



## Chemical alarm cues inform prey of predation threat: the importance of ontogeny and concentration in a coral reef fish

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Prey that respond to inappropriate cues in their assessment of predation risk spend more time performing defensive behaviours and less time undertaking behaviours that promote fitness. Hence, prey should respond to cues that are the best predictors of predation risk relevant to the prey individual. Many fish undergo ontogenetic shifts in habitat and resource use during their lifetime; consequently, prey fish are exposed to a variety of predators at different stages of their development. Also, as relative concentration of the alarm cue represents both spatial and temporal information about a predation event, prey should adjust the intensity of their antipredator response in a threat-sensitive manner. We found that the ambon damselfish, *Pomacentrus amboinensis*, exhibited a threat-sensitive response to damage-released cues of conspecifics, with the magnitude of the response dependent on the cue concentration. Higher cue concentrations (suggesting a greater threat) elicited a stronger avoidance response, while low cue concentrations (weak threats) elicited a weak antipredator response. Moreover, the nature of the response depended on the ontogenetic stage of the cue donor. Reef-naïve recruit individuals exhibited consistent antipredator behaviours when exposed to alarm cue concentrations from individuals of the same ontogenetic stage, modest antipredator responses to juvenile skin extracts, and no antipredator behaviours when exposed to cues from adult fish. Individuals that alter their avoidance response in a manner that reflects the magnitude of risk successfully balance the trade-off between defensive and risky or fitness-promoting behaviours, and natural selection would presumably favour these individuals.

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In aquatic environments where vision is often impaired by complex topography or turbidity, organisms frequently rely on waterborne chemicals to assess the risk of predation (Alemadi & Wisenden 2002). When captured by predators many organisms involuntarily release a chemical signal from their damaged epidermis, which is known to contain a compound that serves an alarm function. The release of an alarm cue from the wounded animal betrays the presence of the predator to surrounding conspecifics (Chivers & Smith 1998; Brown 2003; Mirza & Chivers 2003a). Upon perceiving this chemical alarm cue, nearby conspecifics, and in some instances heterospecifics, display species-specific antipredator behaviours (including dashing, freezing, hiding, schooling) that result in increased survivorship (Brown 2003; Brown & Chivers 2006).

However, there is a threat-sensitive trade-off between antipredator behaviours and foraging benefits (Helfman 1989). During

their lifetime, prey encounter a diverse array of potential predators that have to be discriminated from nonpredators. Prey that responded to every single alarm cue would find themselves always hiding and seldom foraging. The threat-sensitive predator avoidance hypothesis (Helfman 1989) suggests that the most successful individuals will be those that recognize the situations that are associated with high risk. Individuals that accurately adjust their response to the level of threat present, and best balance predator evasion with other fitness-enhancing activities, such as foraging and mating, will get the most possible out of risk assessment and have the highest fitness (Helfman 1989; Wisenden 2003).

Since predators are gape limited and theoretically have dome-shaped size-selection profiles, body size of prey is an important factor that influences the outcome of predator–prey interactions (Rice et al. 1997; Holmes & McCormick 2010). Where a prey organism spans a large size range during ontogeny, there will be a corresponding change in the predator assemblage that can successfully capture and ingest it. Many fish, for instance, increase in size by several orders of magnitude from hatched larvae to adulthood (Werner & Gilliam 1984), which changes a predator's ability to ingest them. When a juvenile grows into an adult, it will be exposed to a new assemblage of predators as it outgrows the

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previous ones; thus it may be counterproductive for juveniles to respond to alarm cues from adults and vice versa.

Previous work on both freshwater and marine communities have demonstrated that the most relevant alarm cues will be those that originate from individuals that are of the same size (Brown et al. 2002; Golub & Brown 2003; Marcus & Brown 2003; Blanchet et al. 2007). For example, Mirza & Chivers (2002) found that small brook char, *Salvelinus fontinalis*, could differentiate alarm cues that were produced by small and large conspecifics, responding more intensely to cues from conspecifics of the same age and size. The study by Mirza & Chivers (2002) suggests that chemical alarm cues, although similar, may change slightly through ontogeny. Contrary to this finding, Carreau-Green et al. (2008) demonstrated that adult fathead minnows, *Pimephales promelas*, exhibited an antipredator response when exposed to chemical alarm cues made from 8 to 17-day-old larvae. At present, little is known about the importance of ontogenetic state to risk assessment behaviours in marine fishes.

Threats are context dependent and the relative risk of each situation needs to be correctly assessed by prey. This risk not only varies over the life of a prey, but also as a predatory attack escalates (Lima & Dill 1990). Predator–prey encounters typically follow a sequence of events in which the prey is detected by a predator, identified, attacked, captured and consumed (Lima & Dill 1990). As the sequence progresses, the risk of the prey being successfully captured and consumed by a predator increases; thus progressively stronger threats should yield progressively stronger antipredator responses (Helfman 1989; Brown et al. 2006). Prey close to another individual that is damaged or captured prey should be exposed to a high concentration of chemical alarm cues, indicating a situation of high risk. Low concentrations either suggest that the predation event occurred further away (as cue concentration decreases with distance) or took place a longer time ago (as alarm cues degrade with time; Brown et al. 2001a,b; Marcus & Brown 2003; Mirza & Chivers 2003b). Similarly, several damaged prey should emit higher concentrations of chemical alarm cues than only one wounded individual, indicating a situation of higher risk (Ferrari et al. 2006). Prey capable of discriminating between low and high risk situations will gain an advantage as they spend more time undertaking fitness-promoting activities, and so be more successful.

The present study examined whether the ontogenetic stage of the alarm cue donor affected the antipredator response of a newly settled damselfish, *Pomacentrus amboinensis*. Moreover, we explored whether individuals adjusted the intensity of their antipredator response to damage-released cues of conspecifics depending on the cue concentration. We predicted that there has been strong selection pressure for prey to respond to cues that best indicate the activity of predators relevant to the ontogenetic stage of the prey. We also predicted that fish would be sensitive to the magnitude of threat and modify the intensity of their response accordingly; higher cue concentrations (i.e. depending on number of cue donors used) would elicit more intense antipredator behaviours.

## METHODS

### Study Species

*Pomacentrus amboinensis* is a common damselfish on Indo-Pacific coral reefs. This species is demersal with a bipartite life history, their pelagic larval phase lasting between 15 and 23 days (Kerrigan 1996). Newly settled fish are 10.3–15.1 mm long (standard length, SL) while adults are significantly larger (50.2–71.3 mm SL; Kerrigan 1996). *Pomacentrus amboinensis* inhabits small patch reefs in sandy areas at the periphery of larger adjacent reefs (Jones

1997) and there is little movement among habitats over its lifetime (McCormick & Makey 1997). Adult *P. amboinensis* are broadly omnivorous (mainly feeding on algae, but also zooplankton), while the juveniles are diurnal planktivores (Kerrigan 1996). The larvae settle onto a range of patch reefs in both shallow and deeper waters (Jones 1997). All *P. amboinensis* used in this experiment were caught around the coral reefs at Lizard Island, northern Australia (14°40'S, 145°28'E). Newly settling *P. amboinensis* were collected with light traps (for design see Meekan et al. 2001) that had been moored overnight above sand away from the reef, while juvenile and adult *P. amboinensis* were collected using a clove oil–ethanol–sea water solution (as an anaesthetic) and hand nets. Fish were transported to the research station in aerated sea water in covered containers to minimize stress. Once at the Lizard Island research station fish were maintained overnight in optimum conditions in the station's flow-through salt water aquaria (12:12 h light:dark period) at 26.5–27.2 °C. Following the initial acclimation period, test fish were transferred to experimental aquaria where they were allowed to acclimate for 24 h before trials began.

All holding tanks were kept inside and covered and had numerous shelters of various sizes on the bottom of the tanks. Adult fish were placed into 60-litre flow-through holding tanks (eight fish per tank) and fed twice daily with commercial fish food pellets, while juvenile *P. amboinensis* were placed in 30-litre flow-through holding tanks (12 fish per tank), and fed twice daily with a mixture of live brine shrimp (*Artemia* sp.) and commercial fish food pellets. Newly settling *P. amboinensis* were placed into 30-litre flow-through sea water aquaria (25 individuals per tank) and fed 36–48 h-old *Artemia* sp. nauplii (1600 nauplii/litre), twice daily.

All research was approved by the James Cook University Ethics Committee and collections were undertaken with a permit from the Great Barrier Reef Marine Park Authority. After the study, the remaining fish were released at the site of capture.

### Stimulus Preparation and Concentration Gradients

While it is known that the chemical that causes the alarm cue response in coral reef fish is located in the epidermis, the chemical identity is presently unknown, as it is for almost all organisms that have been found to have a chemical alarm cue response (Ferrari et al. 2010). Recruits were exposed to chemical alarm cues from three different ontogenetic stages (recruits, juveniles and adults) and three concentrations (low, medium and high) within each stage. To standardize the amount of stimulus cue from the three ontogenetic stages of cue donors, alarm cue concentrations were equated to skin surface area of fish and thus the number of donors varied depending on what concentration was chosen. The skin surface area of an average recruit  $\pm$  SE is  $66.2 \pm 6.6$  mm<sup>2</sup>. We chose skin extract donors from each ontogenetic stage of a similar size.

### Recruit alarm cue preparation

We used one recruit fish for the lowest alarm cue concentration (low, ca. 66 mm<sup>2</sup> skin surface area). To test for an intermediate effect of the cue concentration on the strength of the antipredator response of fish we used two recruit *P. amboinensis* per alarm cue (medium, 132 mm<sup>2</sup>). To exaggerate the cue concentration and examine whether there was a threshold above which there was no increase in antipredator behaviour, we used four skin extract donors per cue (high, 264 mm<sup>2</sup>). Recruits were killed using cold shock (in accordance with the guidelines set by Lizard Island Research Station for fish of this size). With this method individuals are placed for 20 s in a sea water ice slurry (it causes almost instantaneous death) as this is the quickest way of killing fish of this minute size without damaging their bodies. The flank of each recruit was superficially cut six times. The total cue area was rinsed

with 15 ml of salt water and filtered to remove any solid matter (e.g. scales) prior to being used in the experiment. In total, we used 140 recruit fish to prepare skin extracts for the different treatments.

#### *Juvenile alarm cue preparation*

In addition to testing fish for a concentration gradient we wanted to examine how the ontogenetic stage of cue donors influences the response of recipients. We collected skin extracts from a total of 35 juveniles and calculated the amount of cue according to the surface area of juvenile skin (mean  $\pm$  SE total skin surface area =  $506.1 \pm 25.1$  mm<sup>2</sup>). Preparing the skin extracts in this way ensured that the relative concentration (cm<sup>2</sup> of skin per ml water) was equivalent to each recruit's sized unit of skin depending on the 'concentration' (low, medium and high) regardless of donor size. Juvenile fish were humanely killed by a single blow to the head in accordance with the guidelines set by James Cook University. After their immediate death the appropriate amount of skin (depending on the cue gradient) was superficially cut and rinsed in 15 ml of salt water.

#### *Adult alarm cue preparation*

As mentioned above, the concentration of the adult cue was calculated according to the surface area of fish (surface area =  $1037.9 \pm 74.4$  mm<sup>2</sup>). Adults were killed by a quick blow to the head to ensure death and several superficial incisions (corresponding to the recruit's sized skin surface area) were cut down each flank; fish were then rinsed in 15 ml of salt water equivalent to each cue concentration (low, medium and high). In total, 20 adult *P. amboinensis* were collected, euthanized, prepared and rinsed through filter paper (to remove any remaining tissue). We could not use fewer fish because of the requirement of preparing skin extracts within 40 min of the start of the trial, as extracts may lose potency if frozen or stored for longer periods of time (Smith 1989).

#### *Observation Tanks*

All experiments and behavioural observations were conducted in transparent 28-litre aquaria (30  $\times$  40 cm and 25 cm deep) with a constant flow of sea water (0.4 litres/min) until the commencement of trials. A single air stone was placed at the back corner of each tank with a plastic tube 1.5 m long (for stimulus injection) fixed to the airline with the end attached approximately 1 cm above the air stone allowing for rapid dispersal of extracts in the aquaria. Artificial branching corals were placed along the opposite side of the aquaria creating vertical shelters ca. 12 cm high. The substratum consisted of a layer of sand 2 cm thick. Dye trials showed that it took approximately  $57.2 \pm 2.9$  s (mean  $\pm$  SE) for the extract to disperse through the tank after the injection.

#### *Experimental Procedure*

Prior to experimentation, test fish (one per tank) were acclimated in the test aquaria for 24 h to allow for recovery from the stress of capture. To minimize the effect the observer had on the test fish, observations were conducted from behind a black plastic barrier. Before a trial, the water flow was stopped and 20 ml (ca. 2640 nauplii) of extra *Artemia* sp. was added to the aquaria to stimulate feeding. After food had been injected, test fish were left to feed for 2 min before the experiment started, to avoid biasing the data, as fish will feed more when food is first introduced. To remove any stagnant water from within the stimulus injection tube, 60 ml of water was drawn out and discarded. An additional 60 ml of salt water was drawn out and kept within a disposable syringe.

Fish were observed from the long side of the test aquaria. The behaviour of a single *P. amboinensis* was recorded for the 10 min prestimulus period. Immediately following the prestimulus period, 15 ml of the experimental stimulus was injected into the tank, followed by 60 ml of the previously obtained salt water (to ensure that the stimulus had been flushed into the tank). The behaviour of the fish was then recorded for a further 10 min. For each stimulus we conducted 20 replicates.

The behavioural response of naïve recruit *P. amboinensis* to one of three concentrations (low, medium and high) from three ontogenetic stages (recruit, juvenile and adult) was tested, with 20 replicates per treatment. In addition, two control treatments were conducted: skin extracts from a heterospecific fish (*Apogon cyanosoma*; Apogonidae), and a salt water 'blank' control. *Apogon cyanosoma* were used as a control for the effect of adding the scent of any damaged fish into the treatment tank. This species was chosen as they are ecologically very different from the focal species, phylogenetically distant, and it was unlikely that *P. amboinensis* would innately respond with an antipredator response to the chemical release from the apogonid epidermis. Owing to their large size (surface area of  $123.7 \pm 9.3$  mm<sup>2</sup>, mean  $\pm$  SE) one apogonid was used for each alarm cue (in total 20 individuals were used to prepare skin extracts).

#### *Behavioural Assay*

The behavioural response to experimental stimuli was quantified by recording a total of four different behavioural traits for each fish during the trials: number of feeding strikes, time spent in shelter, number of movements in and out of shelter, and distance from shelter. Feeding strikes were recorded as the total number of strikes (successful or unsuccessful) made by the individual during each 10 min observation period. At 15 s intervals, the fish's horizontal and vertical distance from shelter was recorded. Test aquaria had been divided up into a series of zones, both vertical and horizontal using black lines on the side of the tank from which observations were taken. The first zone was set at the outside edge of the branching coral shelter, and the zones continued at 50 mm intervals away from shelter, giving a total of eight horizontal and four vertical zones. This allowed the calculation of the average distance from shelter. Whenever a test fish moved in and out of shelter it was recorded and the total amount of time the test fish spent in shelter was determined during each observation period.

#### *Statistical Analysis*

We calculated changes in the four behaviours between the 10 min pre- and poststimulus periods. A one-factor multivariate analysis of variance (MANOVA) was carried out to compare differences in recruit behaviour between the 11 stimulus treatments (recruit, juvenile and adult skin extracts all at three concentrations, plus a heterospecific and salt water control). A two-factor analysis (ontogeny and concentration) was not undertaken because of the complicating addition of two control treatments. One-factor ANOVAs were then performed to identify significant differences within individual behaviours. A Bonferroni correction was employed to account for nonindependent tests (adjusted alpha = 0.0125), and significance was interpreted in relation to this adjusted significance level. Significant differences were then examined using Tukey's honestly significant difference (HSD) means comparison tests. The assumptions of normality and homogeneity of variance were examined and met prior to carrying out MANOVA and ANOVA.

## RESULTS

There was an overall difference in recruit behaviour between the concentration, ontogenetic stage and control treatments (MANOVA: Pillai's = 0.86,  $df = 40, 836$ ,  $P < 0.001$ ). Univariate ANOVAs also revealed significant differences in behavioural responses between treatments for all variables (Table 1).

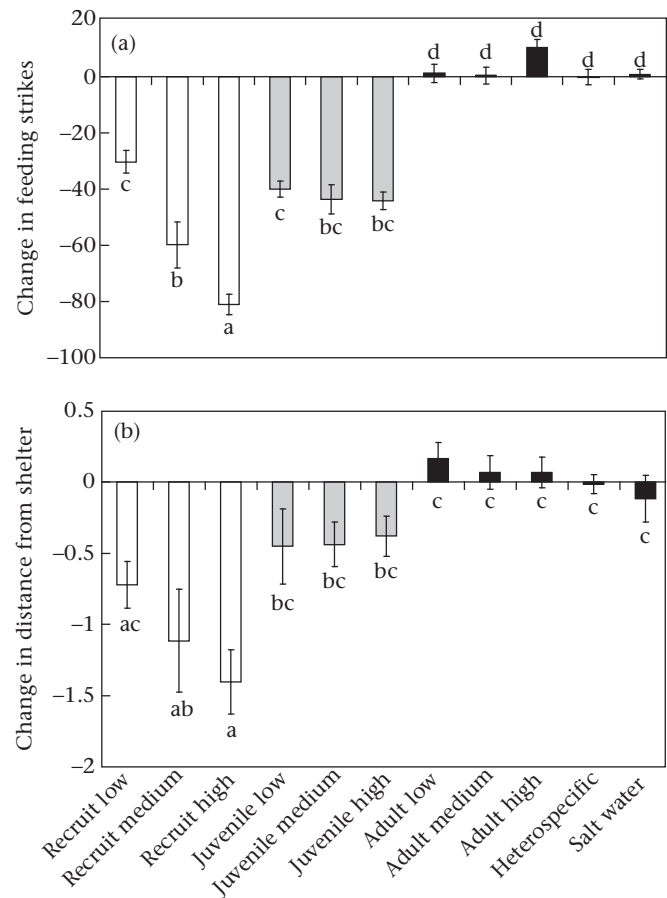
Recruit *P. amboinensis* exhibited a significant increase in anti-predator behaviour when exposed to the two higher concentrations of conspecific skin extracts from individuals of the same ontogenetic stage in all the behavioural traits (Tukey's HSD tests:  $P < 0.005$ ; Figs 1, 2): specifically, a decrease in feeding strikes and distance from shelter (Fig. 1a, b) and an increase in shelter use and time spent in shelter (Fig. 2a, b). They responded with a graded response to the different concentrations of recruit *P. amboinensis*, exhibiting the most intense antipredator behaviour when exposed to the highest concentration of skin extracts (Tukey's HSD:  $P < 0.005$ ; Figs 1, 2).

Recruits also responded to skin extracts from juvenile conspecifics with a significant decrease in feeding strikes (Tukey's HSD:  $P < 0.001$ ), but there was no significant change in shelter use, time spent in shelter and distance from shelter compared to the two control treatments (Tukey's HSD:  $P > 0.2$ ; Figs 1, 2). This behaviour was similar to that exhibited by recruits when exposed to cues from the recruit low cue concentration (Figs 1, 2). Hence, although they appeared to move closer to shelter when exposed to these cues (Fig. 1), there was some overlap with the two control treatments. In contrast to recruit skin extracts, there was no consistent graded response exhibited by recruits exposed to juvenile cues.

Recruits did not exhibit significant changes in behavioural response relative to the two control treatments when exposed to all three concentrations of skin extracts from adults (Tukey's HSD:  $P > 0.5$ ; Figs 1, 2). Similarly to the cue from juveniles, there was no evidence for a graded response to the different concentrations of adult skin extracts.

## DISCUSSION

Our study suggests that newly settled damselfish not only successfully detect damage-released chemical alarm cues, but also use this chemical information to assess relative risks to optimize their antipredator response. Evidence suggests that prey fish have a finely tuned threat recognition system, distinguishing between different concentrations of cues (i.e. cues from multiple donors) as well as between different ontogenetic stages of conspecifics. These findings support research undertaken on species from freshwater environments (Brown et al. 2001b; Mirza & Chivers 2002; Golub & Brown 2003; Marcus & Brown 2003). Reef-naïve individuals exhibited consistent antipredator behaviours when exposed to



**Figure 1.** Mean change  $\pm$  SE in behaviour in (a) number of feeding strikes and (b) distance from shelter by newly settled *P. amboinensis* between the 10 min pre- and poststimulus periods for fish exposed to one of three alarm cue concentrations (low, medium and high) from three ontogenetic stages (recruit, juvenile and adult conspecifics). Heterospecific and salt water stimuli were controls. Letters above or below bars represent Tukey's HSD groupings of means. Bars with different letters are significantly different from each other at  $\alpha = 0.0125$ .

alarm cues from individuals of the same ontogenetic stage. Although newly settled fish decreased feeding when exposed to juvenile cues, there was no graded response, and they either ignored or failed to detect the cue from adult fish. Taken together, this result clearly indicates an ontogenetic switch in the use of conspecific alarm cues for risk assessment in *P. amboinensis*. Furthermore, this study also suggests that the relative concentration of skin extract provides information regarding local predation risk; when exposed to damage-released cues from a higher number of individuals, cue receivers consistently exhibited stronger antipredator responses.

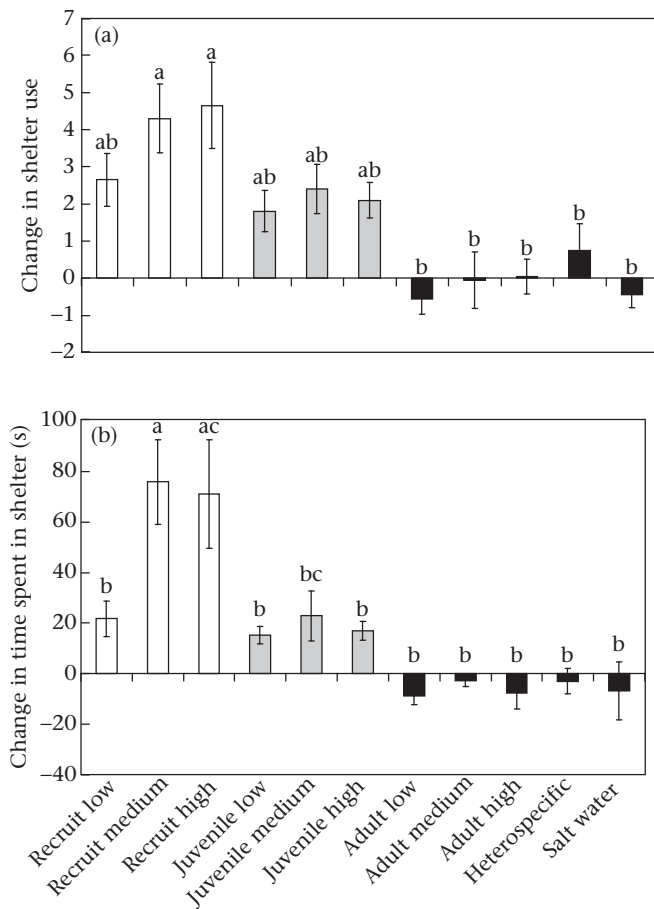
In freshwater systems, the switch in response to cues from individuals of different ontogenetic stages is suggested to be regulated by a threat-sensitive trade-off between antipredator behaviour and benefits obtained when foraging (Brown et al. 2002; Mirza & Chivers 2002; Quirt & Lasenby 2002; Golub & Brown 2003; Marcus & Brown 2003). In these examples, fish exhibit ontogenetic shifts in response to conspecific chemical alarm cues to avoid cannibalism (Brown et al. 2002; Golub & Brown 2003; Marcus & Brown 2003). Invertebrate-feeding young of the year large-mouth bass, *Micropterus salmoides*, and green sunfish, *Lepomis cyanellus*, show a typical antipredator response to damage-released cues of a heterospecific fish of the same prey guild, finescale dace, *Phoxinus neogaeus* (another invertebrate feeder). However, older fish of the same species that have undergone a diet shift (now being

**Table 1**

Results of univariate analysis of variance testing the equality of response of recruit *Pomacentrus amboinensis* to various treatments

	Source of variation	df	Mean square	F	P
Feeding	Treatment	10	18 380.60	55.95	<0.001
	Error	209	328.50		
Shelter use	Treatment	10	66.59	6.89	<0.0001
	Error	209	9.66		
Time (s)	Treatment	10	18 101.16	8.79	<0.0001
	Error	209	2058.69		
Distance	Treatment	10	5.20	7.33	<0.0001
	Error	209	0.71		

Controls were salt water and heterospecific skin extracts and treatments were conspecific skin extracts from three ontogenetic stages (recruit, juveniles and adults) at three concentrations (low, medium and high). The definitions of behaviours tested are given in the text.



**Figure 2.** Mean change  $\pm$  SE in behaviour in (a) number of times entering shelter and (b) time spent in shelter (s) in newly settled *P. amboinensis* between the 10 min pre- and poststimulus periods for fish exposed to one of three alarm cue concentrations (low, medium and high) from three ontogenetic stages (recruit, juvenile and adult conspecifics). Heterospecific and salt water stimuli were controls. Letters above or below bars represent Tukey's HSD groupings of means. Bars with different letters are significantly different from each other at  $\alpha = 0.0125$ .

piscivores) react to the same cues with a foraging response suggesting that the trade-off between defensive behaviours and other activities that promote fitness (such as foraging) depend upon the nature of the chemical information available (Brown et al. 2001a; Golub & Brown 2003). On the other hand, findings by Carreau-Green et al. (2008) suggest that the chemical composition of alarm cues in a freshwater fish (*P. promelas*) do not change with ontogenetic stage. They propose that alarm cues of larvae are not contained within specialized cells in the epidermis, but somewhere else in the bodies of developing fish. However, the response to chemical alarm cues would depend on the life cycle of the species in question; longer lived taxa that undergo major ontogenetic shifts in habitat and resource use during their life would benefit from adjusting the alarm cue response so that they respond most to ecologically relevant cues. Clearly, future studies on coral reef fish would benefit from performing histology on the different ontogenetic stages to examine the size and abundance of club cells.

Damselfish that inhabit tropical coral reefs do not switch to piscivory at any of their life stages. We argue that the difference in the change in behaviour to skin extracts from different ontogenetic stages comes about as a response to gape-limited predators and a perceived reduction in predation risk. Newly settled damselfish are exposed to a completely different suite of predators (smaller opportunistic fish such as dottybacks (Pseudochromidae) and

lizardfish (Synodontidae)) to their adult conspecifics (preyed upon by larger piscivores such as trout (Serranidae) and some of the larger wrasses (Labridae)) and may be better served allocating their antipredator response to cues that are relevant to them, in this way accurately assessing predation risk and responding accordingly (Holmes & McCormick 2010). Olfactory discrimination allows the recruits to maximize food intake instead of avoiding predators when these feed on a different ontogenetic stage to themselves (e.g. Alemadi & Wisenden 2002). When a juvenile is being consumed, which is more similar in size to the recruit than an adult, newly settled fish tend to decrease foraging behaviour and avoid the area associated with the alarm cue, although not strongly and not in a graded fashion. This suggests that the production of the cue starts to change in nature during the juvenile life phase.

Recent research has shown that a common predator (reef dottyback, *Pseudochromis fuscus*) of newly settled *P. amboinensis* can differentiate between the chemical cues of adult and recruit *P. amboinensis*, further suggesting that there is a change in the skin extract composition with ontogenetic stage (O. M. Lönstedt, M. I. McCormick & D. P. Chivers, unpublished data), but whether this is due to the chemical alarm cue formation or an inclusion of additional chemicals that indicate age is unknown. Currently, there is a paucity of information regarding the production mechanism of chemical alarm cues (Brown et al. 2004). Brown et al. (2001b) suggested that damage-released chemical alarm cues are produced as by-products of metabolism. As a result, the formation of these cues may be influenced by an individual's recent diet. As newly settled damselfish change diet from being planktivorous to omnivorous, feeding on algae but also zooplankton (Job & Shand 2001), the chemical alarm cue formation may also change. We know from previous studies that individuals reared on a higher food ration produce a greater number of the club cells that supposedly hold the alarm cue than individuals deprived of food (Wisenden & Smith 1997, 1998; McCormick & Larson 2008). Similarly, individuals fed a higher quality diet produce an alarm cue that elicits stronger antipredator behaviours in conspecifics (Brown et al. 2004). There clearly is some energetic cost associated with the production of these cues, and diet quality and quantity affect the production of the active substance (Brown et al. 2004; McCormick & Larson 2008). It is hypothesized that alarm cues initially evolved to protect the fish against infections, or as a predator attractant, and only secondarily acquired a role as an alarm cue (Mathis et al. 1995; Chivers et al. 1996, 2007; O. M. Lönstedt, M. I. McCormick & D. P. Chivers, unpublished data). Both of these functions would enhance the survival of the cue sender, one through defence against pathogens and the other by bringing about the speedy arrival of a secondary predator that gives the prey a chance to escape. It is possible that individuals in low body condition simply cannot afford to divert energy into maintaining the alarm substance, although producing and responding to alarm cues increase survivorship (Chivers et al. 1996; Mirza & Chivers 2003a).

Apart from the type of predator, the circumstances or context of any dangerous situation influences avoidance success by prey, and therefore needs to be correctly assessed. Our study supports the findings of others from freshwater systems (Mirza & Chivers 2002, 2003b; Brown et al. 2006) that the relative concentration of alarm cues provides information about the distance of the predatory attack. As such, a lower concentration indicates that the predator poses a lower threat. On the other hand, higher levels of skin extracts indicate higher risk situations as the predator(s) is in the immediate vicinity, and responding to these cues increases the chance of survival. In the present study, we used more donors (or a larger amount of skin surface area) to produce cues of higher concentrations, which elicited stronger antipredator responses in focal fish. Individuals that in this way respond more strongly to skin extracts

released from more than one fish should benefit from increased antipredator responses. Exhibiting less risky behaviours in response to skin extracts from a single cue donor compared to when additional individuals are being consumed would be advantageous as a greater amount of alarm cue (i.e. higher number of individuals being preyed upon) would potentially equate to more predators being in the area, or imply enhanced feeding activity. In either case, individuals that can identify and correctly respond to the level of risk present would be at an advantage, as the balance between predator avoidance behaviours and other fitness-enhancing activities is optimized (Helfman 1989; Blanchet et al. 2007).

The current results strongly support a threat-sensitive trade-off in the use of alarm cues with respect to both concentration gradients and ontogeny: prey response may not only be species specific, but also specific to particular life stages. There appears to be a strong selection pressure to respond to cues that are the best predictors of predators that are relevant to the ontogenetic stage of the prey fish. Newly settled *P. amboinensis* responded with typical antipredator behaviours to cues from conspecific prey fish of the same ontogenetic stage, but either ignored or could not detect the cues from adults. There was also a graded response to the concentration of cues from conspecifics of a similar stage, which suggests that the amount of cue present in the water provides information on the level of threat posed, as predicted from the threat-sensitive hypothesis (Helfman 1989). Future studies should investigate whether similar responses occur in the natural environment of the fish, and whether adult damselfish respond to cues from juveniles or newly settled individuals.

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