



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



Ryan A. Ramasamy^{a,*}, Bridie J. M. Allan^a, Mark I. McCormick^a, Douglas P. Chivers^b, Matthew D. Mitchell^c, Maud C. O. Ferrari^c

^a ARC Centre of Excellence for Coral Reef Studies, College of Science and Engineering, James Cook University, Townsville, Queensland, Australia

^b Department of Biology, University of Saskatchewan, SK, Canada

^c Department of Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, SK, Canada

ARTICLE INFO

Article history:

Received 27 January 2017

Initial acceptance 7 March 2017

Final acceptance 30 August 2017

MS. number: 17-00095R

Keywords:

acute risk

background risk

coral reef fish

escape response

plasticity

predator–prey interactions

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.

© 2017 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

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

* Correspondence: Ryan A. Ramasamy, ARC Centre of Excellence for Coral Reef Studies, College of Science and Engineering, 1 James Cook Drive, James Cook University, Townsville, Queensland 4811, Australia.

E-mail address: ryan.ramasamy@my.jcu.edu.au (R. A. Ramasamy).

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 ($14^{\circ}40'S$, $145^{\circ}28'E$), 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 \times 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 \times 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 \times 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.

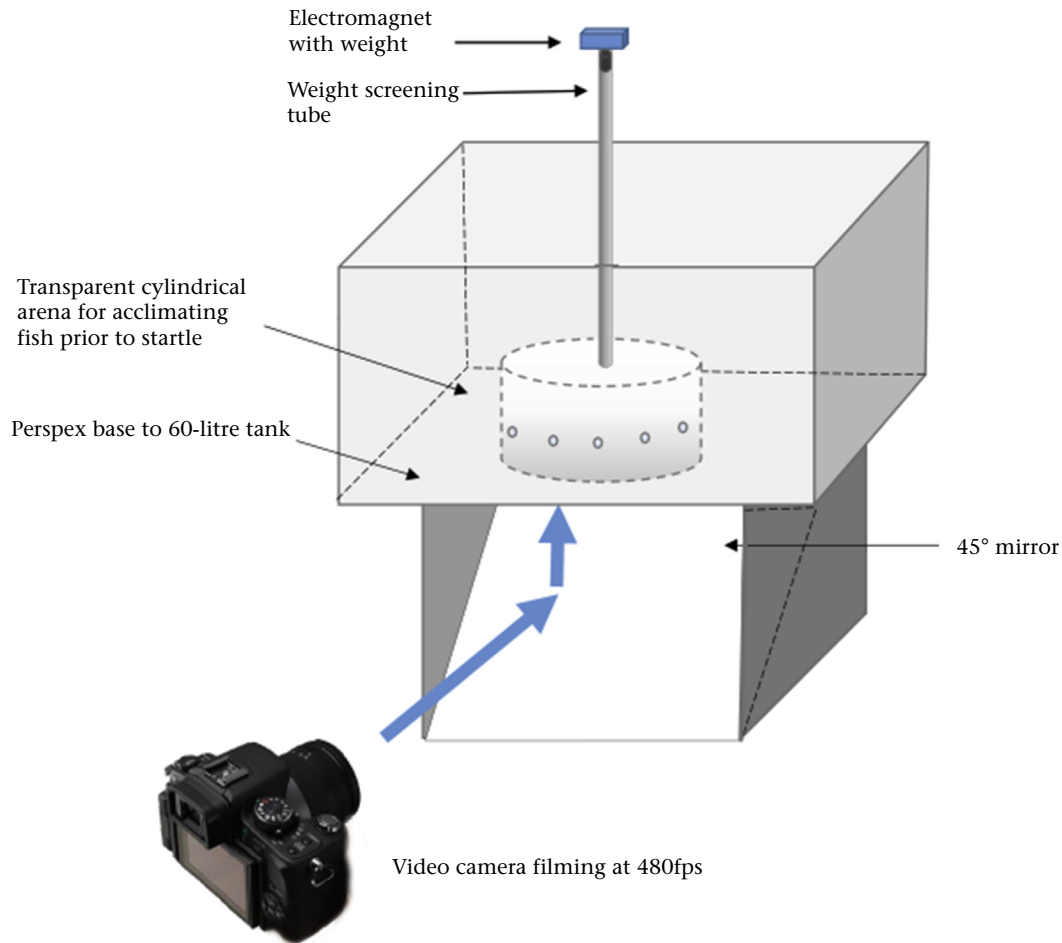


Figure 1. Test arena for testing the escape responses of *A. polyacanthus* juveniles.

(1) Response latency (s) was measured as the time interval between the stimulus touching the water surface and the first detectable movement of the fish during stage 1 (i.e. the commencement of the C-bend).

(2) Response duration (s) was measured as the elapsed time from the start to the end of the escape response (i.e. when the fish has stopped swimming and there is no more forward momentum for 10 ms).

(3) Response distance (m) was a measure of the total distance covered by the fish from the onset of the escape response to the end (i.e. when the fish comes to a halt).

(4) Mean response speed (m/s) was measured as the distance covered within a fixed time (first 29 ms after the initial response), which corresponds to the average duration of the first two tail flips (the first two axial bends, i.e. stages 1 and 2 based on Domenici & Blake, 1997). This period is considered crucial for avoiding predator ambush attacks (Domenici & Blake, 1997).

(5) Maximum response speed (m/s) was measured as the maximum speed reached at any point during the escape response.

(6) Maximum acceleration (m/s^2) was measured as the maximum acceleration within a fixed time (first 29 ms after initial response).

(7) Turning angle ($^\circ$) was measured as the total angle created during stage 1 of the response between the head and the middle of the fish.

Statistical Analysis

All fish tested responded to the stimulus and performed one of two responses. Therefore, data were split into two groups: C-starters (i.e. commencement of the escape response resulting in the individual forming a C-shape) and non-C-starters (i.e. fish that swam backwards away from the stimulus while facing it). Given the variation in the occurrence of C-start responses, we first conducted a logistic regression testing the effect of background risk (low versus high) and acute risk (low versus high) on the occurrence of C-start and non-C-start responses (binomial data).

Given the high level of correlation among the kinematic variables ($r > 0.38$), latency, mean escape speed, maximum escape speed, maximum acceleration and response duration and response distance were reduced to two synthetic variables explaining 83% of total variance using a correlation matrix-based PCA. The first eigenvector (PC1), which explained 66% of the variance, loaded heavily and positively on the speed and acceleration variables and negatively on latency (Table 1). We coined this synthetic variable the 'velocity component' of the escape response. The second eigenvector (PC2) explained 17% of the variance and loaded primarily and positively on response duration and distance, and we referred to this variable as the 'effort component' of the response. These two orthogonal variables were used to test the effect of background and acute risk on the escape performance of

Table 1
Principal component analysis loadings for component 1 (PC1; 66%) and component 2 (PC2; 17%)

	Component 1 (PC1)	Component 2 (PC2)
Latency	−0.701	0.114
Response duration	−0.650	0.688
Response distance	0.637	0.721
Mean response speed	0.971	0.002
Maximum acceleration	0.875	0.016
Maximum response speed	0.966	0.053

the fish. In addition, we also included response type (C-start versus non-C-start) as a factor in the analysis to tease apart variation due to response type (C-starts are typically faster) and risk treatments.

Finally, the turning angle variable, available only for fish performing C-starts, was analysed using a two-way ANOVA to examine the effect of background and acute risk on the response. The data were square-root transformed to improve normality.

RESULTS

Escape Type

The dichotomy between escape types did not appear in the overall analysis as the increased duration and increased distance both loaded positively on PC2, but were opposite on PC1: distance was positively correlated with PC1 scores, while duration was negatively correlated with PC1 (Table 1). There was a significant interaction between background and acute risk on the frequency of fish displaying C-start responses when startled (Wald $\chi^2_1 = 11.1$, $P = 0.001$; Fig. 2). Namely, low-risk background fish exposed to acute high risk were more likely to C-start than those exposed to acute low risk only (acute risk: Wald $\chi^2_1 = 4.9$, $P = 0.028$). However, when high-risk background fish were startled, those exposed to acute high risk were less likely to C-start than those exposed to acute low risk (acute risk: Wald $\chi^2_1 = 6.2$, $P = 0.012$).

Velocity Component of the Escape Response (PC1)

Unsurprisingly, the velocity component of the C-starters' escape response was significantly higher than that of non-C-starters (three-way ANOVA, response type: $F_{1,72} = 168$, $P < 0.001$; Fig. 3). Also, fish displayed higher velocity in the presence of acute high

risk (i.e. alarm cues; acute risk: $F_{1,72} = 4.5$, $P = 0.038$), regardless of the type of response they displayed (response type*acute risk: $F_{1,72} = 2.2$, $P = 0.14$), in both background risk levels (response type*acute risk*background risk: $F_{1,72} = 0.5$, $P = 0.47$). The effect of background risk on escape performance depended on the type of response used (background risk*response type: $F_{1,72} = 8.0$, $P = 0.006$), but not on the acute risk level (background risk*acute risk: $F_{1,72} = 0.2$, $P = 0.65$). High-risk background exposure led C-starters to display greater velocity than those kept under low-risk background conditions (post hoc: $F_{1,34} = 7.7$, $P = 0.009$) but this enhancing effect did not occur for non-C-starters (post hoc: $F_{1,38} = 0.1$, $P = 0.8$).

Effort Component of the Escape Response (PC2)

Overall, the effort component of the escape was higher in the presence of alarm cues (three-way ANOVA; acute risk: $F_{1,72} = 6.3$, $P = 0.015$; Fig. 4), but this effect was not dependent on background risk (acute risk*background risk: $F_{1,72} = 1.4$, $P = 0.24$) or on the type of response performed (acute risk*response type: $F_{1,72} = 0.1$, $P = 0.7$), or on any interaction among those three factors (acute risk*background risk*response type: $F_{1,72} = 0.3$, $P = 0.9$). The enhancing effects of high background risk only occurred for C-starters (background risk*response type: $F_{1,72} = 8.9$, $P = 0.004$), which demonstrated greater effort than their low-risk background counterparts (post hoc: $F_{1,34} = 8.7$, $P = 0.006$). The performance of non-C-starters was not affected by background risk levels (post hoc: $F_{1,38} = 2.5$, $P = 0.12$).

Turning Angle

The two-way ANOVA revealed no statistical difference in the turning angles of the fish performing C-starts (background risk: $F_{1,34} = 1.9$, $P = 0.18$; acute risk: $F_{1,34} = 1.9$, $P = 0.18$, background risk*acute risk: $F_{1,34} = 4.0$, $P = 0.053$, $N = 4–15$ /treatment; Fig. 5).

DISCUSSION

Our results revealed surprising sophistication in the way risk perception modulated escape responses. We found an intriguing pattern of responses when it came to fish displaying C-starts, often considered the most effective way to avoid a predator (Webb, 1981). Fish exposed to high risk, whether through background risk treatment or acute exposure, increased the frequency with which they displayed C-starts, when compared to low-risk controls. This

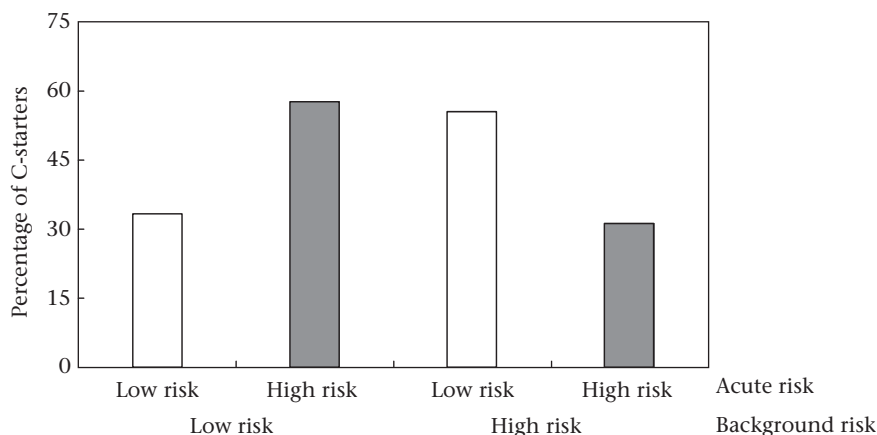


Figure 2. Percentage of fish displaying C-start responses within each treatment group. Fish were maintained under low-risk or high-risk conditions for 4 days (background risk), and were then exposed to an acute stressor (low risk: empty bars; high risk: grey bars) minutes prior to being startled ($N = 15–25$ /treatment).

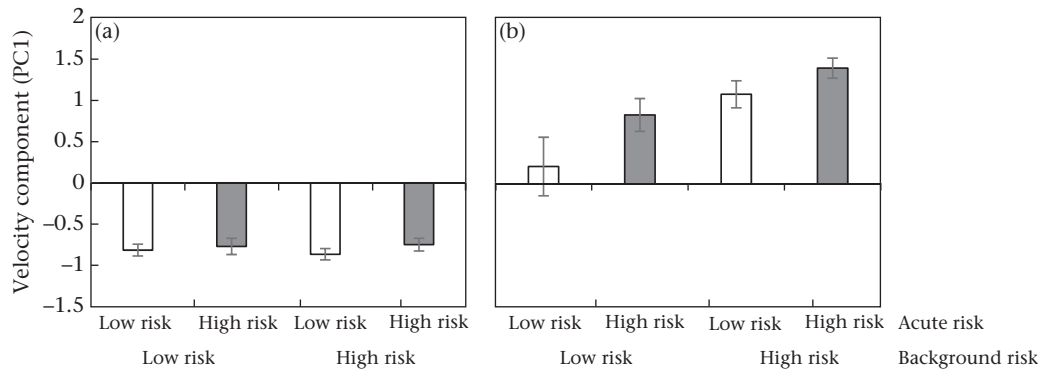


Figure 3. Mean \pm SE scores from the first eigenvector (PC1; explained 66% of total variance) loading on the speed and acceleration aspect of the escape responses in (a) non-C-starters and (b) C-starters. Fish were maintained under low-risk or high-risk conditions for 4 days (background risk), and were then exposed to an acute stressor (low risk: empty bars; or high risk: grey bars) minutes prior to being startled.

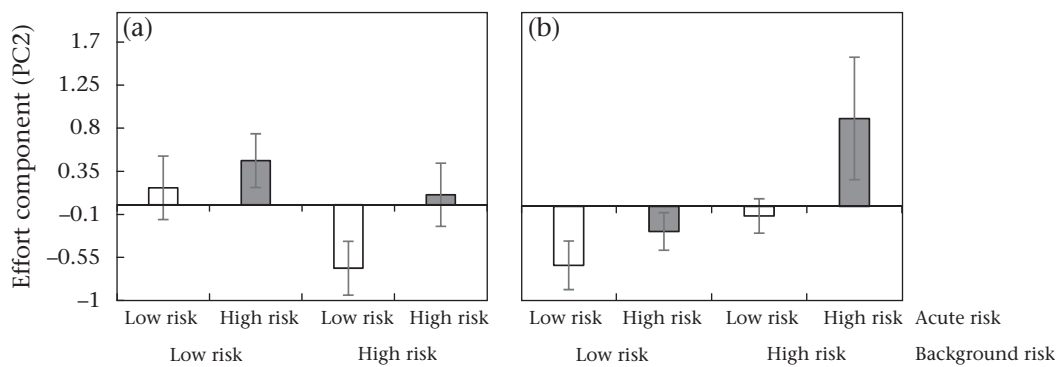


Figure 4. Mean \pm SE scores from the second eigenvector (PC2; explained 17% of total variance) loading on the duration and distance achieved during the escape response in (a) non-C-starters and (b) C-starters. Fish were maintained under low-risk or high-risk conditions for 4 days (background risk), and were then exposed to an acute stressor (low risk: empty bars; high risk: grey bars) minutes prior to being startled.

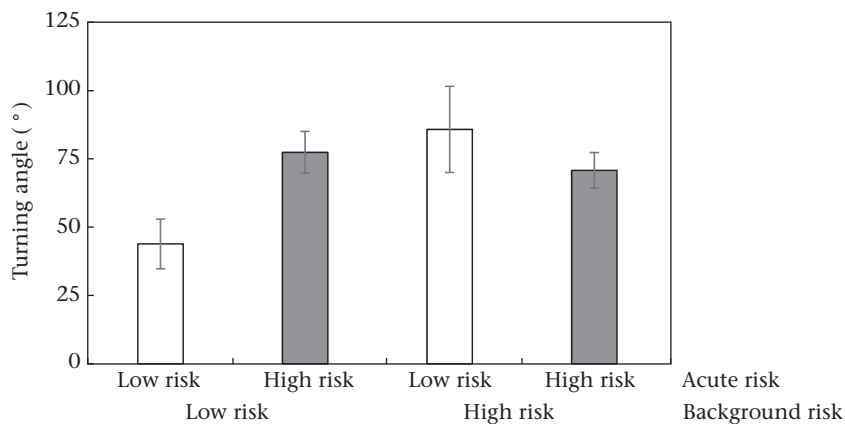


Figure 5. Mean \pm SE turning angle of fish performing C-starts. Fish were maintained under low-risk or high-risk conditions for 4 days (background risk), and were then exposed to an acute stressor (low risk: empty bars; high risk: grey bars) minutes prior to being startled.

result seems logical as C-starts, which are faster in nature than other escape responses, may improve survival odds (O'Steen, Cullum, & Bennett, 2002). However, when presented with both stressors, the proportion of fish displaying C-starts was lower, possibly due to the acclimation to the high-risk background and a change in the way high-risk-exposed fish optimize their escape response.

Fish exposed to both high-risk background and acute high risk are probably expecting an imminent attack. Owing to the high costs

of disrupting normal daily activities, fish from high-risk backgrounds may benefit from setting the threshold for a fast start higher than in a low-risk background. Hence fish under a high-risk background may require more reliable indicators of attacks before C-starting than fish used to a low-risk background. When we investigated the components of their escape responses, we first found that, as expected, C-starters displayed responses that were faster, in terms of response speed when compared to non-C-starters (see Appendix Fig. A1). The effort component, which

encompassed response duration and distance, was affected by both background risk and acute risk conditions, in an additive manner. When we look at each component of this response (Appendix Fig. A1), it is clear that the highest risk group (i.e. high-risk background + acute high risk) displayed C-start responses with the shortest latencies that were among the fastest and longest responses among C-starters, resulting in an escape distance significantly longer than for other groups. The enhanced response would increase the chances of survival from predatory attacks (Ramasamy et al., 2015). This supports Lima and Bednekoff's (1999) risk allocation hypothesis. They explained that animals make decisions based on a reflection of the trade-offs for a given activity. Thus, when exposed to an alarm cue prior to being startled, the high-risk individuals that backed away instead of performing a C-start may have perceived the risk of the alarm cue to be insufficient to employ the high-energy swimming burst. A previous study on Western mosquitofish, *Gambusia affinis*, showed that individuals coexisting with predators performed C-starts more often than *G. affinis* from predator-free populations (Langerhans et al., 2004). Another factor to take into account might be that fish did not perceive the threat of the stimulus weight as strong enough. Eaton and Farley (1975) showed that the intensity of stimuli can affect the latency of the Mauthner cell stimulation. Perhaps the action potential generated by the Mauthner cell was affected due to the weak stimulus that the fish were exposed to, resulting in a reduced escape response.

Risk has been shown to affect the physiology of fishes, especially their levels of stress hormones such as cortisol, and the way they partition energy. A recent review of the influence of predator pressure on the hypothalamus-pituitary-adrenal/hypothalamus-pituitary-interrenal axis (HPA/HPI) indicated that, while acute exposure to predation risk elevated cortisol in most published instances (72 of 99 studies), this was only the case for about 50% (36 of 68 studies) of the cases where vertebrates were exposed to long-term predation threat (Harris & Carr, 2016). Chronic activation of the sensory pathways that inform the HPA/HPI axis about predators may result in classical habituation of neuronal pathways and consequently of behavioural responses (Grissom & Bhatnagar, 2009). This may have occurred for some individuals in response to our high-risk background treatment; a high level of individuality in adrenocortical responsiveness and the capacity to habituate to chronic stress was found in rats, *Rattus norvegicus* (Natelson et al., 1988) and this may also be the case for fishes. Juvenile coral reef fishes have extremely high metabolic rates (Nilsson, Ostlund-Nilsson, Penfold, & Grutter, 2007) and there is likely to be a strong selective pressure to be adaptable to predation threat as reductions in foraging on timescales of hours may have major repercussions for growth and survival due to their relatively low energy reserves. Thus, a risk-driven change in energy allocation may affect the algorithm used to determine when to maximize their escape response, in keeping with the 'Economic Hypothesis' described by Ydenberg and Dill (1986).

For the fish that did not display C-start escapes, we observed a different overall strategy of swimming backwards. For non-C-starters, their escape responses were affected by acute risk conditions, but not background risk (see Appendix Fig. A1). Typically, fish exposed to acute high risk (i.e. alarm cues) responded for longer and escaped further than those exposed to acute low risk (i.e. saltwater). This may be because the non-C-starters represent those fish that have habituated to the high-risk background environment and have effectively reset their judgement of baseline risk. Mirza, Mathis, and Chivers (2006) found juvenile rainbow trout, *Oncorhynchus mykiss*, that were exposed to risky conditions regularly responded with significantly reduced antipredator responses. By backing away from their original position, non-C-starters might have been attempting to maintain visual contact with the source of

the disturbance. Additionally, the lack of a response could be attributed to physiological stress. In treatments involving high-risk background fish paired with acute high-risk cues, these individuals may have had increased levels of cortisol such that it became detrimental to their performance (Maule, Schreck, Bradford, & Barton, 1988; Schreck, 2000). Deepening our understanding of the physiological mechanisms responsible for the variations in escape strategy performances may provide some insights into the impact of environmental factors (food, risk, etc.) on such variation.

Overall, our findings support the notion that escape responses have an inherent level of plasticity that could be driven by abiotic and biotic environmental factors, such as predation. A recent study demonstrated that spiny chromis adjust their level of responsiveness in their escape response to the perceived level of current risk from the sight or smell of a known predator (Ramasamy et al., 2015). Another damselfish, *Pomacentrus chrysurus*, has also been shown to optimize its escape response kinematics based on information concerning the identity of specific predators (McCormick & Allan, 2016). Our results provide further evidence that escape responses are highly plastic, context dependent and species specific (Domenici & Batty, 1997; Domenici, 2010; McCormick & Allan, 2016; Ramasamy et al., 2015; Webb, 1986). While stress (cortisol level) could be a proximate mechanism for the variation seen, the relationship between stress and performance is unlikely to be linear (Schreck, 2000). Fish from high-risk backgrounds may have had greater levels of cortisol priming their 'flight and fight' response system (Henry, 1993). As previously mentioned, those in the highest risk treatment may have increased concentrations of cortisol reaching a level that was detrimental to their performance (Maule et al., 1988; Schreck, 2000). However, the conditions we created would not exceed the predation situation in the wild, where such juvenile fish are exposed to a myriad of predators much more often than three times a day, the risk schedule to which our fish were exposed. Indeed, at the Lizard Island Research Station studies have shown two territorial predators (the lizardfish, *Synodus englemani*, and the dottyback, *Pomacentrus fuscus*) strike at prey on average 1.7 times/h and 10.5 times/h, respectively (Feeney et al., 2012; Sweatman, 1984). Predation on juvenile damselfishes can be intense with upwards of 60–90% mortality at settlement (Almany & Webster, 2006). It is possible that fish that failed to match threats with an appropriate response (i.e. an optimized C-start when there is extreme risk) could be easily preyed upon in nature. The notion that a high-risk background results in lower responses is already well established in the form of the risk allocation hypothesis (Ferrari, Sih, & Chivers, 2009; Lima & Bednekoff, 1999). This model predicts that prey exposed to increased risk, in either intensity or frequency, cannot afford to increase their response intensity linearly, as other competing demands, like foraging, would take precedence over hiding. Instead, prey allocate their maximum antipredator effort during periods of highest perceived risk, and maximize foraging efforts during periods of highest perceived safety. This 'perceived safety' is what can be confusing, as one individual's perception of safety in a particular habitat at a particular time might be another individual's highest risk period, making the two responses appear contradictory. The apparent decrease in performance from the highest risk groups might be couched in light of risk allocation, as an arena devoid of visual predation risk (visual cues being the sensory input with the highest level of reliability) might be considered a 'safe' place for predation-primed fish. Therefore, the decrease in performance may be attributed to fish 'waiting' for a visual cue since alarm cues were not identified as a valid threat. Indeed, the low frequency of C-starts in what could be considered the highest risk group (high background risk plus high acute risk) may be explained by the fact that fish are simultaneously trying to increase the correct response to a real threat (flee when

there is a predator: true positive) and reduce the incorrect response to a fake threat (flee when there is no real risk: false positive).

We have established that background risk influences escape performance for fish tested in isolation. However, the spiny chromis is a schooling fish and individuals located at different places in the school probably perceive their risk of predation differently (Herbert-Read, Buhl, Hu, Ward, & Sumpter, 2015). Future work should consider how background level of risk and acute risk interact in such a dynamic spatial environment.

Results from this study have highlighted the effects of varying levels of risk on escape responses, with background level of risk affecting some response types but not others, while acute risk cues appeared to universally increase fish performance in response to a weight drop stimulus. However, there is little information pertaining to how varying risk histories (e.g. 2 days or 8 days of high-risk conditions instead of 4 days) and ontogenetic stages (i.e. adults instead of juveniles) affect escape responses. Escape responses may be further influenced by these variables as they influence an individual's perceived level of risk. In the spatially and temporally variable environment of a coral reef, it would be interesting to uncover how changes to one's risk regime influence escape responses.

Acknowledgments

We thank the staff at Lizard Island Research Station for logistical assistance during this study. We would also like to thank the Australian Research Council's Centre of Excellence for Coral Reef Studies and the Natural Sciences and Engineering Research Council of Canada for funding this study.

References

- Allan, B. J. M., Domenici, P., McCormick, M. I., Watson, S., & Munday, P. (2013). Elevated CO₂ affects predator-prey interaction through performance. *PLoS One*, 8(3), e58520.
- Almany, G. R., & Webster, M. S. (2006). The predation gauntlet: Early post-settlement mortality in reef fishes. *Coral Reefs*, 25(1), 19–22.
- Bernard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology and Systematics*, 35, 651–673.
- Brönmark, C., & Petterson, L. B. (1994). Chemical cues from piscivores induce change in morphology in crucian carp. *Oikos*, 70, 396–402.
- Brown, G. E., Ferrari, M. C. O., Elvidge, C. K., Ramnarine, I., & Chivers, D. P. (2013). Phenotypically plastic neophobia: A response to variable predation risk. *Proceedings of the Royal Society B: Biological Sciences*, 280(1787), 20122712.
- Chivers, D. P., McCormick, M. I., Mitchell, M. D., Allan, B. J. M., Gonçalves, E. J., Bryshun, R., et al. (2016). At odds with the group: Changes in lateralization and escape performance reveal conformity and conflict in fish schools. *Proceedings of the Royal Society: Biology*, 283. <https://doi.org/10.1098/rspb.2016.1127>.
- Chivers, D. P., McCormick, M. I., Mitchell, M. D., Ramasamy, R. A., & Ferrari, M. C. O. (2014). Background level of risk determines how prey categorize predators and non-predators. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140355.
- Chivers, D. P., Mitchell, M. D., Lucon-Xiccato, T., Brown, G. E., & Ferrari, M. C. O. (2016). Background risk influences learning but not generalization of predators. *Animal Behaviour*, 121, 185–189.
- Chivers, D. P., Zhao, X., Brown, G. E., Marchant, T. A., & Ferrari, M. C. O. (2008). Predator-induced changes in morphology of a prey fish: The effects of food level and temporal frequency of predation risk. *Evolutionary Ecology*, 22, 561–574.
- Domenici, P. (2010). Context-dependant variability in the components of fish escape response: Integrating locomotor performance and behaviour. *Journal of Experimental Zoology Part A: Ecology Genetics and Physiology*, 313A(2), 59–79.
- Domenici, P., & Batty, R. S. (1997). Escape behaviour of solitary herring (*Clupea harengus*) and comparisons with schooling individuals. *Marine Biology*, 128, 29–38.
- Domenici, P., & Blake, R. W. (1997). The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology*, 200(8), 1165–1178.
- Eaton, R. C., & Farley, R. D. (1975). Mauthner neuron field potential in newly hatched larvae of the zebra fish. *Journal of Neurophysiology*, 38(3), 502–512.
- Feeney, W. E., Lönnstedt, O. M., Bosiger, Y., Martin, J., Jones, G. P., Rowe, R. J., et al. (2012). High rate of prey consumption in a small predatory fish on coral reefs. *Coral Reefs*, 31(3), 909–918.
- Ferrari, M. C. O., McCormick, M. I., Allan, B. J. M., Choi, R., Ramasamy, R. A., & Chivers, D. P. (2015). The effects of background risk on behavioural lateralization in a coral reef fish. *Functional Ecology*, 29, 1553–1559.
- Ferrari, M. C. O., McCormick, M. I., Allan, B. J. M., Choi, R., Ramasamy, R. A., Johansen, J. L., et al. (2015). Living in a risky world: The onset and ontogeny of an integrated antipredator phenotype in a coral reef fish. *Scientific Reports*, 5, 15537.
- Ferrari, M. C. O., McCormick, M. I., Meekan, M. G., & Chivers, D. P. (2015). Background level of risk and the survival of predator-naïve prey: Can neophobia compensate for predator naivety in juvenile coral reef fishes? *Proceeding of the Royal Society Biology*, 282, 20142197.
- Ferrari, M. C. O., Sih, A., & Chivers, D. P. (2009). The paradox of risk allocation: A review and prospectus. *Animal Behaviour*, 78, 579–585.
- Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress: Get used to it. *Neurobiology of Learning and Memory*, 92, 215–224.
- Harris, B. N., & Carr, J. A. (2016). The role of the hypothalamus-pituitary-adrenal/interrenal axis in mediating predator-avoidance trade-offs. *General and Comparative Endocrinology*, 230, 110–142.
- Henry, J. P. (1993). Biological basis of the stress response. *American Physiological Society*, 8(2), 69–73.
- Herbert-Read, J. E., Buhl, J., Hu, F., Ward, A. J., & Sumpter, D. J. (2015). Initiation and spread of escape waves within animal groups. *Royal Society Open Science*, 2(4), 140355.
- Langerhans, R. B., Layman, C. A., Shokrollahi, A. M., & DeWitt, T. (2004). Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution*, 58(10), 2305–2318.
- Lima, S. L., & Bednekoff, P. A. (1999). Temporal variation in danger drives anti-predator behavior: The predation risk allocation hypothesis. *American Naturalist*, 153(6), 649–659.
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation – a review and prospectus. *Canadian Journal of Zoology*, 68(4), 619–640.
- Lönnstedt, O. M., McCormick, M. I., & Chivers, D. P. (2013). Predator-induced changes in the growth of eyeballs and false eyespots. *Scientific Reports*, 3, 2259.
- Manassa, R. P., & McCormick, M. I. (2012). Social learning and acquired recognition of a predator by a marine fish. *Animal Cognition*, 15(4), 559–565.
- Marras, S., & Domenici, P. (2013). Schooling fish under attack are not all equal: Some lead, others follow. *PLoS One*, 8(6), e65784.
- Maule, A. G., Schreck, C. B., Bradford, C. S., & Barton, B. A. (1988). Physiological effects of collecting and transporting emigrating juvenile chinook salmon past dams on the Columbia River. *Transactions of the American Fisheries Society*, 117, 245–261.
- McCormick, M. I., & Allan, B. J. M. (2016). Lionfish misidentification circumvents an optimised escape response by prey. *Conservation Physiology*, 4(1). <https://doi.org/10.1093/conphys/cow064>.
- Mirza, R. S., Mathis, A., & Chivers, D. P. (2006). Does temporal variation in predator risk influence the intensity of antipredator responses? A test of the risk allocation hypothesis. *Ethology*, 112, 44–51.
- Moyes, C. D., Schulte, P. M., & Andwest, T. G. (1993). Burst exercise recovery metabolism in fish white muscle. In P. W. Hochachka (Ed.), *Surviving hypoxia: Mechanisms of control and adaptation* (pp. 527–539). Boca Raton, FL: CRC Press.
- Natelson, B. G., Ottenweller, J. E., Cook, J. A., Pitman, D., McCarty, R., & Tapp, W. N. (1988). Effect of stressor intensity on habituation of the adrenocortical stress response. *Physiology and Behavior*, 43, 41–46.
- Nilsson, G. E., Ostlund-Nilsson, S., Penfold, R., & Grutter, A. S. (2007). From record performance to hypoxia tolerance: Respiratory transition in damselfish larvae settling on a coral reef. *Proceedings of the Royal Society: Biology*, 274, 79–85.
- O'Steen, S., Cullum, A. J., & Bennett, A. F. (2002). Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, 56(4), 776–784.
- Palacios, M. M., Killen, S. S., Nadler, L. E., White, J. R., & McCormick, M. I. (2016). Top-predators negate the effect of mesopredators on prey physiology. *Journal of Animal Ecology*, 85(4), 1078–1086. <https://doi.org/10.1111/1365-2656.12523>.
- Preisser, E. L., Bolnick, D. L., & Bernard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86, 501–509.
- Ramasamy, R. A., Allan, B. J. M., & McCormick, M. I. (2015). Plasticity of escape responses: Prior predator experience enhances escape performance in a coral reef fish. *PLoS One*, 10(8), e0132790.
- Schreck, C. B. (2000). Accumulation and long-term effects of stress in fish. In G. P. Moberg, & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications in animal welfare* (pp. 147–158). New York, NY: CABL.
- Sillar, K. T. (2009). Mauthner cells. *Current Biology*, 19(9), 353–355.
- Sweatman, H. P. A. (1984). A field study of the predatory behaviour and feeding rate of a piscivorous coral reef fish the lizardfish, *Synodus nglemani*. *Copeia*, 1, 187–194.
- Webb, P. W. (1981). Responses of northern anchovy, *Engraulis mordax*, larvae to predation by a biting planktivore, *Amphiprion percula*. *Fishery Bulletin*, 79(4).
- Webb, P. W. (1986). Effect of body form and response threshold on the vulnerability of 4 species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). *Canadian Journal of Fish and Aquatic Sciences*, 43, 763–771.
- Ydenberg, R. C., & Dill, L. M. (1986). The economics of fleeing from predators. *Advances in the Study of Behavior*, 16, 229–249.

Appendix

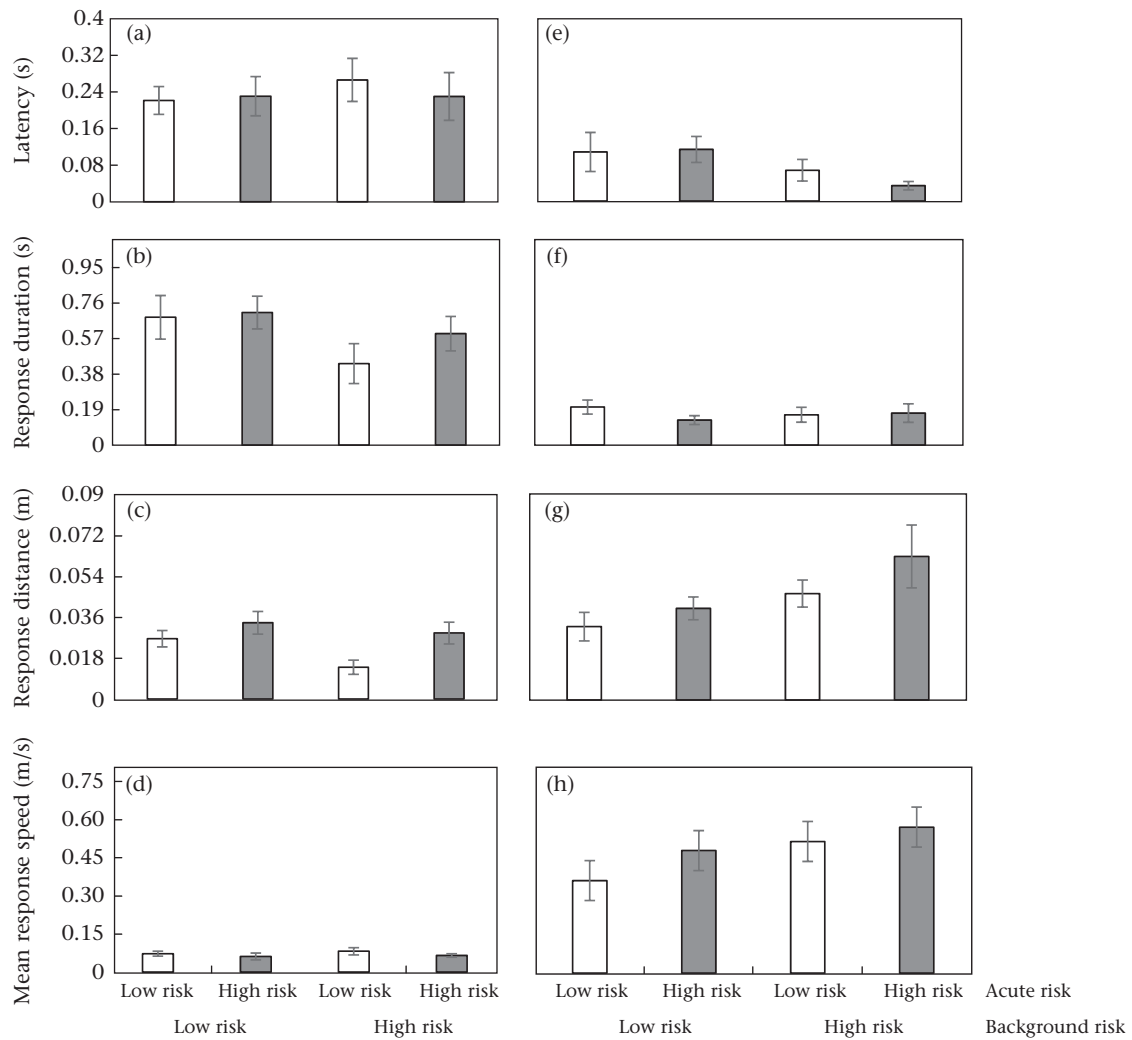


Figure A1. Mean \pm SE (a, e) latency to respond, (b, f) response duration, (c, g) response distance, (d, h) mean response speed, during the escape responses in (a–d) non-C-starters and (e–h) C-starters. Fish were maintained under low-risk or high-risk conditions for 4 days (background risk) and exposed to an acute stressor (low risk: empty bars; high risk: grey bars) minutes prior to being startled.