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Original Article

Familiarity with the test environment improves escape responses in the crimson spotted rainbowfish, *Melanotaenia duboulayi*

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Abstract. Animals that are familiar with their environment have been reported to have greater survivorship for a number of reasons related to their knowledge of the terrain, which they recall from memory. In an initial experiment rainbowfish significantly improved their escape response towards a novel trawl apparatus over a sequence of five runs. Escape latencies were still low during a subsequent exposure 11 months after the initial exposure. While part of the improvement in escape success was certainly due to learning associated with the location of the escape route, it is likely that this was aided by habituation to the tank environment and the experimental protocol. In a follow-up experiment, fish that had been kept in the experimental tank for 3 weeks prior to testing, and had become familiar with the test tank, showed significantly lower escape latencies and escaped more often than fish that were not familiar with the test environment. It is suspected that familiarity with the testing arena decreased stress and enabled individuals to detect novel stimuli and devote more attention to them. These findings have significant implications for experimenters studying aspects of animal cognition, such as learning and memory, in laboratory situations.

Keywords. Familiarity - Rainbowfish - Escape responses - Learning - Cognition

Introduction

The time it takes an animal to successfully escape to safety (escape latency) is a vital statistic when assessing the probability that the animal will survive a predator attack. Animals that are familiar with their surroundings incur a fitness benefit in the form of reduced probability of a successful predator attack (e.g. Brown et al. *2000*). Metzgar (*1967*) provided three hypotheses for this observation: (1) individuals who are familiar with their environment detect danger more readily; (2) animals that know the terrain can escape more effectively; and (3) animals that are unfamiliar with their surroundings may show greater activity levels and hence be more prone to attack.

The work of Windberg (*1996*) on coyotes (*Canus latrans*) in familiar and unfamiliar enclosures showed that coyotes in familiar enclosures showed stronger neophobia towards small and medium-sized visual stimuli. These data suggest that coyotes in familiar environments may be better able to detect novel stimuli and treat them with caution by avoiding them. This could explain the lower capture rates of coyotes within their home ranges compared with transient animals (Windberg and Knowlton *1990*). However, coyotes in unfamiliar enclosures display greater exploratory activity, which, in the wild, may also cause transient animals to be captured more often. Similar observations have been made in rodents (Ambrose *1972*; Daly et al. *1990*; Randall *1993*).

Chipmunks (*Tamias striatus*) that were released within their home range took only half the time, and travelled half as far, to reach a refuge compared to animals that were released outside their home range (Clarke et al. *1993*). Opossums (*Didelphis virginiana*) that were released within their home range preferred known refuges as opposed to the first available refuge during an escape attempt (Ladine and Kissell *1994*). These data suggest that an intimate knowledge of the terrain facilitates predator evasion. Snyder et al. (*1974*) concluded that knowledge of the terrain was only one factor affecting predation vulnerability because of the heavy correlation between knowledge of the terrain and the levels of movement displayed by animals. Movement obviously has a strong impact on the likelihood of detection by predators.

Movement is a cue to which many predators are highly tuned (Brown and Warburton *1997*) and any factor that leads to increased levels of activity is bound to attract predators. When fish are placed in a new environment they show a very similar response to that observed in mice (Ambrose *1972*). Initially they hide or remain motionless, but gradually begin to move about and explore the environment. The level of activity is highly correlated with the degree of novelty (Welker and Welker *1958*). Activity levels drop significantly once the fish have become familiar with the tank environment (Teyke *1989*; Mikheev and Andreev *1993*).

Despite the obvious importance of spatial memory in contexts such as anti-predator behaviour, few people have considered how familiarity with the environment may affect anti-predator behaviour in fish (Warburton *1990*). Furthermore, comparative studies investigating inter- and intraspecific differences in cognitive ability, such as learning and memory, seldom consider differential responses to experimental apparatus. This is particularly pertinent if the subjects are removed from their holding pens and tested in novel enclosures (e.g. a radial maze), as is often the case. This study was designed to investigate the impact of familiarity with the test enclosure on the escape response of the rainbowfish *Melanotaenia duboulayi* confronted by a model trawl apparatus. I first investigate the capacity of rainbowfish to learn how to escape from the trawl and test their ability to recall the information 11 months later. Secondly, I consider the effects of familiarity with the test environment on escape latency. I hypothesised that fish that are familiar with the experimental tank should display significantly faster escape responses than fish that are unfamiliar with the experimental tank.

Methods

Experiment 1: learning and memory retention

Rainbowfish were collected from Amamoor Creek (26°21′ S, 152°40′ E) and transported to The University of Queensland. The fish were maintained in large aquaria for 1 month, by which time they had adjusted to captive conditions. The holding aquaria measured 600 × 800 × 400 mm and contained around 40 fish each. Both natural light and overhead fluorescent globes provided 14:10 h light:dark regime. The water temperature was 22°C and the pH 6.5-7. The fish were fed once per day and their mean length was 52.7±1.1 mm standard length (±SE, n=120). All of the fish were adults and, while the age of the individuals was unknown, it is likely that they ranged from 1 to 3 years old. It is highly unlikely that variation in the age of individuals affected the findings of the experiments in any way. At the end of the 1st month, three female and two male rainbowfish were chosen at random, removed from the holding tank and placed into the experimental tank. A clear glass experimental tank (200 × 30 × 30 cm) was equipped with a model trawl apparatus (see Brown and Warburton *1999a*, *1999b* for details). The depth of the water was maintained at 20 cm. The trawl net had a 2 × 2 cm hole positioned in the centre offering the only escape route through which the fish could swim in order to avoid being trapped as the trawl moved down the long axis of the experimental tank.

After being placed in the experimental tank, the fish were allowed to adjust to the new surroundings for 15 min. The net was drawn from the 100-cm mark along the tank until it was 3 cm from the end, at which point it was held in position for 60 s. The time taken for the net to move from one end to the other was 30 s. Fish that did not escape were allocated the maximum time limit of 90 (30+60) s. The net was then removed and placed back in its original position. This constituted one run. The procedure was repeated a further four times at 2-min intervals, in order to investigate the effect of negative experience (i.e. being trapped) on the fishes' learning ability. For each run the mean time taken for the shoal to escape through the hole and the number of fish that successfully escaped was later recorded from video playback (N.B. only the shoal escape latency data are reported here). After each replicate of five runs the tested fish were placed in a neighbouring storage tank. Five groups of five fish were tested in 1997.

The same fish were tested again 11 months later, using the procedure outlined above. The results of the 1997 and 1998 tests were compared using non-parametric analysis (Kruskal-Wallis and Friedman's tests; SAS Institute *1996*) because the data could not be normalised. The analysis was conducted for each run and for each year separately. This was done because it is not possible to examine a two-way interaction effect using non-parametric statistics.

Experiment 2: Environment familiarity

Twelve months after collection, a group of 30 female fish were placed in the experimental tank for 3 weeks before being tested (N.B. the trawl apparatus had been removed from the tank). These females were labeled as being "familiar" with the test arena. Another group of 30 females were placed in a separate holding tank of and remained in this tank until the beginning of the test runs. None of the fish had been tested previously. The latter fish were labeled as being "unfamiliar" with the test arena. The familiar fish were transferred to a holding tank shortly before the tests began, the test tank was cleaned and the water replaced. Although this may have led to an increase in handling stress the outcome would have been a reduction in the difference between the familiar and unfamiliar fish. A selection of five fish was taken at random from one of the two groups (familiar females and unfamiliar females) and introduced to the experimental tank. Each group underwent the same procedure as outlined in experiment 1. Unfortunately, one of the fish in the familiar group leapt out of the bucket while being transferred to the test tank. This group displayed highly skittish behaviour throughout the five runs and was subsequently removed from the data set. In this experiment the data were distributed normally and were analysed using a repeated-measures ANOVA.

Results

Experiment 1: learning and memory retention

In 1997 the fish showed significantly higher escape latencies (Kruskal-Wallis χ^2 approximation: $\chi^2=10.974$, $df=1$, $P=0.001$) than when tested again in 1998. In 1997 the fish showed significant improvement in the response with repeated exposure (Friedman’s: $\chi^2=9.990$, $df=4$, $P=0.041$). When tested again in 1998, the fish showed relatively low escape latencies from the first run onwards (Friedman’s: $\chi^2=5.015$, $df=4$, $P=0.286$) (Fig. 1). In 1997 nearly all fish showed fast and erratic swimming along the edges of the tank as the trawl approached for the first time. After several runs the fish settled down and began to escape more readily from the trawl. When exposed 11 months later, the fish showed much lower levels of panic (as illustrated by erratic swimming) when confronted with the moving trawl. It should be noted that the data from 1997 have been published previously as part of another set of experiments (Brown and Warburton *1999a*).

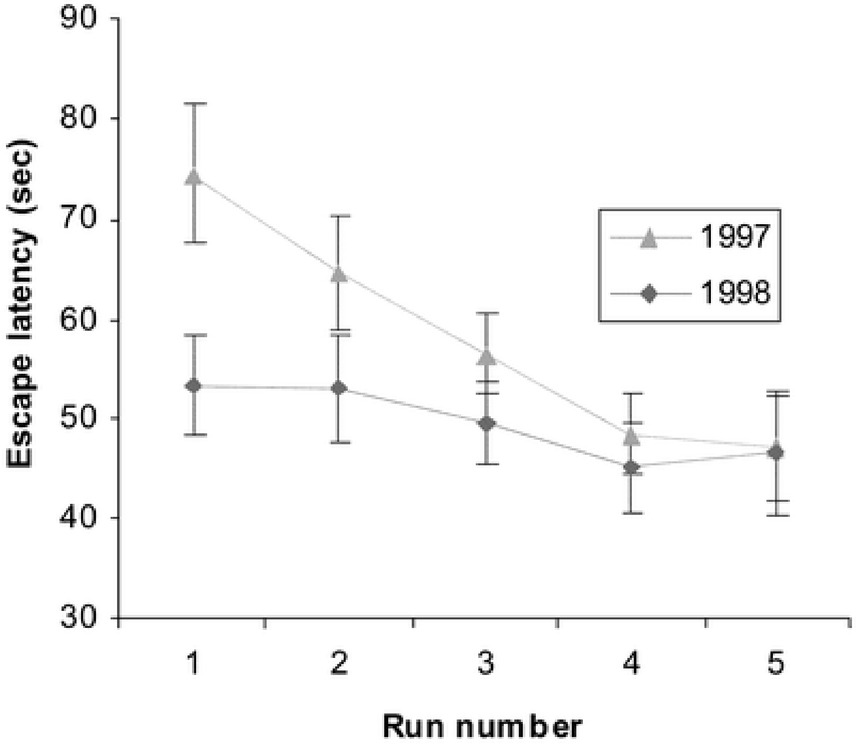

Fig. 1. The mean escape latencies (±SE) of fish escaping through the hole in the model trawl over a series of five runs. Fish were first tested in 1997 and then again 11 months later (1998)

Experiment 2: Environment familiarity

A repeated measures ANOVA showed that fish that were familiar with the experimental tank showed a significantly faster escape response than those that were not familiar with the tank (Fig. 2, Table 1). The difference in escape latency between familiar and unfamiliar fish did not vary significantly with run number. Neither group showed significant improvements in escape latency with repeated exposure to the trawl. Fish that were unfamiliar with the tank showed a relatively high degree of response variability. During the second experiment, the familiar fish appeared to orientate towards the net both during the 15-min settling period and throughout the runs. The unfamiliar fish continually searched for escape routes through the glass walls of the experimental tank when trapped behind the net rather than attempting to escape through the net.

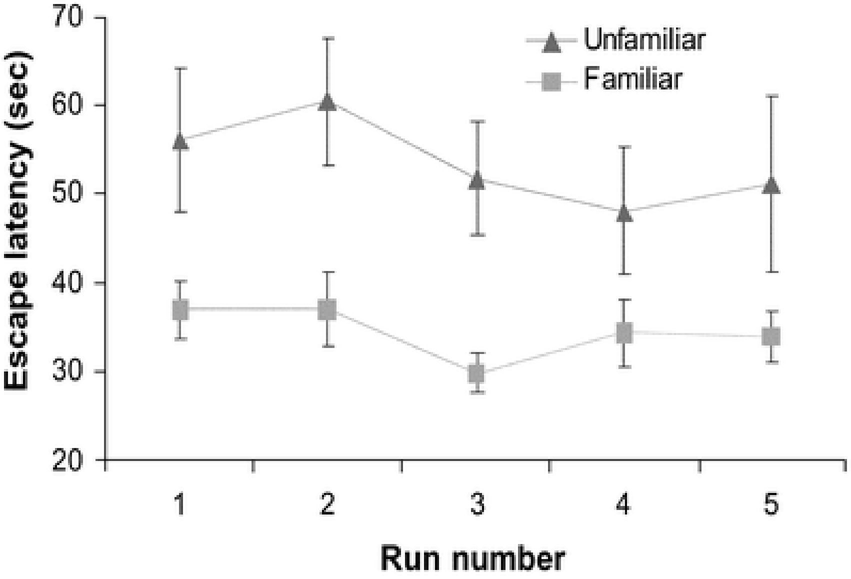

Fig. 2. The mean escape latencies (±SE) of fish familiar and unfamiliar with the test arena. Familiar fish escaped more rapidly than unfamiliar fish from the first run. The difference between the two groups was maintained over the five runs

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Table 1. Results of a repeated-measures ANOVA on the escape latency of familiar and unfamiliar rainbowfish ("Familiarity") in experiment 2. The repeated measure is represented by "Run number" and the interaction term is "Familiarity × Run". Fish that were familiar with the test arena escaped significantly faster than those that were unfamiliar with the test arena

Source	*df*	*F*	*P*
Familiarity	1	12.68	0.006
Run number	4	0.72	0.570
Familiarity × Run	4	0.22	0.914

Discussion

Learning has been intensively studied with respect to fish foraging behaviour (Dill *1983*), migration, orientation and recognition (for reviews see Gleitman and Rozin *1971*; Csanyi et al. *1989*; Kieffer and Colgan *1992*). However, few studies have considered the longevity of predator recognition and avoidance behaviours. The ability of rainbowfish in this study to remember information about a trawl for at least 11 months is comparable to the long-term maintenance of hook shyness in carp and salmon for over a year (Tarrant *1964*; Beukema *1970*). It is also similar to the retention of shuttle-box avoidance displayed by largemouth bass (*Micropterus salmoides*) in a study by Coble et al. (*1985*). Carp (*Cyprinus carpio*), salmon (*Oncorhynchus nerka*) and largemouth bass are relatively long-lived, and although little is known about the longevity of rainbowfish in the wild, it is likely that they rarely reach 3 years of age.

On re-exposure to the trawl apparatus after an 11-month period, rainbowfish showed low escape latencies that were maintained during subsequent runs. Once fish had remembered the location of the escape hole it appears that there was little more they could do to decrease their escape latency. It is highly unlikely that this improved response was due to an increase in the average age of the individuals over the 11-month period between the first and second exposures. While it is well known that the learning and responses of fish improve rapidly early on in development (Godin *1978*; Fuiman *1994*), once fish reach adult size there is no evidence to suggest that further aging improves escape responses except through further experience with the stimuli (i.e. learning). One possible explanation, however, is that the fish may have become accustomed to living in the laboratory and subsequently showed improved escape responses from the first run of their second exposure (i.e. in 1998). This explanation remains to be tested. In the second experiment all fish had been kept in captivity for equally long periods and hence this possibility was effectively controlled for. Another alternative explanation is that the fish had become accustomed to the experimental protocol (they were aware that there was an escape route). This latter explanation has also been considered in experiments using Morris water mazes where prior exposure to the apparatus improves the subject's performance considerably (Tolman *1966*; Perrot-Sinal et al. *1996*). Behavioural observations made throughout the experiments seem to support this idea. During their first exposure to the moving trawl in the first experiment nearly all fish showed signs of panic as signaled by fast and erratic swimming along the edges of the tank. As the number of runs increased this erratic activity decreased. Almost a year later the fish showed similarly low levels of panic when confronted with the moving trawl during the first run indicating that they may have become familiar with the trawl apparatus and/or the experimental protocol. In the first experiment, however, it is difficult to distinguish between the fish recalling the *existence* of an escape route and the fish recalling the *location* of the escape route, since they must first recall that there is an escape route before they recall its location. Experiments where the location of the escape route is manipulated may be able to tease these two factors apart.

It should also be noted that the sex ratio within a shoal might play an important role in determining the shoals response to the trawl apparatus. Mixed shoals (experiment 1) appeared to take longer to escape from the trawl initially, but showed rapid improvement. In contrast shoals containing females only (experiment 2) showed comparatively lower initial escape latencies and little improvement over repeated exposure. In mixed-sex shoals the attention of the fish, particularly the males, is drawn towards members of the opposite sex (and in the case of males, towards one another in the form of aggression) rather than towards the task of escaping the trawl during the early runs. Male guppies show similar blasé attitudes towards predation and tend to focus on sneaky mating attempts while the females are distracted (Magurran and Garcia *2000*). Surprisingly, studies on rainbowfish have shown that shoals consisting of males only were better able to avoid the trawl apparatus than female-only shoals (C. Brown, unpublished work). A difference in life-history strategies between males and females is probably responsible for the observed divergence in avoidance behaviour.

The results of the second experiment, nevertheless, support the test arena familiarity hypothesis and indicate that much of the improvement in the response displayed by the fish during their second exposure to the trawl (1998) can be explained by familiarity with the test tank rather than the fish remembering the exact location of the hole or becoming accustomed to the apparatus or the protocol of the experiment.

It is well known that dispersing animals face increased risk of mortality as they move through unfamiliar habitats (Plissner and Gowaty *1996*). Animals that are familiar with their surroundings may become aware of danger more rapidly since they can detect novelty in their surroundings more quickly. The early detection of novelty allows an animal to then devote its attention towards novel stimuli and subsequently avoid or investigate the object. Fish that were unfamiliar with the test tank had a number of new stimuli to contend with even before the trawl started moving towards them. On the other hand, familiar fish had habituated to the arena over the 3 weeks they had spent in the test tank. The only new thing to the familiar fish was the appearance of the trawl, which they then proceeded to inspect during the 15-min settling period. The orientation and the behaviour of the familiar fish suggested that their attention was focused on the net from the moment they were released into the tank and they subsequently located the escape route with relative ease. These observations support Metzgar’s first hypothesis, which suggests that familiar fish become aware of "danger" more readily.

The unfamiliar fish continually searched for escape routes through the glass walls of the experimental tank rather than through the net. In contrast, familiar fish did not attempt to escape through the glass walls of the tank and confined their search to the net, suggesting that their knowledge of the tank environment may have further contributed to greater escape success. In a natural situation freshwater fish may frequently find themselves in novel surroundings following periods of high flow, either by being physically displaced further downstream by currents or through having their habitat altered by scouring floods. Under these conditions fish must explore the novel environment in order to increase foraging and predator avoidance success. Familiarity with the environment is strongly implicated in the prior residence effect, whereby long term residence of territories are less likely to be evicted from their territory by more dominant individuals than short term residents. It is commonly believed that an intimate knowledge of the territory and its resources means that residents place a higher value on that territory than invading individuals and are therefore willing to escalate conflicts (Cutts et al. *1999*). This willingness to take great risks to defend a territory is manifested in an higher competitive ability than would otherwise be expected by the residents dominance status (or size) alone. Differences in competitive ability in Atlantic salmon (*Salmo salar*) have been shown to have both long- and short-term consequences effecting survival and growth rates and ultimately life-history strategies (Cutts et al. *1999*).

The level of stress displayed by prey animals when under predatory threat may have serious implications for escape performance, but this has not been well documented. Yet the effects of stress on learning and memory are well known in other animals such as laboratory rats and humans (Conrad et al. *1996*; Lupien and McEwen *1997*). Olla and Davis (*1989*) reported that salmon that had had prior experience of predators showed lower levels of stress and higher survivorship when confronted by an attack. Salmon that were suffering from handling related stress also showed lower survivorship when attacked by a predator. This effect was lost after 90 min, when the fish had recovered from handling. In the current study, rainbowfish that were familiar with the experimental arena showed significantly lower escape latencies than unfamiliar fish. It could be that familiar fish were less stressed when released into the experimental tank prior to the movement of the trawl. However, stress and environmental familiarity are intimately linked since familiarity with the environment may lead to lower levels of stress. The three explanations given by Metzgar (*1967*) for the improved survivorship of animals that are familiar with their environment do not exclude the possibility of an underlying stress effect. Future studies should aim to determine how long it takes fish to become familiar with the test arena and how differing levels of familiarity may affect escape responses. The interaction between habitat complexity, familiarity and predator avoidance may prove interesting. Presumably it would take longer to become familiar with more complex environments. At this stage it is unknown what environmental cues the fish are utilizing in order to detect familiar environments, but is seems likely that in the wild they would include both chemosensory and visual cues.

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