Daily variation in behavioural lateralization is linked to predation stress in a coral reef fish

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Behavioural lateralization, the preferential use of one side of the body or one of the limbs, is a trait common in vertebrates, and is often expressed as a turning bias in fishes. Recent studies have demonstrated considerable plasticity in lateralization, inferring the role of predation pressure as a key driver of this plasticity over short periods (i.e. days). Such plasticity may be expected if predator pressure is highly variable through space and time and can provide prey with a distinct advantage. We know that increased turning bias is linked with better escape performance, but we do not know the extent to which prey can adjust this trait to match temporal variation in risk. Here we trained juvenile Ambon damselfish to recognize different temporal patterns of risk throughout the day and asked whether their degree of lateralization changed according to this pattern. Damselfish that were taught that midday was risky showed a stronger turning bias at midday than in the evening, whereas damselfish that were taught that evening was risky showed a stronger turning bias in the evening than at midday. Subsequently, we found that fish exposed to cortisol expressed a greater degree of turning bias. Our results suggest that predation stress may be a key factor determining the degree of behavioural lateralization in vertebrates.

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recognition learning (Chivers et al., 2017). Moreover, increased lateralization is positively correlated with escape performance (Chivers et al., 2016; Dadda, Koohlaas, & Domenici, 2010). However, higher lateralization tendencies are associated with poorer competitive abilities in a coral reef fish (Chivers et al., 2017). Hence, the resulting expression of this trait likely represents a fine balance between the cost and benefits it provides.

One context for which costs appear particularly high is predation. Prey often have a side bias in response to threatening stimuli. Birds and reptiles show an eye preference to look at predators (Koboroff, Kaplan, & Rogers, 2008; Martin, López, Bonati, & Csermely, 2010). For example, some prey have greater detection and escape performance when predators are detected in their left visual field (Austin & Rogers, 2007; Shibasaki, Nagumo, & Koda, 2014). Many show a turning bias in their escape response (Bonati, Csermely, López, & Martin, 2010; Lippolis, Bisazza, Rogers, & Vallortigara, 2002; Yamashita, Naitoh, & Wassersug, 2000). Given that predators are just as likely to appear and attack from either side, one would expect that the trait would be at a selective disadvantage in high-risk environments. However, evidence suggest that under high predation risk conditions, prey tend to display stronger lateralization (Brown, Gardner, & Brathwaite, 2004; Ferrari, McCormick, Allan, Choi, Ramasamy, Johansen et al., 2015). Perhaps faster escape responses associated with increased lateralization explain this paradox. Lateralization is a trait that appears to be highly plastic, as prey exposed to high predation risk show stronger lateralization tendencies than those exposed to low or no risk (Broder & Angeloni, 2014; Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015; Jozet-Alves & Hébert, 2013). Moreover, such an increase in laterality appears to be linked to increased survival during predator—prey encounters (Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015), although the covariance of multiple traits in response to risk makes it difficult to credit the survival benefits to increased lateralization only (Ferrari, McCormick, Allan, Choi, Ramasamy, Johansen et al., 2015). In concert, these studies suggest that the benefit from lateralization in a predation context must outweigh the cost of sensorial asymmetry.

Recent evidence on the inductive aspect of these traits (Broder & Angeloni, 2014; Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015; Jozet-Alves & Hébert, 2013) implies a cost that is avoided under low-risk conditions. How plastic should the trait be in order to be beneficial? Juvenile whitetip damselfish, Pomacentrus chrysurus, exposed to injured conspecific cues for only 4 days showed increased behavioural lateralization tendencies over their low-risk counterparts (Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015). Data on wild-caught yellow-and-blueback fusiliers, Caesio teres, a schooling fish common on coral reefs, indicate that lateralization may in fact change from day to day, as fish showed a gradual decrease in strength of lateralization over the 4 days they were held in the absence of risk (Chivers et al., 2016). In the present study, we investigated the possibility that behavioural lateralization could change in response to diel patterns of predation risk. Several species of prey adjust the intensity of their behavioural response to predators based on the time of day that the predator is actively foraging. For example, woodfrog tadpoles, Lthobates sylvaticus, taught to avoid salamanders, Ambystoma tigrinum, in the morning show much stronger responses to salamanders in the morning than in the evening, whereas tadpoles that were taught that salamanders were a high risk in the evening responded more to salamander cues in the evening than in the morning (Ferrari, Messier, & Chivers, 2008). Similar temporal patterns of behavioural responses to predators are known in lemon damselfish, Pomacentrus moluccensis, that learn foraging patterns of rockcod (Cephalopholis cyanostigma) predators (Bosiger, Lonstedt, McCormick, & Ferrari, 2012).

Using a well-established protocol (Bosiger et al., 2012; Ferrari et al., 2008), we exposed juvenile Ambon damselfish, Pomacentrus amboinensis, to one of two predictable patterns of risk for 9 days, with risk peaking either at noon or in the evening. The fish from both groups were then tested both at noon and in the evening. We hypothesized that, if behavioural lateralization is an inducible trait expressed in response to risk, then the expression of lateralization should follow the risk pattern the fish were exposed to, with stronger turning bias tendencies observed at the time of day that is perceived as the most risky. To further investigate a potential mechanism responsible for this plasticity, we exposed fish to waterborne cortisol, a stress hormone, or to a sham control, and compared their lateralization.

**METHODS**

**Ethical Note**

All work carried herein followed animal care ethics and was approved by James Cook University protocols A2080, A2005. All fish were released at their capture site at the end of the experiment.

**Test Species**

The Ambon damselfish is a common planktivorous reef species found throughout the Indo-Pacific. As juveniles, they are prey for a variety of predators, including wrasses, lizardfishes and dotb Aircrafts. We collected juvenile Ambon damselfish from reefs surrounding the Lizard Island Research Station (14° 40’S, 145° 28’E), Great Barrier Reef, Australia in March 2015. Fish were captured on SCUBA using clove oil and hand-nets. The fish were transported back to the laboratory and held in 30-litre flow-through tanks, where they were fed brine shrimp and pellets three times per day.

**Experiment 1: Temporal Variation in Behavioural Lateralization**

The goal of the first experiment was to test whether fish exhibit temporal variation in their degree of behavioural lateralization in response to variation in diel predation risk.

**Risk pattern**

We used a well-established technique to create predictable variation in predation risk (Ferrari & Chivers, 2009; Ferrari, Manek, & Chivers, 2010), exposing fish to injured conspecific cues (i.e. alarm cues) at the time of day that was risky, and exposing them to water (low-risk disturbance) at the time of day that was less risky. We created to two opposite risk patterns to control for natural diel variation in antipredator responses in fish.

Alarm cues are chemicals located in the skin of many aquatic species and are known to elicit dramatic antipredator responses when detected by nearby conspecifics. Given that these cues can only be released in the water column via mechanical damage to the skin, as would occur during a predator attack, they represent a highly reliable indicator of risk (reviewed in Ferrari, Wisenden, & Chivers, 2010). A wide variety of taxa are known to possess and respond to these alarm cues, including our test species (Ferrari et al., 2011). Alarm cues were prepared by sacrificing five fish (via cold shock followed by pithing) and making eight superficial vertical cuts on either side of the body using a scalpel. The fish were then rinsed in 40 ml of sea water. Each risk exposure consisted of injecting 5 ml of this solution in the tank, for a final concentration of two cuts per litre, a concentration known to elicit overt antipredator response in our test species (Chivers, McCormick, Mitchell, Ramasamy, & Ferrari, 2014). A 5 ml injection of water served as a low-risk exposure.
Groups of four fish were placed in 16, 5-litre flow-through plastic tanks. Eight tanks were randomly allocated to the ‘high—low’ risk pattern, while the other eight were allocated to the ‘low—high’ risk pattern. The ‘high—low’ risk group received a high risk at noon (three injections of alarm cues between 1100 and 1300 hours) and low risk in the evening (three injections of water between 1600 and 1800 hours). The ‘low—high’ risk group received the opposite risk pattern. Successive injections were separated by a minimum of 30 min to ensure each injection was perceived as a different risk event by the fish. This pattern was maintained for 9 days. The following day, each fish was tested for their behavioural lateralization tendencies at noon and in the evening.

**Behavioural lateralization assessment**

At the end of the treatment period, we conducted two behavioural lateralization trials for each fish. One trial was done during the noon risky period (1100–1300 hours) and one during the evening risky period (1600–1800 hours). To control for carryover effects from repeated testing, half of the fish from each tank were first tested at noon, while the other half were first tested in the evening.

We used a detour test following the methodology of Ferrari, McCormick, Allan, Choi, Ramasamy, and Chivers (2015) following a design from Bisazza, Facchin, Pignatti, and Vallortigara (1998) to test for behavioural lateralization. Briefly, the test chamber consisted of an opaque PVC tank (60 × 30 × 15.4 cm) with a runway in the middle (25 × 3 × 12 cm), and at both ends of the runway (3 cm ahead of the runway), an opaque barrier (12 cm long × 12 cm height) was positioned perpendicular to the orientation of the runway. Water in the tank was 6 cm deep. At the start of each trial, a single fish was introduced into the middle of the runway and left undisturbed for 2 min. The fish was then gently manoeuvred to the starting point of the runway. The fish swam along the runway until it faced the barrier, at which time it had to make a decision to turn left or right around the barrier. To account for any possible asymmetry in the set-up, tests were carried out alternately on the two ends of the runway (Bisazza et al., 1998). To avoid fish taking a ‘familiar route’, the fish entered the runway from a different side from which they exited. Turning was scored by direct observation. The criterion used for scoring was the first turning direction taken by the fish when exiting from the runway. Ten consecutive tests were conducted for each fish. To avoid changes in water temperature and dissolved oxygen levels, both of which have been found to influence neural function (Domenici, Lefrancos, & Shingles, 2007), the tank water was changed every five trials. Water temperature in the experimental tank was maintained at 27–28 °C.

To compare the groups with respect to their left—right preference in the detour test, we calculated a relative lateralization index (L_R) according to the following formula (Bisazza et al., 1998): 

\[ L_R = \frac{(n_{right} - n_{left})}{(n_{left} + n_{right})} \times 100 \]

This index ranges from −100 (completely left-biased) to +100 (completely right-biased). We then calculated the absolute lateralization index (L_A) for each fish, which is simply the absolute value of L_R. The L_A index ranges from 0 (an individual that turned in equal proportion to the right and to the left, no bias) to 100 (an individual that turned in the same direction in all 10 trials). While L_A focused on population-level lateralization (which was not our primary interest and for which we had no a priori hypothesis), L_R allowed us to compare the strength of the lateralization (irrespective of its direction) among groups at the individual level.

**Statistical analysis**

We performed a two-way repeated measures ANOVA, investigating the effect of risk peak time (noon versus evening) on the absolute lateralization of fish tested both at noon and in the evening. The data met assumptions of homoscedasticity. We tested 10 fish in the noon-risk peak group and 15 fish in the evening-risk peak group, for a total of 50 trials. Our sample size was reduced because of an emergency evacuation of the research station due to Cyclone Nathan.

**Experiment 2: Cortisol Effects on Lateralization**

The goal of this experiment was to explore a potential proximate mechanism for the previous results. We tested whether exposure to cortisol, a known stress hormone in fish, could alter the lateralization strength in fish.

**Cortisol exposure**

Groups of three randomly selected fish were placed in each of 30, 1-litre tanks, which contained three pieces of plastic pipe as shelter and an airstone. Half of the tanks were randomly allocated to the cortisol treatment and filled with cortisol water, while the other half was allocated to the sham treatment. The fish were left in that water for 48 h, during which time they were fed twice per day with Artemia.

The cortisol exposure was achieved by preparing batches of cortisol-enhanced water at a concentration of 1 μg/ml of water. This concentration was chosen as it was previously demonstrated to raise cortisol level in fish larvae and, in damselfish, to a level that is considered biologically relevant (Ayson, Kaneko, Hasegawa, & Hirano, 1995; Gagliano & McCormick, 2009; McCormick, 1998, 1999). We measured 0.0045 g of cortisol (17-hydroxycorticoosterone; Sigma–Aldrich, St Louis, MO, U.S.A.) placed into a 120 ml beaker and added 1 ml of 95% ethanol to help dissolution. We added 50 ml of sea water and a stir bar and left the solution to spin for 3 h, to let the alcohol evaporate. Additional sea water was added to reach a final volume of 4.5 litres. A sham batch was prepared following similar steps, with the exception that cortisol was not added.

Following the exposure phase, fish were tested, in a random order, for their lateralization tendencies, following the same bioassay outlined for experiment 1.

**Statistical analysis**

We performed a two-way blocked ANOVA, testing the effect of cortisol (sham versus cortisol) and blocking for testing day (random factor), on the absolute lateralization of fish. We tested 25 fish in each cortisol treatment.

**RESULTS**

**Temporal Variation**

The absolute lateralization scores of the fish were affected by an interaction between risk peak time and testing time (F_{1,23} = 25.9, P < 0.001; Fig. 1). Fish conditioned to the noon-risk peak displayed a stronger lateralization score at noon than in the evening (paired t test: t_9 = 3.2, P = 0.01), while fish conditioned to the evening-risk peak displayed a stronger lateralization score in the evening than at noon (t_{14} = 4.0, P < 0.001). Figure 2 reveals no clear pattern of population-level side preference for right-versus left-side bias.

**Cortisol Manipulation**

Fish displayed a higher lateralization score when exposed to cortisol than when exposed to the sham (F_{1,47} = 6.2, P = 0.016; Fig. 3). Testing day had a significant effect (F_{1,47} = 6.7, P = 0.012).
and in the evening.

Our first experiment indicates that perceived predation risk is enough to induce the change in lateralization. Indeed, no risk cues were given immediately prior to or at the time of testing. Mechanistically, we set out to test the hypothesis that stress would be, if not the cause, at least a part of the mechanism behind variation in the trait expression. The results of experiment 2 indicate that exposure to cortisol, a stress hormone in fish, leads to an increase in the strength of lateralization, as compared to a sham control. Given that cortisol was the only factor differing between the two groups, the difference can only be attributed to the presence of cortisol. This is the first causal link established between stress and lateralization expression. However, stress is a known factor altering brain chemistry (Braastad, 1998), so this link should not be extremely surprising. For instance, a recent study by Byrnes, Pouca, and Brown (2016) discussed the correlation between stress reactivity and lateralization, indicating that fish that were more lateralized tended to score higher in the stress reactivity scale than those that were not, supporting the existence of a link between stress level and lateralization. While their study pointed towards inter-individual variability, ours provides evidence for intra-individual variability as well. However, a recent paper (Ocklenburg, Korte, Peterburs, Wolf, & Güntürkün, 2016) reviewed the literature linking stress to changes in laterality, and concluded that empirical evidence for hormonal control of laterality was unfortunately lacking. We believe that this study will provide further empirical evidence to create a concrete link between stress and laterality.

Lateralization is thought to reflect the loss of symmetry in the processing of information or tasks between our two hemispheres. Our results provide evidence that, although the brain does not reorganize itself on an hourly basis, the expression of this asymmetry is not as fixed as previously thought. This phenotypic plasticity, just like for any other trait, is a reflection of the cost–benefit trade-offs linked to the expression of the trait. During times where the benefits override the costs, the trait is expressed. In the case of predation risk, the trait would appear to provide a survival benefit.
(Ferrari, McCormick, Allan, Choi, Ramasamy, & Chivers, 2015), and hence should be expressed, even in the absence of present risk indicators, since prey tend to err on the side of caution. However, the expression of this trait appears to have a cost. Recent evidence suggests that highly lateralized fish are more likely to lose competitive interactions against nonlateralized fish (Chivers et al., 2017). What is unknown is how the ‘stress’ from different contexts comes into play. Future work needs to characterize stress responses in light of diet variation in risk as well as the competitive environment in which the individuals find themselves. With this information, we can start to dissect the mechanism behind the variation seen in the expression of lateralization.

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References

