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Juvenile coral reef fish alter escape responses when exposed to changes in background and acute risk levels

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The response of prey to predation threats is often plastic and can vary with the individual's perceived level of threat. To determine whether prey escape responses can be modulated by background levels of risk or short-term acute risk, we maintained juvenile damselfish, *Acanthochromis polyacanthus*, under high- or low-risk background conditions for several days and then exposed them to an acute risk (high-risk alarm cues or a low-risk saltwater control) minutes prior to startling them with a mechanical disturbance. Fish responded in one of two ways: they either made a C-start escape response or backed away from the threat. While exposure to either background high risk or acute high risk increased the proportion of C-starters, surprisingly the frequency of C-starters decreased when background high risk and acute risk types were combined. Exposure to an acute high-risk cue increased the escape performance for both types of escape responses. However, when the acute high-risk cue occurred within high-risk background conditions, this only increased the performance of C-start escape responses. Non-C-starters reacted similarly in both background risk conditions. Background risk and acute risk acted in a simple additive manner, as seen by the lack of interaction between the two factors. Results showed that escape responses are amplified as the level of perceived risk increases.

Predation risk plays a major role in shaping prey populations. Predators can impact prey through direct interactions resulting in mortality. However, they can also have an indirect effect influencing life history characteristics, morphology, physiology and behaviour (Brönmark & Petterson, 1994; Bernard, 2004; Chivers, Zhao, Brown, Marchant, & Ferrari, 2008; Ferrari, McCormick, Allan, Choi, Ramasamy, Johansen, et al., 2015; Lönnstedt, McCormick, & Chivers. 2013; Preisser, Bolnick, & Bernard, 2005; Palacios, Killen, Nadler, White, & McCormick, 2016), any one of which has the potential to influence the ability of the prey to escape an attack.

Predator–prey interactions follow a well-described sequence of events, from detection to capture or escape. Within this sequence, there are steps where both the predator and prey can optimize their success (Domenici & Blake, 1997; Lima & Dill, 1990). Our study focuses on the crucial step when prey must escape from a predator after an attack has been initiated. Some escape responses, such as C-start responses, are short, anaerobically powered swimming bursts elicited by the activation of a large pair of reticulospinal neurons called the Mauthner cells (Domenici & Blake, 1997; Moyes, Schulte, & Andwest, 1993), found in fish and amphibians (Sillar, 2009). Such escape responses involve a number of stages in a sequence. Stage 1 consists of the formation of the C-bend (i.e. the preparatory stroke), stage 2 consists of the return flip of the tail associated with forward acceleration (i.e. the propulsive stroke) and stage 3 consists of the continuous swimming or coasting after stage 2 (Domenici & Blake, 1997).

To undertake a successful escape, prey will use all information available to them. This information can be visual (i.e. sight of a predator), chemical (i.e. predator odour or chemical alarm cues), auditory (i.e. hearing a predator) and/or mechanosensory (i.e. movements detected by the lateral line in fish). Behavioural history (i.e. prior experiences that affect future behaviour) has also been shown to affect the mechanics (i.e. kinematics) of the escape response (e.g. Langerhans, Layman, Shokrollahi, & DeWitt, 2004; Ramasamy, Allan, & McCormick, 2015) and suggests that, rather

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than being hardwired, components of the fast start response are under cognitive behavioural control (Ramasamy et al., 2015) and are context dependent (Chivers, McCormick, et al., 2016; Domenici, 2010; McCormick & Allan, 2016). Given the strong influence of predation history on behaviour and the development of integrated antipredator phenotypes (Ferrari, McCormick, Allan, Choi, Ramasamy, Johansen, et al., 2015), our present study examined the role that background risk and acute risk have on escape responses. To do this, we used the coral reef damselfish spiny chromis, *Acanthochromis polyacanthus* (Pomacentridae) to ask: (1) does background risk history influence an individual's escape performance and (2) does the addition of information on current (i.e. acute) risk affect the escape responses of individuals exposed to different risk histories? To test this, juvenile fish were captured, and given two background risk treatments using damage-released cues from conspecifics (i.e. alarm cues), known to elicit an antipredator response in this species (Manassa & McCormick, 2012). Minutes prior to being startled, these fish were also exposed to a low- or high-risk stimulus and the resulting escape behaviour of the fish was analysed. We predicted that fish that were exposed to conditions with the highest risk (i.e. high background and acute risk) would exhibit the greatest escape responses (i.e. shorter latencies, higher escape velocities and longer response distances). This prediction is based on findings by Ramasamy et al. (2015), who showed that juvenile coral reef fish amplified their escape responses as the level of threat increased when exposed to a known predator.

METHODS

Study Species

Five schools of juvenile *A. polyacanthus* (18.37 ± 0.21 mm), a reef-associated brooding planktivore commonly found on the Great Barrier Reef, Australia, were captured using hand nets and clove oil while on SCUBA near the reefs surrounding the Lizard Island Research Station (14°40′S, 145°28′E), northern Great Barrier Reef, in March 2015. The fish were transported to the laboratory, randomly divided into 12 equal groups and held in 3-litre flow-through tanks (43 × 32 cm and 31 cm high), where they were conditioned to a high- or low-risk background. During this period, fish were fed *Artemia* sp. three times per day for 4 days.

Conditioning Regime

The goal of this experiment was to test the effect of background and acute risk on the escape response of a coral reef fish. We used a well-established methodology to create difference in background risk. High-risk background was created by introducing a solution of alarm cues into the conditioning tanks three times per day for 4 days (Brown, Ferrari, Elvidge, Ramnarine, & Chivers, 2013; Chivers, Mitchell, Lucon-Xiccato, Brown, & Ferrari, 2016). Prey organisms exposed to this risk regime, whether freshwater or marine fish or amphibians, have been shown to alter their behaviour (expression of neophobia, degree of behavioural lateralization, learning of predators and nonpredators), physiology (physiological recovery after stress) and survival (using multiple predators; Chivers, McCormick, Mitchell, Ramasamy, & Ferrari, 2014; Ferrari, McCormick, Meekan, & Chivers, 2015; Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015). We crossed background risk (low versus high) with an acute risk treatment (low versus high) in a 2 × 2 design. Fish ($N = 72$) were equally divided into a series of 12 tanks (3 litres, six fish per tank). Fish in half of the tanks were exposed to elevated risk for 4 days while the remainder were exposed to a low-risk control. The alarm cue solution was prepared minutes prior to being used, by making six vertical cuts on each side of four, freshly euthanized (using cold shock, in accordance with James Cook University animal ethics guidelines, permit: A2005) donor conspecific fish and then rinsing the fish in 60 ml of saltwater. We injected 10 ml of this alarm cue solution into the conditioning tanks, which gave a concentration of 2 cuts/litre once injected. This concentration has been shown to elicit strong antipredator responses in coral reef fishes (Chivers et al., 2014; McCormick, Allan, Choi, Ramasamy, Johansen, et al., 2015). The timing of the three injections occurred randomly between 0800 and 1800 hours, with a minimum of 1.5 h between consecutive injections. Low-risk conditions were obtained by injecting 10 ml of saltwater on the same time schedule as the high-risk treatment.

Escape Response Assay

After fish had been in one of the two risk treatments for 4 days, we conducted an escape response assay to test whether the response of fish was affected by background risk or the presence of current acute risk. A single fish was placed into our test arena to isolate the individual escape response. The test arena consisted of a transparent circular acrylic arena (200 mm diameter × 70 mm height) contained within a large opaque-sided plastic tank (585 × 420 mm and 330 mm high; 60 litres) with a transparent Perspex bottom to allow responses to be filmed from below (Fig. 1). The circular acrylic arena was large enough not to affect the response distance of the fish. The few fish that swam into the wall were removed from the analysis. To minimize vertical displacement of the prey during the escape response, the water level was set at 60 mm. Following a 3 min acclimation period, 20 ml of either high-risk (i.e. alarm cue) or low-risk (i.e. saltwater) acute cue was introduced into the arena through a plastic tube above the water. Alarm cues were produced fresh (2 cuts/litre). The individual fish were exposed to the cue for 2 min before an escape response was elicited. We followed the methods described in other escape response studies (Allan, Domenici, McCormick, Watson, & Munday, 2013; Marras & Domenici, 2013; Ramasamy et al., 2015) in which a tapered metal weight was released from above the water surface. The metal weight was controlled by a piece of fishing line that was long enough to allow the tapered tip to lightly touch the surface of the water but not hit the bottom of the tank. To remove the possibility of fish responding to the visual cue of the approaching stimulus, the weight was released through a white PVC tube (40 mm diameter × 550 mm length) suspended above the experimental arena, with the bottom edge sitting 10 mm above the water level.

To standardize the distance between the test subject and the stimulus, fish were only startled when they moved to the middle portion of the tank, and no forward momentum was seen. This also allowed the individual to move in any direction. There was no statistical difference in the distance between fish and the PVC tube between treatments (background risk: $F_{1,73} = 0.1$, $P = 0.75$; acute risk: $F_{1,73} = 0.08$, $P = 0.78$; background risk∗acute risk: $F_{1,73} = 1.04$, $P = 0.31$). Escape responses were recorded at 480 frames/s as a silhouette from below obtained through pointing the camera (Casio EX-ZR1000) at a mirror angled at 45°. The water in the experimental arena was changed after each trial. Kinematic variables associated with the escape response were analysed using ImageJ (http://rsbweb.nih.gov/ij/), with a manual tracking plug-in. Each fish was tracked using a point directly behind the fish's eye, which corresponds to the thickest part of the body. We chose to standardize tracking based on this point as it is the most stable and easiest to track due to the small size of subjects. The following kinematic variables were measured.