The responses of prey fish to temporal variation in predation risk: sensory habituation or risk assessment?

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Predation is an important selection pressure acting on prey behavior. Although numerous studies have shown that when predation risk is high, prey tend to increase vigilance and reduce foraging effort, until recently, few studies have looked at how temporal patterns of risk influence the trade-off between foraging and antipredator behavior. The risk allocation hypothesis predicts that prey should respond strongly to predators that are usually absent, as they can meet their energy demands during safe periods. In contrast, if predators are almost always present, prey need to forage actively even though predators are present, a counter-intuitive prediction for many behavioral ecologists. This decrease in antipredator behavior on increasing exposure to risk has thus far been attributed to sensory habituation. Using cichlids, we show that sensory habituation is likely not the proximate explanation for the reduction in antipredator behaviors in this system. Such responses may rather be the result of adaptive decision making. Key words: antipredator behavior, foraging, habituation, risk allocation, trade-offs. [Behav Ecol 21:532–536 (2010)]

Predation is a pervasive selection force influencing many aspects of an individual’s behavior, such as when and where to forage or which mate to choose (Lima and Dill 1990). The acquisition of high-quality food items or habitats is often associated with an increased risk of predation; hence, animals have to optimize their behavior in the face of the trade-off between the conflicting demands of resource intake and predator avoidance (Sih 1986). For the past 30 years, behavioral ecologists have attempted to understand the way in which prey weigh the costs of predation against other benefits when choosing which behavioral options to pursue. A great number of studies have provided evidence that prey are indeed able to adjust their behavior in such a way (reviewed by Lima 1998). Although these studies have allowed a tremendous advancement in our understanding of decision making by prey, the temporally variable nature of predation has often been overlooked in research on decision making (Lima and Bednekoff 1999; reviewed by Ferrari et al. 2009).

Predation risk can vary over a range of temporal scales, that is, on a moment-to-moment basis or on a daily, lunar, or seasonal cycle (Sih et al. 2000; Sullivan et al. 2005). A number of studies have reported prey altering their behaviors due to such cycles. Kotler et al. (2002), for example, showed that many rodents alter their foraging cycles according to lunar cycles, avoiding foraging during periods of full moon light to avoid nocturnal predators. Ferrari and Chivers (2009) showed that tadpoles avoid salamander predators more at times of day when the predator is more likely to be foraging. Predators with different foraging cycles will impose different “pressures” on prey individuals. Prey could encounter predators on an infrequent basis or alternatively could be chronically exposed to them. This aspect of predation has only recently received attention. The risk allocation hypothesis (Lima and Bednekoff 1999) was the first model to predict the effects of the recent history of predation risk on prey decision making and has since become one of the most influential papers in behavioral ecology (reviewed by Ferrari et al. 2009). According to this model, prey should adjust their antipredator behavior, and conversely foraging effort, according to the intensity and the duration of risk encountered by prey. One prediction of this model is that prey exposed to long bouts of risk should decrease their overall vigilance toward predators because long periods of sustained vigilance will result in unacceptable decreases in energy intake. The predicted negative relationship between predator exposure and antipredator response has been experimentally documented in a number of systems (e.g., Hamilton and Heithaus 2001; Sih and McCarthy 2002; Van Buskirk et al. 2002; Pecor and Hazlett 2003). Not all studies have supported the risk allocation model, but much of this may be explained by how well the test conditions fit the model’s key assumptions regarding energy limitation and the requirement that prey know their environmental risk regime (Ferrari et al. 2009). Convict cichlids (Amatitlania nigrofasciata) have become a model species to test the risk allocation predictions (Brown, Rive, et al. 2006; Ferrari, Rive, et al. 2008). For example, Brown, Rive, et al. (2006) exposed convict cichlids to a low (once a day) or high (3 times a day) frequency of risk for 3 consecutive days, introducing injured conspecific cues (alarm cues [ACs]) in the tank to mediate predation risk. When subsequently exposed to injured conspecific cues, cichlids in the high-frequency treatment showed a lower antipredator response than those from the low-frequency risk.

Such results support the risk allocation model, but some authors have highlighted the difficulty in assessing whether the waning of antipredator responses over time represents...
a higher level of information processing or simply the animals’ habituation to risk (Hamilton and Heithaus 2001; Sih and McCarthy 2002; Brown, Rive, et al. 2006). In the “risk assessment” scenario, the decision-making center receives accurate information from the sensory receptors, and the decrease in behavioral response on detection of predation stimuli is an active process resulting from complex decisions making from a higher cognitive center. In the “sensory habituation” scenario, the sensory receptors used to detect the predation stimuli become fatigued and lose their sensitivity as the frequency of exposure to the stimuli increases. This results in information loss from the receptors to the processing center, either in terms of intensity or in terms of frequency, which in turn results in a lower behavioral response by the prey.

Here, we tested whether the waning of antipredator responses observed on frequent exposures to predation risk occurs due to sensory habituation to a given stimulus or whether it is due to risk assessment across risk situations. One key distinction between the 2 scenarios is that 2 stimuli perceived and/or processed by the same sensory apparatus could both be subject to sensory habituation, whereas cues perceived by different sensory systems should not be subject to sensory habituation. For example, habituation to one type of chemical stimulus may prevent responses to another type of chemical stimulus, but habituation to a chemical stimulus should not affect responses to a visual stimulus.

In this study, groups of convict cichlids were preexposed to conspecific ACs either once (low frequency) or 3 times (high frequency) a day for 3 days. Following this preexposure phase, cichlids were tested for their antipredator response and foraging effort when exposed to a control of water, conspecific ACs (cue used in the preexposure), conspecific disturbance cues (DCs) (novel chemical cue), or a simulated attack by an aerial predator (novel visual cue [VC]). Following from the risk allocation model, we predicted that cichlids from the high-frequency risk treatment should display lower intensities of antipredator response to conspecific ACs, and inversely higher foraging efforts, than those from the low-frequency risk treatment. If the waning observed is a result of sensory habituation to conspecific ACs, risk frequency should only affect the responses of cichlids to conspecific ACs and should not affect their responses to other novel cues (DCs and VCs). However, if the waning in the response is indeed the result of adaptive allocation of vigilance or effort, then risk frequency should affect the responses of cichlids to all risk-related stimuli and not only to the stimulus used in the preexposure.

METHODS

Test fish

Juvenile convict cichlids (standard length [SL]: mean ± standard deviation [SD] = 3.42 ± 0.79 cm) were descendants of laboratory cichlids crossbred with wild-caught cichlids from Costa Rica. Cichlids were held in aerated 110-l aquaria filled with dechlorinated tap water (ca. 26 °C, pH 7.0–7.5) under a 12:12 h light:dark cycle and fed ad libitum twice daily with commercial flake food and brine shrimp (Artemia spp.).

Test stimuli

Damage-released chemical ACs were used to create background risk and as a test stimulus (see below). Twenty donor cichlids (5.29 ± 0.69 cm SL) were humanely killed by cervical dislocation in accordance with the Canadian Council on Animal Care. Skin fillets were removed from either side of the donor cichlids and placed into 200 ml of chilled glass-distilled water. Skin fillets were then homogenized and filtered through polyester filter floss to remove any remaining tissue. We collected 197 cm² of skin, which we then diluted to a final volume of 1795 ml with the addition of distilled water. This concentration is known to elicit reliable increases in antipredator behavior in juvenile convict cichlids (Brown et al. 2004; Brown, Bongiorno, et al. 2006). ACs were frozen in 20-ml aliquots at −20 °C until required.

DCs were collected following the methodology of Vavrek et al. (2008). Twenty-four hours prior to stimulus collection, 10 donor cichlids (4.95 ± 0.83 cm SL) were placed into a 20-l glass aquarium filled with 15 l of dechlorinated tap water at similar temperature and pH conditions as the holding tanks and containing a charcoal corner filter. One hour prior to stimulus collection, the filter was turned off. We slowly passed a fish predator model (yellow/green realistic fish predator model, 15 cm SL) attached to a glass rod through the tank 20 times, taking care not to contact donor fish to avoid potentially releasing damage-released ACs into the tank. Qualitative observations suggest that the donor fish did indeed react to the model predator with a strong antipredator response (dropping to the substrate and sholing in a corner of the tank). Vavrek et al. (2008) and Ferrari, Vavrek, et al. (2008) have likewise shown that juvenile convict cichlids respond to DCs collected in this manner. One minute after model presentations, the tank water was gently mixed, and 200 ml of tank water was removed to be used as DCs. DCs were collected fresh for each day of trials and were used within 30 min of collection. Donors were used only once and then returned to the stock population.

Creating background levels of risk

Conditioning tanks consisted of a series of opaque white plastic basins (48 × 38 × 20 cm) filled with 25 l of dechlorinated tap water. Water conditions were as described above for holding tanks, except conditioning basins were not filtered. Each basin was equipped with an overflow valve, approximately 5 cm from the upper edge to allow for water changes.

To create differing levels of background risk, we exposed 12 groups of 20 juvenile convict cichlids to conspecific ACs either once per day (low frequency risk) or 3 times per day (high frequency risk) for 3 consecutive days. We injected 25 ml of ACs in a series of 3 equal pulses separated by a 5-min period. Injections were given at 1100 h for the low-frequency treatment and 0800, 1100, and 1400 h for the high-frequency treatment. We delivered the ACs in a series of discrete pulses to better simulate natural patterns of risk (Foam et al. 2005). Ten minutes after the final pulse, we slowly flushed each basin with approximately 25 l of dechlorinated tap water. Water changes were conducted on all basins, regardless of frequency treatment to control for general disturbance. During the conditioning phase, cichlids were fed ad libitum twice daily with commercial flake food. Food was never given less than 1 h from the most recent conditioning. Approximately 1 h following the final conditioning on day 3, 8 pairs of size-matched cichlids (16 of 20 fish) were transferred to test tanks (see below) for behavioral observations. The remaining 4 fish were extras; extra fish ensured that we could properly size match the pairs. Two of the 8 pairs from each basin were subsequently tested for response to each of 4 cues (water, conspecific ACs, DCs, or VCs). We conducted 6 conditioning blocks in each frequency treatment for a total of 12 replicates for each frequency × stimulus type treatment combination.

Testing phase

Twenty-four hours prior to being tested, cichlids were transferred into 37-l test tanks (same temperature and lighting as mentioned above), filled with 35 l of dechlorinated tap water,
and containing a gravel substrate but no filter. The tanks were also equipped with an air stone positioned on the back wall of the tank. A second (ca. 2-m long) piece of tubing was anchored beside the air stone and was used to inject the stimulus into the tanks. Three sides of the tanks were wrapped in black plastic to ensure visual isolation from neighboring tanks. In addition, the tanks were positioned behind a black plastic observation blind to minimize disturbance of the observer on the fish. Cichlids were fed flake food the evening before testing and 1 h prior to testing. We fed enough food to ensure that some flakes, once saturated, settled to the substrate, providing foraging opportunities during observation periods.

Trials consisted of a 5-min prestimulus and a 5-min poststimulus injection observation period. Immediately prior to a trial, we withdrew and discarded 60 ml of tank water from the stimulus injection tube to ensure that the tube was clean. We then withdrew and retained an additional 60 ml of water, which were subsequently used to completely flush the stimulus into the tank. Following the prestimulus observation period, we injected 10 ml of distilled water (control), AC, or DC and slowly flushed it into the test tank with the retained tank water. A fourth treatment was the presentation of a visual stimulus. Our visual predator model consisted of a plastic bird head with a long beak mounted on a 45-cm plastic rod. Following the prestimulus period, we positioned the predator model along the front edge of the tank and slowly dipped the model 3 times to the substrate. We then removed the predator model from sight of the test tank. To control for any possible mechanical disturbance associated with the removal of water through the stimulus injection tube, we withdrew 120 ml of tank water for tanks exposed to the VCs as well.

During both the prestimulus and the poststimulus observation periods, we recorded time spent moving and the frequency of foraging attempts as indicators of an antipredator response. Both of these behaviors are common antipredator responses in a variety of taxa including fishes (review Lima 1998; Brown, Bongiorno, et al. 2006; Brown, Rive, et al. 2006). By reducing activity and foraging, the prey decreases its probability of being detected by a predator and is able to maintain higher levels of vigilance. For time spent moving, we arbitrarily selected 1 of 2 cichlids and recorded the total time not stationary. Typically, both cichlids are either moving or stationary at the same time. For foraging attempts, we quantified the behavior of both test fish. We defined a foraging attempt as pecking at the substrate with the body inclined at an angle greater than 45° to the substrate (Grant et al. 2002). We conducted a total of 12 observations per treatment combination (N = 96). Observations were made blind with respect to the risk frequency treatment.

Statistical analysis
For both time spent moving and the frequency of foraging attempts, we calculated the difference between the pre and poststimulus observation periods. Time spent moving and frequency of foraging attempts are correlated (Pearson’s r = 0.628, P < 0.001); hence, we analyzed the 2 variables simultaneously using a multivariate analysis of variance (MANOVA) approach. “Conditioning block” was introduced in the analyses as a nesting factor but was subsequently removed as it never had a statistically significant effect (nest: P > 0.3; nest × stimulus: P > 0.5). The following analyses were performed to answer the following questions: “Do fish display antipredator responses to the predation cues?” A MANOVA was performed using “stimulus type” (water, ACs, DCs, or VCs) and “frequency of risk” (1× or 3×) as fixed factors. The responses of cichlids to water were compared with the 5 other predation cues using simple contrasts.

“Is there an effect of risk frequency and/or cue type on the responses of cichlids to predation cues?” A similar MANOVA was performed on the 3 predation cues only. If the response pattern of cichlids is a result of sensory habituation, a significant interaction should be found between risk frequency and cue type. If the response pattern of cichlids is a result of a complex higher level decision process, then we should not find any interactions between risk frequency and cue type, as risk frequency should always have the same effect on the responses of cichlids, regardless of the cues mediating the risk. The second approach purposely excluded the water treatment because risk frequency is not expected to affect the responses of fish to water, so retaining water in the analysis would automatically lead to a frequency by cue interaction (as above). In all cases, data met the assumptions of homoscedasticity.

RESULTS
Responses of cichlids to predation cues
The MANOVA revealed a statistically significant effect of frequency (Pillai’s trace: F<sub>9,87</sub> = 11.8, P < 0.001) and stimulus (F<sub>6,176</sub> = 14.5, P < 0.001) and a significant interaction between the 2 factors (F<sub>6,176</sub> = 2.4, P = 0.03; Figure 1). Simple contrasts indicated that the responses of cichlids to predation cues were all significantly different from the responses to water (movement: F<sub>3,40</sub> = 29.8, P < 0.001, all P < 0.001; foraging: F<sub>3,40</sub> = 16.6, P < 0.001, all P < 0.005).

Interaction between frequency and cue type
The MANOVA revealed a statistically significant effect of frequency (Pillai’s trace: F<sub>9,80</sub> = 19.7, P < 0.001) and stimulus (F<sub>2,87</sub> = 9.4, P < 0.001) but no significant interaction between the 2 factors (F<sub>3,122</sub> = 1.0, P = 0.42). Regardless of cues, cichlids from the low-frequency treatment displayed stronger responses to predation cues than those from the high-frequency treatment. Tukey post hoc test indicates that cichlids responded with similar intensity to ACs and VCs (both P > 0.6; Figure 1), although showing a weaker response to DCs (both P < 0.001; Figure 1).

DISCUSSION
Our results provide clear evidence that the waning in antipredator response observed in cichlids exposed to increased frequency of predation risk cannot be explained by sensory habituation. Increased risk frequency resulted in decreased responses of cichlids not only to conspecific ACs but also to DCs and avian VCs. Because cichlids were exposed to DCs and avian VCs for the first time, sensory habituation cannot explain this decrease in response in the high-frequency treatment. These results provide insights into the considerable controversy over whether or not prey animals are making “behavioral decisions” to adjust antipredator behaviors as opposed to decreasing their response to a particular cue due to sensory habituation (Hamilton and Heithaus 2001; Sih and McCarthy 2002; Pecor and Hazlett 2003).

Sensory habituation is considered to be stimulus specific. Because the chemical composition of ACs and DCs is still unknown (Chivers and Smith 1998; Ferrari et al. 2010), it is difficult to assert that these 2 cues are not detected by the same sensory receptors and firing the same neurons. The consensus is that DCs involve a pulse release of urinary ammonia (Kiesecker et al. 1999). All chemical ACs identified to date from aquatic organisms have been proteinaceous (Rittschof 1990). The scarcity of work on the chemical characterization of ACs stands in sharp contrast to the amount of work done on the ecological role these cues play in the
aquatic systems. However, there is no doubt that our bird model is processed through a different sensory route. In our experiment, we could not distinguish whether the response of cichlids to the aerial predator was solely a result of VCs or a combined response to visual and mechanical cues. Support for the risk allocation hypothesis has been found in a variety of taxa, including mammals, fishes, damselflies, and gastropods (reviewed by Ferrari et al. 2009). In a recent study, Rodriguez-Prieto et al. (2008) found that risk frequency affected the flight initiation distance of wild blackbirds not only to pedestrians but also to novel radio-controlled vehicles. They concluded that the blackbird antipredator responses were the result of both habituation and risk assessment. However, their novel stimulus was very similar to the “normal” pedestrian stimulus, that is, an approaching visual object. The similarity of the stimuli may explain why they could not rule out habituation as a possible explanation.

Our results indicate that the processing of information related to overall risk level in cichlids has to occur at a higher and more complex level than the stimulus receptor. Multisensory risk-related information seems to be integrated and processed together. This is in concordance with the sensory complementation hypothesis (Lima and Steury 2005), whereby prey that are provided with multiple sources of risk information make more accurate risk-sensitive responses to predators. Understanding the processing of risk-related information and the role of multisensory inputs in prey decision making will be a fascinating area of research for both behavioral ecologists and neuroethologists. We particularly encourage comparative studies directed at differentiating the risk assessment scenario from the habituation scenario. Future research should attempt to address whether there is a level of biological complexity where sensory habituation becomes replaced with adaptive cognitive responses in the prey. Understanding how animals perceive temporal patterns of risk has important implications for explaining the role of trait-mediated indirect interactions in predator–prey systems.

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