) found multiple bivalve, gastropod and holothuroid species respond according to the level of threat posed by various predators. The novelty of our findings lies in the stage in development at which the response occurs in the study species. The early post-settlement period for coral reef fish is characterized by extremely high levels of predation. As such, there is significant pressure placed on individuals both to avoid predatory encounters directly and to maximize growth to decrease the amount of time vulnerable to gape-limited predators (Holmes & McCormick 2010a). Our results show that reef fish possess a well-developed sensory system

during this early period of physiological development, being able to use cues from multiple sensory sources to acquire information on local predation risk, and respond accordingly.

Although it is clear that naïve newly settled fish possess the ability to detect and react to predatory cues from multiple sources, the nature of both the responses and the sensory cues themselves suggest that independent of one another their utility may be limited. What may be most critical is the ability of naïve individuals to couple these cues together into knowledge that can be directly applied to the new system. In a study of threat-sensitive predator avoidance by slimy sculpins, *Cottus cognatus*, Chivers et al. (2001a, b) concluded that sensory cues might perform very different roles, with chemical cues functioning to warn that a predator is in the vicinity, and visual cues used to make an accurate assessment of the predation risk. Although the cues potentially have a similar function in coral reef fishes, it is also possible that the pairing of cues during this early period works to fine tune the visual identification of relevant predatory species. This theory is supported by McCormick & Manassa (2008), who found that *A. semipunctatus* had the ability to differentiate between predatory and nonpredatory visual cues independently during later life stages. In making this link, our study adds considerably to current information regarding the mechanisms of threat detection, and the role of predatory cues during transitional periods.

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