

# Friend or foe? The role of latent inhibition in predator and non-predator labelling by coral reef fishes

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**Abstract** In communities of high biodiversity, the ability to distinguish predators from non-predators is crucial for prey success. Learning often plays a vital role in the ability to distinguish species that are threatening from those that are not. Many prey animals learn to recognise predators based on a single conditioning event whereby they are exposed to the unknown predator at the same time as alarm cues released from injured conspecifics. The remarkable efficiency of such learning means that recognition mistakes may occur if prey inadvertently learn that a species is a predator when it is not. Latent inhibition is a means by which prey that are pre-exposed to an unknown species in the absence of negative reinforcement can learn that the unknown animal is likely not a threat. Learning through latent inhibition should be conservative because mistakenly identifying predators as non-predators can have fatal consequences. In this study, we demonstrated that a common coral reef fish, lemon damselfish, *Pomacentrus moluccensis* can learn to recognise a predator as non-threatening

through latent inhibition. Furthermore, we showed that we could override the latent inhibition effect by conditioning the prey to recognise the predator numerous times. Our results highlight the ability of prey fish to continually update the information regarding the threat posed by other fishes in their vicinity.

**Keywords** Learning · Latent inhibition · Anti-predator behaviour · Threat-sensitivity · Risk assessment · Predator recognition

## Introduction

Learning allows individuals to make decisions based on prior experience, providing them with a means to respond to changes and fluctuations in the environment in a way that will increase their fitness and survival (Davey 1989). Through learning, prey individuals are able to identify new predators, respond to changes in predator community structure and assess predation risk as it fluctuates in space and time (Kelly and Magurran 2003; Lima and Dill 1990). As a result, prey can fine-tune their anti-predator behaviour to match the current risk and thus balance the costs of predator avoidance with fitness promoting behaviours, such as foraging and mating (Lima and Bednekoff 1999). The ability to assess local predation risk through learning increases an individual's chances of survival (Mirza and Chivers 2003).

Chemosensory information provides prey from aquatic environments with a reliable source of information to assess predation risks within their local environment. Chemical alarm cues, released from the skin of an injured conspecific following a predation event, provide a reliable indication of an increase in the current level of predation risk. Detection of alarm cues elicits dramatic short-term

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increases in antipredator behaviour amongst a diverse range of aquatic taxa, enhancing survival (Ferrari et al. 2010). In the absence of innate predator recognition, alarm cues can be used to learn a novel predator's identity through associative learning (Chivers and Smith 1998). Learning occurs when individuals detect the predator odour (conditioned stimulus) paired with an alarm cue (unconditioned stimulus) (Chivers and Smith 1998). Subsequent encounters with the predator odour provide individuals with an early warning about presence of a predator in their environment. Given the reliability of chemical alarm cues as indicators of predation risk, pairing an alarm cue with any novel stimulus (e.g. non-predatory goldfish, Chivers and Smith 1994) results in the stimulus being recognised as a predation threat. To date, there are no studies that show that the pairing of a chemical alarm cue with a novel stimulus fails to label the stimulus as a predation threat (Ferrari and Chivers 2006a).

The persistence of chemical alarm cues and dispersive nature of chemicals in water (Hazlett 1999) means that conspecifics from a wide area have the potential to detect alarm cues and associate them with any local novel odour. If any novel stimulus can be labelled as a predation threat by pairing it with a chemical alarm cue, the potential exists for individuals to falsely learn to recognise irrelevant cues as a risk. Responding to non-threatening odours will be detrimental to an individual's fitness. Benefits gained from learning predator identities will be negated if individuals do not possess mechanisms to prevent irrelevant cues from being perceived as a threat (Davey 1989). One such mechanism, latent inhibition, is known from the psychological literature (Shettleworth 1998). Latent inhibition occurs when an individual is exposed to a neutral (i.e. without positive or negative reinforcement) odour repeatedly, labelling it as irrelevant or inconsequential, prior to a conditioning event. As a result, the association between the odour and the unconditioned stimulus is retarded (Kaplan and Lubow 2001; Lubow and Moore 1959). Latent inhibition has shown to be effective in preventing non-predators being labelled as a threat for crayfish *Orconectes virilis* and *O. rusticus* (Acquistapace et al. 2003), fish (fathead minnows, *Pimephales promelas*, Ferrari and Chivers 2006a) and amphibians (Ferrari and Chivers 2009, 2011).

Most aquatic prey species lack innate recognition of their potential predators (Chivers and Smith 1998); hence, latent inhibition has the potential to prevent novel predators from being correctly identified, if their odours were detected in the absence of a risk context. In aquatic environments, ontogenetic shifts in habitats are common (George 1981; Manzur et al. 2010; Mumby et al. 2004), resulting in a number of prey species encountering novel predator communities. There is therefore a high chance that an individual will encounter cues from a potential predator

multiple times prior to a conditioning event. For instance, prey may encounter the odour of opportunistic predators several times prior to a predation event; predators that undergo an ontogenetic switch in prey species preference and cryptic ambush predators are likely to be present in an area a long time before a predation event occurs. The threat sensitive learning hypothesis suggests that individuals should continuously learn about predators in order to respond to fluctuations in the threat posed by them (Ferrari and Chivers 2006b). Given the potential consequences associated with a failure to learn the identity of predators, individuals that have experienced predators in previously non-threatening contexts should be able to reverse this initial inhibition and alter the risk assessment associated with a predator.

Coral reefs support a diverse and abundant assemblage of fishes, comprising many species of predators and non-predators. After an initial planktonic phase, juvenile reef fish return to reefs and must rapidly learn to identify which fish represent a threat. A recent study demonstrated that prey fish on coral reefs are very adept at learning predators through pairing novel odours with chemical alarm cues (Larson and McCormick 2005) and can simultaneously learn multiple predators from a single predation event (Mitchell et al. 2011). The results highlight the potential for non-predators to be wrongly identified as a predation threat if they are present at the time of a predation event. The present study investigated whether latent inhibition is an advantageous mechanism in preventing or modulating learning for a common coral reef fish, the lemon damselfish, *Pomacentrus moluccensis*. Specifically, we address three questions: (1) Do juvenile reef fish have an innate antipredator response to predator odours? (2) Can latent inhibition prevent the learning of a novel odour in coral reef fishes? (3) If latent inhibition prevents the learning of novel odours, can the effects be reversed as an individual's experience with the odour and its associated threat increases?

## Methods

### Study species

Lemon damselfish, *Pomacentrus moluccensis* (family Pomacentridae), is a common planktivorous fish found in association with coral reefs throughout the Indo-Pacific region and the Great Barrier Reef, Australia. After an 18–21 day planktonic phase, they settle to the reef at a size of ~10 mm in length (Wellington and Victor 1989). *P. moluccensis* juveniles are preyed upon by multiple predators, including the brown dottyback, *Pseudochromis fuscus* (Pseudochromidae) (Beukers and Jones 1997).

*P. fuscus* are small (maximum size ~10 cm total length) crypto-benthic predators, common on reefs (Messmer et al. 2005). They are found in areas of high coral cover or rubble in association with damselfish.

#### Collection and maintenance

Fish were collected at Lizard Island, northern Great Barrier Reef (14°40'S, 145°28'E) between November and December 2009. *P. moluccensis* recruits were collected prior to settling using light traps (small trap design, Meekan et al. 2001) deployed overnight, 50–100 m away from the reef edge. As *P. fuscus* is a benthic-associated predator (Messmer et al. 2005), collecting *P. moluccensis* recruits from the pelagic environment ensured that they should be naïve to *P. fuscus*. Recruits were maintained in a 60-l aquarium (64 × 41 × 40 cm) supplied with aerated sea water and maintained at ambient sea water temperatures (29°C) under a 14:10 light:dark photoperiod. Fish were fed ad libitum twice a day with freshly hatched *Artemia* sp. and supplemented with 5/8 NRD marine food pellets (Spectrum Aquaculture).

*Pseudochromis fuscus* were collected on scuba from the lagoon at Lizard Island using hand nets and anaesthetic clove oil mixed with alcohol and sea water. The fish were maintained as described above in 32-l aquaria (43 × 32 × 30 cm). Fish were fed twice a day with thawed bait squid.

#### Stimulus preparation

Fresh alarm cues were prepared daily prior to the conditioning phase (see below). One *P. moluccensis* per treatment was killed by a quick blow to the head and placed in a plastic disposable dish. Using a clean scalpel blade, 15 superficial cuts were made along each flank of the fish. Fish were rinsed with 15 ml of sea water, and the solution was filtered through filter paper to remove any solid material.

*Pseudochromis fuscus* odour was prepared from two individuals (57 and 79 mm standard length (SL)) maintained in a 32-l flow-through aquaria (43 × 32 × 30 cm). They were fed twice per day for 2 days and then starved for 2 days to remove any potential alarm cues present in their guts. On day 4, the fish were moved into an aerated 32-l stimulus collection tank containing 10 l of sea water and left undisturbed for 6 h. The fish were then returned to their original tanks, and the water from the stimulus collection tank was bagged and frozen in 30-ml aliquots.

#### Observation tanks

Conditioning and pre-exposure were done in 3-l flow-through aquaria (11 × 18 × 12 cm), containing a 2 cm layer

of sand and an airstone. Recognition trials were conducted in 13-l flow-through aquaria (36 × 21 × 20 cm; mean flow: 0.6 l/min). Tanks contained a 3 cm layer of sand, a small shelter (terracotta pot; 5 cm diameter) at one end and an airstone at the opposite end. A feeding tube and stimulus tube were attached to the airstone tube to aid rapid dispersal of the food and chemical stimuli, whilst minimising disturbance to the fish. The front of each tank was marked with a 4 × 6 grid. Tanks were surrounded on three sides with black plastic to visually isolate the fish and a black plastic curtain was hung in front to create an observation blind.

#### Experimental overview

Recent studies have suggested that some larval reef fish may have an innate recognition of some predator odours (Dixon et al. 2010; Vail 2009). Hence, the first part of our study investigated whether our larvae displayed an innate recognition to the predator species we used in the subsequent experiment (question 1). The second part looked at latent inhibition and its potential reversal (question 2 and 3).

#### Innate predator recognition

This experiment consisted of two phases, a conditioning phase followed by a testing phase. After acclimating overnight in individual observation tanks, individual *P. moluccensis* were conditioned for 1 h with either 30 ml *P. fuscus* odour paired with 15 ml alarm cue or with 45 ml saltwater. The flow-through system was turned off for the duration of the conditioning phase. The following day, fish were tested for their response to either *P. fuscus* odour or saltwater, using the behavioural assays: feeding rate, distance from shelter and time spent in shelter. This produced three different treatments ( $n = 15$  fish per treatment): conditioned with *P. fuscus* odour + alarm cue and tested with *P. fuscus* odour; conditioned with saltwater and tested with either *P. fuscus* odour or saltwater. The saltwater conditioning and saltwater recognition trials allowed us to control for both the conditioning procedure and the injection process. Comparison of the saltwater control with the *P. fuscus* odour conditioning tested for an innate response to *P. fuscus* and assuming a non-response, controlled for the introduction of an unknown odour. Finally, comparison of the alarm cue with *P. fuscus* odour cue conditioning indicated a conditioned antipredator response to *P. fuscus* odour.

#### Latent inhibition and reversal

Questions 2 and 3 were investigated using fish that were not used in the innate recognition trials. Both question 2 and 3 were examined using a single experiment, but the questions

were answered separately. This allowed comparison amongst treatments and ensured that there was no temporal or recruitment pulse effect on the trials.

The experiment for latent inhibition consisted of three phases: a pre-exposure phase, a conditioning phase and a testing phase (Supplementary Fig. 1). Fish were placed in individual 3-l tanks and acclimated over night. During the pre-exposure phase, individuals were exposed to either 30 ml of *P. fuscus* odour (6PO) or 30 ml of saltwater (6SW) twice a day for 1 h over a 3-d period, representing six exposures in total. Following this, individuals were conditioned with either *P. fuscus* odour paired with an alarm cue (PO + AC—true conditioning) or *P. fuscus* odour paired with saltwater (PO + SW—false conditioning). The next day, individuals were tested for their response to PO alone. This  $2 \times 2$  design allowed us to test for the effect of pre-exposure (pre-exposed to saltwater or predator odour) and conditioning cue (with saltwater or alarm cues) on the responses of fish to the predator odour. We predicted that fish that received the false conditioning (PO + SW) would not recognise the predator as threatening, regardless of the pre-exposure cues they received. Fish pre-exposed to saltwater (6SW) and conditioned with alarm cues (PO + AC) should successfully learn to recognise the predator as threatening, while the fish pre-exposed to the predator odour (6PO) should not (i.e. the latent inhibition group).

To assess the potential for reversal, we pre-exposed the fish to predator odour (6PO) and conditioned them either twice (2PO + AC), three times (3PO + AC) or four times (4PO + AC). The day following the last conditioning event, individuals were tested for their response to PO alone. As treatments were randomised, pre-exposures and conditioning were staggered to allow testing to be done on the same day. We used the ‘latent inhibition’ group from above (6PO (PO + AC)) and the classical conditioning group from above (6SW (PO + AC)) as negative (response inhibited) and positive (full anti-predator response) controls, respectively. Although a complete  $2 \times 4$  design, testing for the effect of pre-exposure (saltwater or alarm cues) and number of conditioning (1–4) on the responses of fish to predator odour would have been more rigorous, logistic and animal limitations prevented us from doing so. We predicted that, as the number of conditioning events increased, the fish should respond to the predator odour with an increasing intensity. A total of 124 fish were tested in the seven treatments.

#### *Conditioning and pre-exposure protocol*

The pre-exposure and conditioning treatments followed the same protocol. Individual *P. moluccensis* were allowed to acclimate over night before receiving their first treatment between 1000 and 1100 h the following day. Fresh

*P. fuscus* odour was defrosted each morning and allowed to reach ambient sea water temperature. Prior to stimulus injection, the flow-through system was turned off before the treatment period to prevent the stimuli being flushed out. To remove any stagnant water in the injection tubes, 20 ml of sea water were removed and discarded, a further 20 ml were then removed. After a few minutes, we injected the relevant amount of the various stimuli, followed by 20 ml of sea water to flush the tube. The fish were left undisturbed for 1 h before the flow-through system was turned back on, to flush the tanks. We conditioned a total of 191 fish across the three experiments, up to 48 fish per day.

#### *Recognition trials*

Trials were conducted between 0730 and 1430 h, the day after conditioning. Each trial consisted of a 5-min feeding period, a 5-min pre-stimulus observation and 5-min post-stimulus observation. Prior to the start of the trials, saltwater for stimuli injection was removed following the above protocol, a further 60 ml was also removed from the feeding tube. The flow-through system was then turned off. At the start of the 5-min feeding period, we injected 2.5 ml of food (an *Artemia* solution containing ~250 individuals per ml), followed by 20 ml of seawater (to completely flush the food into the tank), allowing the fish to reach a stable feeding rate before the pre-stimulus observation. At the start of the pre-stimulus observation, an additional 2.5 ml of food was introduced and flushed with 20 ml of saltwater. Following the pre-stimulus observation period, we injected 2.5 ml of food, flushed with 20 ml of saltwater, followed by 30 ml of stimulus odour (*P. fuscus* odour or saltwater) flushed with 20 ml of saltwater.

#### *Behavioural bioassay for all experiments*

The behaviour of the fish was observed during the pre- and post-observation periods. We quantified three response variables: foraging rate, time in shelter and distance from shelter. Decreased foraging rate, distance from shelter and increased shelter use are well known antipredator responses in a number of prey species, including coral reef fishes (Ferrari et al. 2010; Holmes and McCormick 2010). The foraging rate included all feeding strikes irrespective of whether they were successful at capturing prey. Time in shelter (in seconds) was defined as total time that the fish spent within one body length of the terracotta pot. For distance from shelter, the horizontal and vertical locations of the fish in the tank were recorded every 15 s, using the grid drawn on the side of the tank. The position of the fish in the tank was then converted into a linear distance from shelter using the dimensions of the grid squares ( $57 \times 42$  mm) and Pythagoras’ theorem.

## Statistical analysis

The data for the three questions were analysed using separate statistical analyses. The three behaviours were not independent from each other; thus, we analysed them together using a MANOVA approach. We used the change in behaviour from the pre-stimulus baseline (post – pre) as our raw data in the analyses.

To test for innate recognition of predators by damselfish larvae (question 1), we performed a one-factor MANOVA, followed by ANOVAs and Tukey's HSD post hoc comparisons on individual variables to compare the responses of the fish to saltwater, predator alone and to predator odour following conditioning.

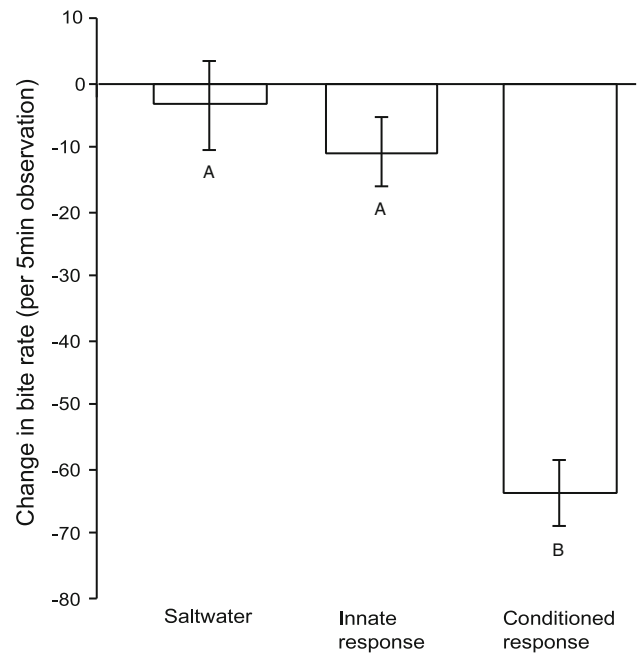
To test for latent inhibition, we performed a  $2 \times 2$  MANOVA followed by ANOVAs and unequal n HSD post hoc comparisons on individual variables, looking at the effects of pre-exposure (saltwater vs. predator odour) and conditioning cues (saltwater vs. alarm cues) on the responses of the fish to predator odour.

To test for latent inhibition reversal, we performed a one-factor MANOVA followed by ANOVAs and unequal n HSD post hoc comparisons on individual variables, comparing the responses of five experimental groups: the latent inhibition group (6PO (PO + AC)—negative control), the classical conditioning group (6SW (PO + AC)—positive control) and the three groups of fish receiving increasing numbers of conditioning events (6PO followed by 2PO + AC, 3PO + AC or 4PO + AC). Data for the variables foraging rate and distance from shelter were normal and homoscedastic. The data for time spent in shelter were 4th root transformed for the innate predator recognition question, while the latent inhibition and latent inhibition reversal data were  $\text{Log}_{10}$  transformed to meet assumptions of normality and homoscedasticity.

## Results

### Innate predator recognition

There was a significant effect of treatments on the response of *P. moluccensis* to the predator odour (MANOVA,  $F_{6,80} = 13.7$ ,  $P < 0.001$ ). Univariate exploration revealed that only foraging rate was affected by the treatments (foraging,  $F_{2,42} = 48.12$ ,  $P < 0.0001$ , time in shelter,  $F_{2,42} = 3.21$ ,  $P = 0.055$  and distance from shelter,  $F_{2,42} = 0.39$ ,  $P = 0.679$ ). Post hoc tests revealed that individuals conditioned with saltwater and tested for their response to saltwater did not vary in their foraging rate from those tested for their response to *P. fuscus* odour ( $P = 0.201$ , Fig. 1). However, after being conditioned with *P. fuscus* odour paired with alarm cue, individuals exposed to the *P. fuscus* odour alone



**Fig. 1** Change ( $\pm$ SE) in foraging rate for the damselfish *Pomacentrus moluccensis* in response to three treatments: tested for response to saltwater after being conditioned with saltwater (saltwater control), tested for response to *Pseudochromis fuscus* odour after being conditioned with saltwater (innate response) and tested for response to *P. fuscus* odour after being conditioned with *P. fuscus* odour paired with an alarm cue (conditioned response). Letters below bars indicate unequal n HSD groupings ( $\alpha = 0.05$ )

displayed a reduction in foraging rate compared to the other two treatments, indicative of an antipredator response ( $P < 0.001$ , Fig. 1). The lack of response to *P. fuscus* odour after conditioning with saltwater suggests that individuals do not possess and innate recognition of *P. fuscus* odour.

### Latent inhibition

The two-factorial MANOVA revealed that there was no interaction between pre-exposure and conditioning cue ( $F_{3,61} = 1.40$ ,  $P = 0.251$ ), but there was a significant effect of both pre-exposure ( $F_{3,61} = 4.29$ ,  $P = 0.008$ ) and conditioning cue ( $F_{3,61} = 5.27$ ,  $P = 0.003$ ) on the responses of fish to the predator odour, suggesting the importance of latent inhibition. The univariate tests indicated that both foraging rate (pre-exposure,  $F_{1,63} = 9.46$ ,  $P = 0.003$  and conditioning cue,  $F_{1,63} = 11.51$ ,  $P = 0.001$ ) and time in shelter (conditioning cue,  $F_{1,63} = 6.82$ ,  $P = 0.011$ ) were affected by the treatments. Unequal n HSD post hoc analysis revealed that individuals showed a significantly greater reduction in foraging rate after being conditioned with the 6SW (PO + AC) treatment, compared to the 6PO (PO + AC) ( $P = 0.005$ ) and two control treatments (6SW (PO + SW),  $P = 0.002$  and 6PO (PO + SW),  $P < 0.001$ ;

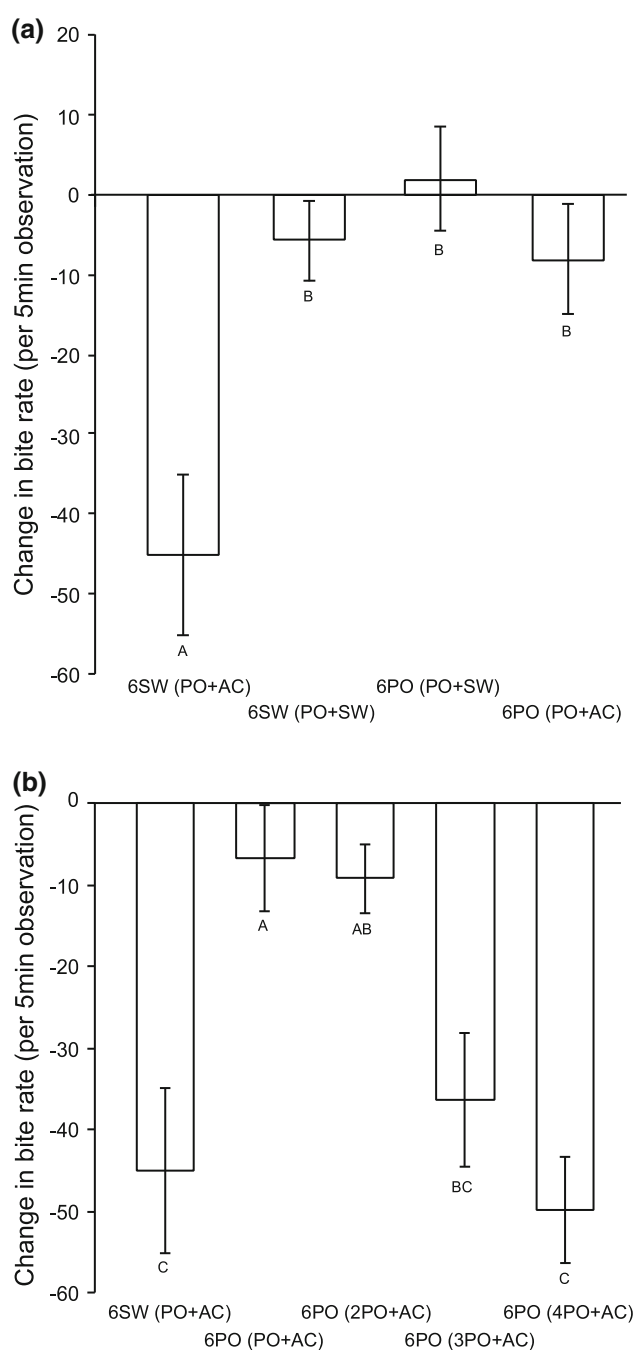
Fig. 2a). Individuals from the 6PO (PO + AC) treatment did not differ in their response to the PO stimulus compared to the two controls [6SW (PO + SW),  $P = 0.99$  and 6PO (PO + SW),  $P = 0.81$ ], with individuals from all three treatments showing little difference in their behaviour during the pre- and post-stimulus observations. Unequal n HSD post hoc analysis for time in shelter revealed that there was only a significant difference between individuals from the 6SW (PO + AC) treatment and from the control 6PO (PO + SW) ( $P = 0.046$ ). There was no difference between individuals from the 6SW (PO + AC) treatment and from the latent inhibition treatment, 6PO (PO + AC;  $P = 0.732$ ).

#### Latent inhibition reversal

There was a significant effect of treatment on the responses of individual *P. moluccensis* to predator odour (MANOVA,  $F_{12, 190.8} = 3.45$ ,  $P < 0.001$ ). The univariate results showed that only foraging rate was affected by the treatments (ANOVA foraging,  $F_{4,76} = 7.08$ ,  $P < 0.001$ , time in shelter,  $F_{4,76} = 1.96$ ,  $P = 0.108$  and distance from shelter,  $F_{4,76} = 1.92$ ,  $P = 0.116$ ). Post hoc analysis revealed significant differences between treatments (Fig. 2b). Individuals that received one or two conditionings with PO + AC failed to override the effects of latent inhibition, as they did not respond to the introduction of PO with an antipredator response. However, after receiving three or four conditionings, individuals displayed an antipredator response equal to the control.

#### Discussion

This study clearly demonstrates that prior knowledge of an odour is an effective method to prevent learning irrelevant odours as a predation threat when they are detected in unison with an alarm cue. When *P. moluccensis* experienced six exposures to the odour of *P. fuscus* prior to the coupling of the odour with a conspecific alarm cue they did not respond to *P. fuscus* odour as a predation threat during subsequent encounters. This result confirms that latent inhibition is an advantageous mechanism for preventing irrelevant odours from being learnt as a risk in marine organisms, adding to findings from amphibians (Ferrari and Chivers 2009, 2011), freshwater fishes (Ferrari and Chivers 2006a) and freshwater invertebrates (Acquistapace et al. 2003). Furthermore, by using a predator known to feed on juvenile reef fishes, we were able to demonstrate that, in the absence of an innate antipredator response to predator odours, latent inhibition has the ability to prevent prey from learning the identity of potential predators. However, as an individual's experience with the odour and the associated



**Fig. 2** Change ( $\pm$ SE) in foraging rate for *Pomacentrus moluccensis* in response to *Pseudochromis fuscus* odour, testing for: **a** latent inhibition and **b** latent inhibition reversal. Individuals were pre-exposed to either *P. fuscus* odour or saltwater six times prior to being conditioned with *P. fuscus* odour paired with an alarm cue or saltwater. Letters below bars indicate unequal n HSD groupings ( $\alpha = 0.05$ )

risk increases, they are able to learn to recognise the odour as a risk, reversing the effects of latent inhibition. To our knowledge, this is the first study to demonstrate the reversal of latent inhibition in a predator/prey context. Our study showed that learning occurs in a graded way reflecting the relative increase in risk associated with the odour and

highlights that individuals learn about predators in a way that is far more complex than a simple one time conditioning with an unknown odour and an alarm cue. The fish are able to learn in a way that can identify and isolate odours that are irrelevant, but they are able to alter their responses when the context of information associated with those odours changes.

For risk assessment in aquatic environments, the benefits of latent inhibition are obvious. Individuals from environments containing a diverse array of species, such as coral reefs, are constantly surrounded by odours from a range of predators and non-predators. Following a predation event, alarm cues are released into the water column. Due to the dispersive nature of chemicals in water and the persistence of alarm cues (Hazlett 1999), there is the potential for conspecifics from a wide area to detect these cues and associate them with ambient odours. Latent inhibition allows individuals to filter out ambient odours and learn only the specific odour associated with the alarm cue as a threat. However, as demonstrated here, in the absence of an innate antipredator response to predators, latent inhibition will prevent an individual learning the identity of a predator if its odour is detected several times prior to a predation event. The consequences of such inhibition could be fatal. Situations where an individual may encounter a predator several times before encountering it in a predation event are likely to be common, e.g., *P. fuscus* is a cryptic ambush predator and will be present in an environment for some time prior to its first capture of a prey individual. Other situations may include unsuccessful predation events where no alarm cues is released, opportunistic predators that only feed periodically on the focal species, predators where only part of the population target the focal species and when either the predators or prey undergo an ontogenetic switch in prey species preference or trophic group.

Individuals are able to reverse the effects of latent inhibition as they gain more information regarding risks associated with the predator. Individuals exposed to *P. fuscus* odour prior to conditioning were able to overcome the inhibitory effects and learn to respond to *P. fuscus* odour with an antipredator response after three conditionings with *P. fuscus* odour paired with an alarm cue. This result is unsurprising given the potential consequences of permanently inhibiting an individual's ability to learn about predators and the dynamic nature of the environment. Predation risk in species-diverse environments will be highly variable spatially and temporally. As a result, it is adaptive for individuals to be able to respond to changes in the risk associated with a specific odour, even if this means reversing a previously fixed perception.

Individuals appeared to display a threat-sensitive antipredator response to *P. fuscus* odour with increasing numbers of conditionings, following pre-exposure. As the

predation risk associated with the *P. fuscus* odour increased with further conditionings, so did the antipredator response. Whilst individuals conditioned twice with *P. fuscus* odour paired with alarm cue were still inhibited, individuals conditioned three times displayed an antipredator response that was significantly greater than those conditioned once, and the strongest antipredator response was recorded by individuals that received four conditionings. A previous study showed that, after several conditionings with predator odour paired with an alarm cue of varying strength, fathead minnows displayed an antipredator response to a predator odour that matched the intensity of the most recent conditioning event, rather than integrating the accumulated knowledge into an average response (Ferrari and Chivers 2006b). The disparity between the previous study and our own may reflect the fact that fathead minnows were responding to varying degree of risk, whereas *P. moluccensis* had to overcome truly conflicting information. The graded anti-predator response displayed by *P. moluccensis* likely reflects prey gradually learning to pay attention to the *P. fuscus* odour after being conditioned to ignore it or the gradual resolution of the conflicting information represented by the *P. fuscus* odour.

Whilst this study highlights the importance of latent inhibition for individuals learning about local predation risks, it also demonstrates the importance of an individual's experience outside of a conditioning context. To date, most studies have focused on how a single conditioning affects an individual's ability to learn. Such conditioning over simplifies natural conditions. A useful avenue of study will be to investigate how an individual's numerous interactions with other species may affect its ability to learn and retain the recognition of a specific predator. Indeed, the clear conditioning regimes used in the present experiment were used to test for latent inhibition and any potential overriding effects. Under natural conditions, however, it is likely that individuals showing latent inhibition effects towards a specific predator will encounter the predator odour paired with an alarm cue interspersed with encounters with the predator odour alone. In future studies, it is therefore important to know over what time frame these processes occur and how other cues, such as visual and diet cues, aid in resolving such conflicts of information.

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**Ethics statement** This research was undertaken with approval of the James Cook University Animal Ethics Committee (permit: A1067) and according to the University's Animal Ethics Guidelines.

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