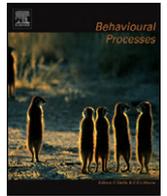




Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc



Smell, learn and live: The role of chemical alarm cues in predator learning during early life history in a marine fish

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ARTICLE INFO

Article history:

Received 10 July 2009

Received in revised form 1 January 2010

Accepted 24 January 2010

Keywords:

Chemical cue

Anti-predator behaviour

Recognition

Learning

Marine fish

ABSTRACT

The speed with which individuals can learn to identify and react appropriately to predation threats when transitioning to new life history stages and habitats will influence their survival. This study investigated the role of chemical alarm cues in both anti-predator responses and predator identification during a transitional period in a newly settled coral reef damselfish, *Pomacentrus amboinensis*. Individuals were tested for changes in seven behavioural traits in response to conspecific and heterospecific skin extracts. Additionally, we tested whether fish could learn to associate a previously novel chemical cue (i.e. simulated predator scent) with danger, after previously being exposed to a paired cue combining the conspecific skin extract with the novel scent. Fish exposed to conspecific skin extracts were found to significantly decrease their feeding rate whilst those exposed to heterospecific and control cues showed no change. Individuals were also able to associate a previously novel scent with danger after only a single previous exposure to the paired conspecific skin extract/novel scent cue. Our results indicate that chemical alarm cues play a large role in both threat detection and learned predator recognition during the early post-settlement period in coral reef fishes.

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1. Introduction

The ability of individuals to detect and respond to a perceived threat will have a large influence on who is lost from a population. This may be particularly important during encounters with potential predators, when the adoption of certain behaviours (e.g. decreased foraging, increase in shelter use) may significantly decrease vulnerability (Sih, 1986; Spieler and Linsenmair, 1999; Blumstein et al., 2002; Hunter et al., 2007). As such, the capacity to detect and react appropriately to a predation threat early in the predation process will presumably be advantageous to survival (Wisenden et al., 1999; Mirza and Chivers, 2002, 2003). The use of anti-predator behaviour by prey individuals can however be energetically costly, with time spent avoiding predation reducing the amount of time spent on foraging and reproductive activities (Sih, 1980; Lind and Cresswell, 2005; Fievet et al., 2008). From a fitness perspective, it is therefore also advantageous to be able to distinguish a 'possible' threat from an 'actual' threat, so as to reduce the time spent reacting to false alarms.

One mechanism for early detection and recognition of a predation threat is through the use of alarm cues (Sih, 1986; Martin and Lopez, 2005; McCormick and Manassa, 2008). In aquatic organisms, chemical cues produced by damaged individuals have been shown to provide an early warning to conspecifics of a predation threat (Chivers and Smith, 1998; Gonzalo et al., 2007; Wisenden, 2000; Mirza and Chivers, 2002). By recognizing such cues and adopting anti-predator behaviours, others in the general vicinity or within the group will greatly increase their chance of survival. One major limitation to this mechanism however, is that the production of the cue is secondary to an event having already occurred. If group sizes are small, this means that there is still a relatively high probability of being the target of the primary predation event. As a result, a more definitive survival advantage would be gained from the ability to detect and recognize a potential predator cue before the initial attack.

The use of alarm cues to facilitate learning of primary predator cues has been well documented in a range of different animal taxa (e.g. flatworms, Wisenden and Millard, 2001; insects, Wisenden et al., 1997; amphibians, Woody and Mathis, 1998; Gonzalo et al., 2007; mammals, Herzog and Hopf, 1984; and fish, Chivers et al., 1995). This is particularly so in the case of fishes, with conspecific alarm cues and predator scents known to play a role in teaching naïve prey the identity of potential predators (Magurran, 1989; Chivers and Smith, 1994, 1995; Brown et al., 1997; Mirza

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and Chivers, 2000; Smith et al., 2008). In making the direct connection between the predator cue and danger, prey are able to adopt anti-predator behaviours whenever the predator is within detectable distance, increasing an individual's probability of surviving an encounter. The importance of this learning process may be particularly heightened during periods of high mortality, when the speed with which the connection is made by prey may determine who survives (Spieler and Linsenmair, 1999).

In comparison to freshwater systems, we currently know very little about the use of chemical alarm cues amongst marine fishes. What little published work has been done has focused entirely on adult life stages (Smith and Smith, 1989; Smith, 2000; Larson and McCormick, 2005; McCormick and Larson, 2007; McCormick and Larson, 2008; McCormick and Manassa, 2008), with no attention being given to earlier or transitional life stages when predation pressures are significantly higher. The scope for the use of alarm cues in predator avoidance and learning processes is particularly high during the period immediately following settlement to the reef environment in coral reef fishes. During this stage, 'reef naïve' individuals are subjected to extremely high levels of predation (Doherty et al., 2004; Almany and Webster, 2006) and must rapidly learn to recognize and react appropriately to predation threats in a completely novel system. A recent study by McCormick and Holmes (2006) has shown that individuals are able to acquire knowledge from prior predation experiences during this period and translate this into a survival advantage during future predatory encounters. Such a mechanism of associating a stimulus with a biologically relevant behaviour is known as Releaser-Induced Recognition Learning (Sobuski, 1990; Sobuski et al., 1990). Although it is possible that the coupling of chemical alarm cues plays some role in this process due to the mechanism's prior documentation in later life stages, it is largely unknown at what stage in development the mechanism becomes functional (for exceptions see Alemadi and Wisenden, 2002; Carreau-Green et al., 2008; Mathis et al., 2008).

This study investigated the use of chemical alarm cues in the anti-predator response of a common coral reef damselfish, *Pomacentrus amboinensis*, during the period immediately following settlement. Using aquarium-based observation trials, we examined whether chemical cues released by damaged conspecifics and heterospecifics elicited an anti-predator response amongst individuals, and if so, how the behavioural response was characterized. Additionally, we examined whether conspecific alarm cues were a mechanism by which fish could learn the scent of novel predators. Due to the extreme predation pressures placed on fish during this life history period, and the ecological importance of a rapid learning mechanism, individuals were given a single exposure to a paired alarm/predator scent cue, before being retested with the predator scent alone the following day.

2. Materials and methods

2.1. 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 saltwater aquarium system at Lizard Island Research Station was used to conduct all experiments, while fish were collected from surrounding waters.

The common damselfish *P. amboinensis* was used 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. *P. amboinensis* has

a pelagic larval phase of between 15 and 23 days and settles at 10.3–15.1 mm standard length (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 and Doherty, 1994).

2.2. Fish collection

Settlement stage *P. amboinensis* were collected using light traps (see Meekan et al., 2001 for design) moored overnight close to the reef crest, and transported back to the Lizard Island Research Station at dawn. Fish were collected using this method so as to keep them naïve to all reef-based processes and influences. All fish were maintained in 25 L flow through aquaria systems for ~24 h, and fed newly hatched *Artemia* sp. twice per day ad libitum to allow for recovery from the stress of capture. Growth during this period was negligible.

2.3. Experimental aquaria

Experiments were conducted in transparent 12 L (320 × 230 × 200 mm) aquaria with flow through seawater. Two artificial branching *Acropora* corals (moulded resin; item no. 21505; Wardleys/TFH, Sydney; dimensions: 14 × 11.5 × 5 cm) 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 potential chemical cues 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 approximately 12L:12D photoperiod, with a constant flow of seawater until the commencement of trials. Observations were conducted from behind a black plastic barrier to avoid human disturbance.

2.4. Stimulus preparation

Potential conspecific chemical alarm cues were prepared using skin extracts taken from settlement stage *P. amboinensis* collected in light traps. Potential heterospecific alarm cues were prepared using skin extracts taken from adult *Apogon cyanosoma* (Apogonidae), collected using clove oil from surrounding reefs. All collected *A. cyanosoma* were maintained in aquaria for 24 h prior to experiments to remove any residue clove oil. This extract was used to test behavioural changes that may result from exposure to an extract of any injured fish. *A. cyanosoma* live in similar reef habitat to *P. amboinensis*, but are ecologically and phylogenetically distinct. The donor fish were sacrificed by cold shock and placed into a clean petri dish. A fresh scalpel blade was used to make seven superficial vertical incisions along each flank. Incisions damaged the skin but caused minimal flesh damage. Extracts were standardized by body area, meaning that two sacrificed *P. amboinensis* were rinsed in 15 mL of seawater for each conspecific extract, while a single *A. cyanosoma* was rinsed in the same amount of seawater for each apogonid extract. Extracts were then filtered through filter paper (125 mm Ø, qualitative 1) to remove any solid particles (e.g. scales), and drawn into a disposable 60 mL syringe for injection into the experimental aquaria. All extracts were used within 20 min of preparation, as they may lose potency if frozen or stored for long periods (Smith and Smith, 1989; Ferrari et al., 2007a, b).

Novel predator scents were prepared using adult dottybacks, *Pseudochromis fuscus*, collected from surrounding reefs. *P. fuscus* is a known predator of *P. amboinensis* once settled to the reef habitat.

However, at the point of capture in this study, *P. amboinensis* are yet to settle, and hence are assumed to be completely naïve to any cues or potential stimuli produced by *P. fuscus*. Immediately following collection, *P. fuscus* were placed into acclimation aquaria for 36 h so that any fecal matter could pass through the system and prevent contamination of the final stimulus. Individual fish (mean standard length $57.8 \text{ mm} \pm 5.6 \text{ SE}$) were subsequently placed into separate 6 L aquaria with fresh seawater for four days. The seawater was not changed during this period, but aquaria were provided with constant aeration. Fish were not fed throughout the entire process, again to prevent fecal matter from contaminating the final stimulus. At the end of this period, the water was drawn from the aquaria and frozen in 60 mL portions until used in experiments. At the end of the experimental period *P. fuscus* were fed ad libitum a commercial fish diet and released at the point of capture two days later.

2.5. Experiment 1 – Presence of conspecific alarm cue

The behavioural response of fish to three different stimulus treatments was tested: a conspecific skin extract, a heterospecific skin extract, and a saltwater control. The heterospecific extract was used to determine whether there were any generalized behavioural responses to any injured fish, while the saltwater stimulus allowed us to control for any behavioural changes resulting from the injection of any stimulus into the experimental aquaria. Trials consisted of a 10 min pre-stimulus and a 10 min post-stimulus observational period.

Individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 24 h. Flowing seawater 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. 60 mL of saltwater was slowly drawn out of the stimuli injection tubing using a disposable syringe and discarded to remove any stagnant water from within the line. An additional 60 mL of saltwater was drawn out and kept within the disposable syringe.

The behaviour of the single fish within each aquaria was first recorded for a 10 min pre-stimulus period. At completion, 15 mL of the experimental stimulus (conspecific skin extract, heterospecific skin extract or saltwater control) was injected into the tank via the injection tubing, immediately followed by the 60 mL of saltwater previously removed from the aquaria to flush the stimulus into the aquaria. The behaviour of the fish was then recorded for a further 10 min period. Dye trails (using food colouring) showed that it took a mean of 58 s (± 4.2 s) for the stimulus to disperse evenly throughout the aquaria. Behavioural observations were carried out by two observers sitting behind a black plastic barrier, so as to avoid disturbing fish during the trials.

A total of seven different behavioural traits were recorded for each fish during each observational period: (1) number of feeding strikes; (2) number of 'bobs'; (3) time spent in shelter; (4) number of movements into/out of shelter; (5) amount of horizontal movement outside of shelter; (6) distance from shelter; and (7) 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. 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, juvenile *P. amboinensis* undertake a bobbing behaviour, consisting of short, sharp vertical movements in the water column. It is possible the behaviour serves a purpose in either predator detection or avoidance and as such, it was recorded in this study.

Every 15 s the fish's horizontal distance from shelter and vertical position in the water column was recorded. If the fish was within

the confines of the branched shelter, its horizontal position was recorded as 0. From this data, measures of time spent within shelter, average distance from shelter and average vertical position within the water column were obtained. To obtain an estimate of space use, in the form of movement in and out of shelter and horizontal movement outside of shelter, the experimental aquaria was divided up into a series of horizontal zones. The first zone was set at the outside edge of the branching coral shelter, and they 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.

A new fish was used for each separate observation trial and water was changed in each aquaria after the completion of each trial. A total of 45 observation trials were conducted over the duration of the experiment, 15 from each of the three stimulus treatments.

2.6. Experiment 2 – Learning of a novel predator scent

The second experiment aimed to identify whether naïve fish were able to learn to associate a novel scent with danger, after previously being exposed to a potential alarm cue paired with the novel scent. This experiment used new fish collected in light traps and consisted of three separate observational periods conducted over three days. On the first day, fish were exposed to a single paired presentation of both a skin extract (fish were conditioned with either a conspecific or heterospecific stimulus treatment) and a novel predator scent (i.e. the *P. fuscus* tank water, not previously associated with danger). On the second day, fish from both condition treatments were exposed to the novel predator scent only, to determine whether they had learned to associate the cue with potential danger after a single exposure on the previous day. On the third day, fish were exposed to a saltwater control, to determine whether any behavioural changes were a result of a learned response to the injection process (Mathis and Smith, 1993).

As in Experiment 1, individual *P. amboinensis* were placed into experimental aquaria and left to acclimate for 24 h prior to the first trial day. The maintenance, feeding and experimental regime followed that of Experiment 1, with the same 10 min pre-stimulus and 10 min post-stimulus observation protocol used on each day. Seven behavioural traits (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) were recorded during these observation periods, as in the previous experiment.

Immediately prior to the observation period on each day, 60 mL of saltwater was drawn out of the stimuli injection tubing using a disposable syringe, and discarded to remove any stagnant water from within the line. An additional 60 mL of saltwater was drawn out and kept within the disposable syringe. The water flow was again turned off and 10 mL of extra *Artemia* sp. was added to the aquaria to stimulate feeding.

The chemical stimulus injected into the aquaria between the pre and post-stimulus observation periods, differed on each of the three observation days. During trials conducted on the first day, fish were exposed to one of the following pairs of stimuli: 15 mL conspecific skin extract (potential alarm cue) and 15 mL *P. fuscus* scent (novel cue) – "Conspecific-paired cue"; or 15 mL heterospecific skin extract (control for alarm cue) and 15 mL *P. fuscus* scent – "heterospecific-paired cue." On the second day of trials, fish from both the "conspecific-" and "heterospecific-paired cue" treatments were exposed to 15 mL of the predator scent only – "conspecific + predator cue" and "heterospecific + predator cue." During trials conducted on the third day, all fish were exposed to 15 mL of saltwater control only – "conspecific-control cue" and

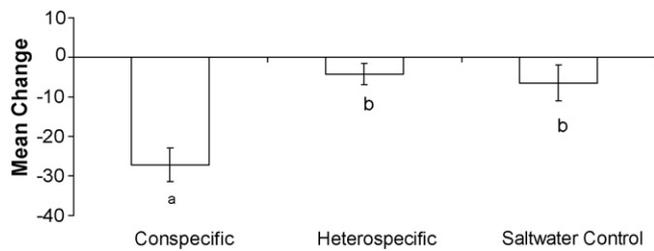


Fig. 1. The behavioural response of newly metamorphosed *Pomacentrus amboinensis* to conspecific alarm cues. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the three chemical cue treatments tested in Experiment 1: Conspecific skin extracts, heterospecific skin extracts and Saltwater Control. Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (a), bobs (b), movement in/out shelter (c), movement between zones (d), time in shelter (e), distance from shelter (f), position in water column (g). The circles represent 95% confidence intervals.

“heterospecific-control cue.” On each occasion, the injection of the stimulus was immediately followed by the injection of the 60 mL of saltwater previously retained, in order to flush the stimulus through the injection line. Between trial days all fish were fed twice daily on *Artemia* sp., and water flow was returned to all aquaria to ensure that all residue stimulus was flushed from the system.

The three day trial process was conducted on a total of 30 separate fish over the duration of the experiment, 15 from each of the two original conditioning treatments (i.e. conspecific and heterospecific).

2.7. Analyses

Changes in the seven prey behaviours were calculated between pre and post-stimulus observation periods. These values were compared between treatments within each experiment using a multivariate analysis of variance (MANOVA). Canonical discriminant analyses (CDA’s) 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 further explore the differences between treatments one-way ANOVA’s were used to identify significant differences within individual behaviours of interest. Any differences were further examined using Tukey’s HSD means comparison tests. Residual analysis was used to examine the assumptions of normality and heterogeneity of variances.

3. Results

3.1. Experiment 1 – Presence of conspecific alarm cue

There was a significant difference in the overall change in behaviour in response to the chemical stimulus among the three alarm cue treatments (conspecific, heterospecific and saltwater control; Pillai’s Trace_{14, 70} = 0.6531, *p* = 0.0078). The CDA visually shows a clear separation of the three treatments into two distinct groups with respect to the seven behavioural measurements, with the conspecific treatment being separate from the heterospecific and saltwater control treatments (Fig. 1). This differentiation is largely due to differences in the number of feeding strikes, with a suggestion that the number of movements in/out of shelter may also play a minor role. This suggestion was statistically confirmed by the results of the ANOVA’s, with exposure to the conspecific alarm cue causing a significantly greater change in the number of feeding strikes than the heterospecific and saltwater controls

Table 1
 Summary of one-way ANOVA’s comparing changes in behaviour for each of the three chemical cue treatments (conspecific, heterospecific and saltwater control) during Experiment 1. * denotes significance at *p* = 0.007 (incorporating Bonferroni correction).

Behaviour	Source	DF	MS	F	<i>p</i>
Feeding	Treatment	2	2275.954	10.839	0.0002*
	Error	40	209.97		
Bobs	Treatment	2	82.689	0.47	0.6284
	Error	42	176.006		
Movement (in/out shelter)	Treatment	2	115.467	1.983	0.1504
	Error	42	58.235		
Movement (between zones)	Treatment	2	80	0.5	0.6101
	Error	42	160		
Time in shelter	Treatment	2	25.956	0.803	0.4546
	Error	42	32.311		
Distance from shelter	Treatment	2	0.325	0.379	0.6868
	Error	42	0.858		
Height in water column	Treatment	2	0.177	0.171	0.8431
	Error	42	1.031		

(*F*_{2, 40} = 10.839, *p* = 0.0002; Table 1). The introduction of the conspecific skin extract caused a large reduction in the number of feeding strikes (mean 27.21 strikes 10 min⁻¹ less), whilst both the heterospecific and saltwater control cues caused only minor reductions (mean 4.27 and 6.5 strikes 10 min⁻¹ less, respectively; Fig. 2). There was however no significant difference in the change in number of movements in/out of shelter between treatments (*F*_{2, 42} = 1.983, *p* = 0.1504).

3.2. Experiment 2 – Learning of a novel predator scent

We found a significant difference between the overall pre and post-stimulus behaviour between the six chemical cue treatments (Pillai’s Trace_{35, 380} = 0.6175, *p* = 0.0307). The CDA shows the chemical cue treatments to be split into two groups with respect to the seven behavioural measurements, with the “conspecific-paired cue,” and the “Conspecific+Predator Cue” from the conspecific treatment separated from the other four (Fig. 3). This treatment separation indicates three important results. First, the introduction of conspecific skin extracts had changed the fish’s behaviour, with a reduction in activity. Second, that once fish had been exposed to the novel predator scent and a conspecific skin extract together, they reacted to the predator scent on its own with a similar reduc-

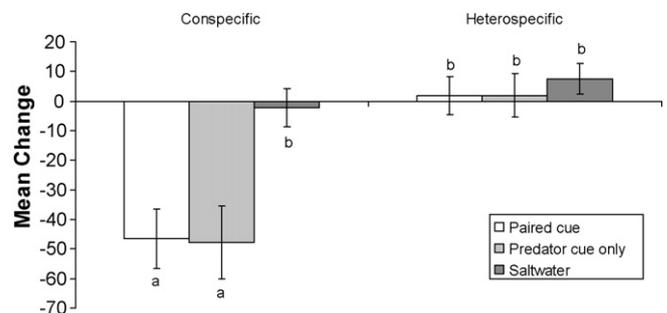


Fig. 2. Comparison of the mean change in the number of feeding strikes between pre and post-stimulus periods made by *Pomacentrus amboinensis* tested in each of the three chemical cue treatments (conspecific, heterospecific and saltwater control) in Experiment 1. Alphabetic notations (a and b) designate significantly different groups as determined by Tukey’s HSD means comparisons tests.

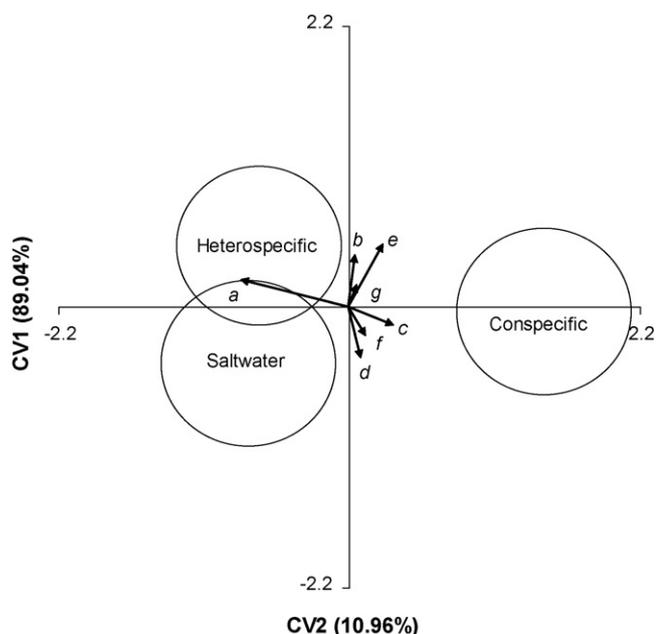


Fig. 3. Importance of chemical alarm cues to learning predator scents. A canonical discriminant analysis compares the behavioural changes between pre and post-stimulus periods for the six chemical cue treatments tested in Experiment 2: conspecific-paired (CPa: day 1 conspecific cue + predator scent), conspecific-predator (CPr: day 1 conspecific cue + predator scent, day 2 predator cue), conspecific-control (CC: day 1 conspecific cue + predator scent, day 2 predator cue, day 3 saltwater control), heterospecific-paired (HPa: day 1 heterospecific cue + predator scent), heterospecific-predator (HPPr: day 1 heterospecific cue + predator scent, day 2 predator cue) and heterospecific-control (HC: day 1 heterospecific cue + predator scent, day 2 predator cue, day 3 saltwater control) (see Section 2 detailed description). Vectors represent the direction and intensity of trends in the prey behaviours: feeding strikes (a), bobs (b), movement in/out shelter (c), movement between zones (d), time in shelter (e), distance from shelter (f), position in water column (g). The circles represent 95% confidence intervals.

tion in activity. Third, that fish exposed to the novel predator scent without being conditioned with the conspecific skin extract did not change their behaviour. The differentiation between groups again appears to be largely due to differences in the number of feeding strikes, although the time spent in shelter also appears to play a minor role. The ANOVA's again confirm this suggestion, with a significant difference being shown in the change in number of feeding strikes between treatments ($F_{5, 84} = 10.572, p < 0.0001$; Table 2). In this case, individuals used in both the 'conspecific-paired cue' and the 'conspecific-predator cue' trials from the conspecific treatment had significantly fewer feeding strikes after being exposed to the stimulus (mean 46.53 and 47.8 strikes 10 min^{-1} less, respectively), than those used in the other four treatments (Fig. 4). Changes in the number of feeding strikes in response to the 'saltwater cue,' from the conspecific treatment, and the heterospecific 'paired cue,' 'predator scent only cue' and 'saltwater cue' treatments were negligible. There was no significant difference in the change in time spent in shelter among treatments ($F_{5, 81} = 0.92, p = 0.4722$).

4. Discussion

The ability to recognize and alter behaviour in response to a predation threat will greatly increase an individual's probability of survival (Sih, 1986; Brown and Laland, 2003). Although anti-predator responses can vary between groups, species and ecosystems, they typically involve decreases in foraging rate and space use, grouping/schooling behaviour, and/or an increase in shelter usage (Stauffer and Semlitsch, 1993; Chivers and Smith, 1998; Griffiths et al., 1998). The results of this study show that

Table 2
 Summary of one-way ANOVA's comparing changes in behaviour for each of the six chemical cue treatments (conspecific-paired, conspecific-predator, conspecific-control, heterospecific-paired, heterospecific-predator and heterospecific-control; see Section 2 for detailed description) during Experiment 2. * denotes significance at $p = 0.007$ (incorporating Bonferroni correction).

Behaviour	Source	DF	MS	F	p
Feeding	Treatment	5	10244.95	10.572	<0.0001*
	Error	84	969.06		
Bobs	Treatment	5	12046.1	1.031	0.4053
	Error	78	11682.3		
Movement (in/out shelter)	Treatment	5	223.842	1.663	0.1529
	Error	81	134.567		
Movement (between zones)	Treatment	5	1842.122	1.455	0.2136
	Error	81	1265.658		
Time in shelter	Treatment	5	57.341	0.92	0.4722
	Error	81	62.308		
Distance from shelter	Treatment	5	3.647	1.408	0.2301
	Error	81	2.59		
Height in water column	Treatment	5	2.483	1.426	0.2236
	Error	81	1.74		

the newly settled reef fish *P. amboinensis*, significantly alters its behaviour in response to a conspecific chemical alarm cue, by decreasing its feeding rate. In addition, individuals were able to learn and respond to a previously novel predator cue by pairing it with the conspecific alarm cue. After only a single exposure to the novel predator cue paired with a conspecific cue, fish showed a behavioural change consistent with the first experiment (reduction in feeding rate) when retested with the predator cue only. The lack of behavioural change in response to the novel predator scent during the heterospecific treatments indicate that fish had no

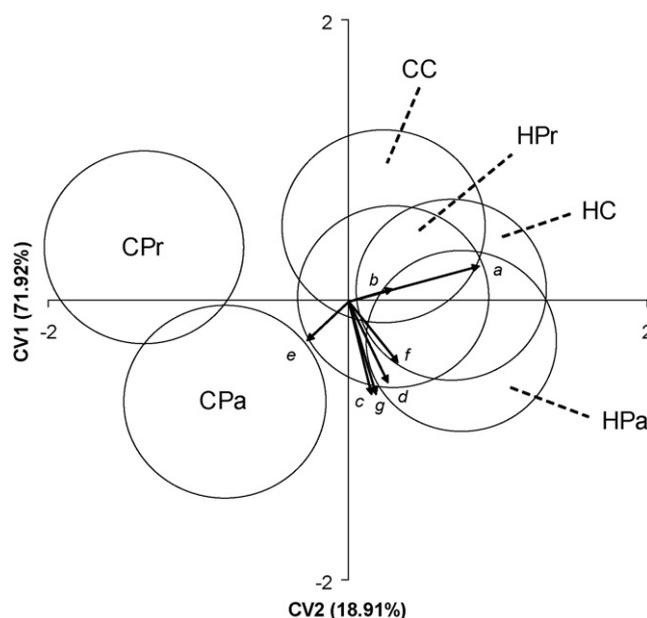


Fig. 4. Mean change in the number of feeding strikes between pre and post-stimulus periods made by *Pomacentrus amboinensis* tested in each of the six chemical cue treatments (conspecific-paired, conspecific-predator, conspecific-control, heterospecific-paired, heterospecific-predator and heterospecific-control) in Experiment 2. Alphabetic notations (a and b) designate significantly different groups as determined by Tukey's HSD means comparisons tests.

innate response to the novel scent on its own. Our findings show that alarm cues may play a key role in the learning process of naïve recruits during the hazardous early post-settlement period in coral reef fishes.

A recent study found that experience acquired through exposure to predation during the early post-settlement period significantly increased an individual's probability of survival in the field (McCormick and Holmes, 2006). The results of the current study suggest that this process of acquired learning may be largely due to the pairing of conspecific alarm cues with a predator scent. The pairing of this cue potentially allows prey to identify when a predator is in the general vicinity, through olfactory reception, and initiate anti-predator behaviours at an earlier stage in the predation process. Although this relationship has been identified during the larval stage in amphibians (Kiesecker et al., 1996; Gonzalo et al., 2007), as well as in later life stages in a number of freshwater (e.g. Brown et al., 1997; Mirza and Chivers, 2000; Smith et al., 2008) and one marine fish (Larson and McCormick, 2005), this is the first time that it has been identified at such an early and critical life stage in the marine environment.

The presence of this learning mechanism during this early juvenile stage is of particular significance for a number of reasons. The period immediately after settlement from the larval life stage is characterized by extremely high levels of mortality for organisms with complex life histories, with estimates of mortality of over 56% within the first two days (Gosselin and Qian, 1997; Almany and Webster, 2006). For coral reef fishes, much of this mortality is caused by small site attached fish predators (Carr and Hixon, 1995; Holbrook and Schmitt, 2002; McCormick and Meekan, 2007). The speed with which individuals are able to acquire knowledge of these predators, and react accordingly, will have a large influence on who is lost and who persists within a population (Wisenden et al., 1997; Brown and Chivers, 2006). Our study indicates that newly settled fish are able to make this link after a single exposure to the paired chemical cue, suggesting that this mechanism provides an extremely effective method of knowledge acquisition during this early period. The fact that this knowledge is acquired, rather than hereditary, is also advantageous, as it allows individuals to mould their anti-predator responses to the particular suite of predators around them. Given that many coral reef fish possess a highly dispersive pelagic larval stage, and that the composition of predatory communities can differ greatly over both local and regional scales (Holmes and McCormick, 2006), the predator community into which individuals of the same species settle has the potential to differ considerably. The ability to adapt to variation in predation threat through a single exposure to the predator cue, rather than be constrained by genetically inherited anti-predator responses, would be advantageous to both fitness and survival (Brown and Chivers, 2006).

The response of newly settled *P. amboinensis* to conspecific skin extracts involved a substantial and significant reduction in feeding rate. However, it did not affect shelter use or movement patterns, two other behavioural changes commonly linked to anti-predator responses. For example, in a study of the behaviour of western toad (*Bufo boreas*) tadpoles, Kiesecker et al. (1996) found a reduction in activity and an increase in shelter use in response to olfactory predation cues. Similarly, Hirvonen et al. (2007) noted a decrease in activity and increase in shelter use by the noble crayfish (*Astacus astacus*) when in the presence of the odour from the predatory European eel (*Anguilla anguilla*). This lack of change with respect to these other behaviours in our study may be related to the newly metamorphosed state of the fish that are not only naïve to reef-based predators, but also to their benthic environment. It is perhaps not surprising therefore that newly metamorphosed *P. amboinensis* do not show significant retreat to shelter within a foreign and novel 3-dimensional environment.

There will be strong selective pressure on settling larvae of all organisms to learn to identify and avoid the predators in their new environment; individuals who survive initial, unsuccessful predation attempts and learn from this experience will have a survival advantage (Kristensen and Closs, 2004; McCormick and Holmes, 2006). Detailed field observations on the ontogenetic behavioural shifts that occur immediately around settlement have shown that many species go through a transition period of hours to weeks while they adopt behaviours displayed by juveniles (McCormick and Makey, 1997). More extensive anti-predator responses are predicted to develop with greater experience with the benthic habitat and community. In an investigation of variation in the response of fathead minnow (*Pimephales promelas*) to alarm cues, Pollock et al. (2006) found behavioural reactions to differ with ontogeny. When exposed to the alarm cue, larger/older individuals exhibited significantly increased anti-predator responses in comparison to smaller/younger minnows during the non-breeding season. This same relationship may be present in the marine system, with younger newly settled individuals displaying a weaker anti-predator response in comparison to older individuals of the same species. As experience of predatory events increases with age, so to will the knowledge of appropriate anti-predator behaviours.

Within aquatic systems, chemical cues have been shown to play a large role in both knowledge acquisition and knowledge transfer amongst individuals within close social groups (e.g. social learning; Brown and Laland, 2003; Ferrari et al., 2007a, b). Given the rapidity and efficiency with which knowledge of a predation threat was acquired by individuals through chemical means in this study, it is reasonable to suggest that chemical signals may play a wider role within the community ecology of newly settled fish outside of this function (e.g. social learning, habitat selection, foraging). However, to date this remains largely unstudied in marine systems. Our study adds significantly to our current understanding and provides an insight into the mechanisms of knowledge acquisition and predator recognition during a high mortality period in marine fishes.

Acknowledgements

We wish to thank J. Moore, A. Villacorta Rath and S. De Jong for their assistance in the field. Comments made by S. Wilson and D. Dixon on the manuscript were greatly appreciated. The staff of the Lizard Island Research Station (a facility of the Australian Museum) provided logistical support. This project was funded through grants supplied by the Australian Research Council Centre of Excellence for Coral Reef Studies and Lizard Island Doctoral Fellowship program. Research was conducted under the JCU animal ethics guidelines.

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