CHAPTER 5 Familiarity with the test environment improves escape responses in the rainbowfish, *Melanotaenia duboulayi*.

5.1 ABSRACT

Animals that are familiar with their test 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. Rainbowfish significantly improved their escape response towards a novel trawl apparatus over a sequence of five runs. Escape latencies were still low 11 months after the first exposure. While part of the improvement in escape success was probably 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. 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. Familiarity with the environment enables individuals to detect novel stimuli and devote more attention towards them. Familiarity may also lead to lower levels of stress, which may improve escape responses.

5.2 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. Metzgar (1967) provided three hyphotheses for this observation: 1) familiar individuals become aware of danger more readily, 2) animals that know the terrain can escape more effectively, 3) unfamiliar animals may show greater activity levels and hence be more prone to attack.

Windberg's (1996) work 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). These observations lend some support to Metzgar's first hypothesis. However, coyotes in unfamiliar enclosures also display greater exploratory activity, which in the wild, may also cause them to be captured more often (Metzgar's third hypothesis).

Chipmunks (*Tamias striatus*) that were released within their home range took only half the time, and traveled half as far, to reach a refuge compared to animals which were released outside of their home range (Clarke *et al.* 1993). Opossums (*Didelphis virginiana*), which were released within their home range, preferred known refuges to the first available refuge during an escape attempt (Ladine and Kissell 1994). These data suggest that an intimate knowledge of the terrain facilitates more effective predator evasion (Metzgars□ second hypothesis).

Daly *et al*. (1990) found that kangaroo rats (*Dipodomys merriamu*) that moved relatively long distances were more likely to be attacked by predators than those staying closer to home. Similarly, Randall (1993) proposed that rodents moving through familiar terrain avoid predation more successfully. However, Snyder *et al*. (1976) concluded that knowledge of the terrain was only one factor affecting predation because of the heavy influence of knowledge of the terrain on the levels of movement displayed by animals. Ambrose (1972) concluded that increase in exploratory activity caused higher predation rates in mice unfamiliar with their environment. Movement rates obviously have 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 tank. 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 (Tyke 1989, Mikheev and Andreev 1993).

Each of these studies provides evidence to support hypotheses 2 and 3, but, with the possible exception of Windberg's work, to my knowledge there are virtually no data to support Metzgar's awareness hypothesis (hypothesis one). Similarly, despite the obvious importance of spatial memory in contexts such as anti-predator behaviour, few people have considered how familiarity with the environment may impact on anti-predator behaviour in fish (Warburton 1990).

This study was designed to investigate the impact of environmental familiarity on the escape response of the rainbowfish *Melanotaenia duboulayi* confronted by a model trawl. I first describe the capacity of rainbowfish to learn how to escape from the trawl and their ability to maintain the improved response for extended periods of time. Secondly, I consider the effects of familiarity with the test environment on escape latency. I hypothesised that fish that are familiar with the test environment should display significantly faster escape responses than fish that are unfamiliar with the test tank.

5.3 METHODS

5.3.1 Experiment 1: Learning and memory retention

Rainbowfish were collected from Amamoor Creek (26E21'S, 152E40'E) and transported to The University of Queensland. The fish were maintained in large aquaria for one month, by which time they had adjusted to captive conditions. The holding aquaria measured 600 by 800 by 400 mm and contained around 40 fish each. Both natural light and overhead fluorescent globes provided 14:10 L:D regime. The water temperature was 22EC 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 (± standard error). The age of the individuals was unknown but it is likely that they ranged from one to three years old. At the end of the first month, 3 female and 2 male rainbowfish were chosen at random, removed from the holding tank and placed into the experimental tank. The experimental tank measured 200 by 30 by 30 cm and was equipped with a pulley system which allowed a vertical net to be pulled along the long axis of the tank (i.e. a model trawl) as described in Chapter 2. The depth of the water in the tank was maintained at 20 cm and the temperature at 22EC. The net had a mesh size of 1cm and completely blocked the tank with the exception of a 2cm by 2cm hole. The hole was positioned in the centre of the net. The fish could use the hole to avoid being trapped as the net was dragged from one end to the other.

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 three cm from the end, at which point it was held in position for 60 seconds. The time taken for the net to move from one end to the other was 30 sec. Fish that did not escape were allocated the maximum time limit of 90 (30+60) sec. 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 two 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 recorded. 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.

Eleven months later the same fish were tested again, using the procedure outlined above. Non-parametric analysis (Kruskal-Wallis Test; SAS Institute Inc. 1996) was conducted on the data because they were not normally distributed. 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.

5.3.2 Experiment 2: Environment familiarity

Fish that did not take part in Experiment 1 were used in Experiment 2. Twelve months after collection, a group of 30 female fish were placed in the experimental tank for three weeks. The trawl had been removed from the tank prior to the introduction of these fish. These females were labeled as being familiar" with the test environment. Another group of 30 females were placed in a separate holding tank and remained in this tank until the beginning of the test runs. These later fish were labeled as being unfamiliar with the test environment. The familiar fish were transferred to a holding tank moments before the tests began. Although this may have lead to an increase in handling stress the resultant outcome would be to reduce the difference between the familiar and unfamiliar. 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 5 runs and were subsequently removed from the data set. The order in which each of the groups was tested was randomised. The data were analysed using a repeated measures analysis.

5.4. RESULTS

5.4.1 Experiment 1: Learning and memory retention

In 1997 the fish showed significantly higher escape latencies ($X^2 = 10.974$, df = 1, Prob > $X^2 = 0.001$) than when tested again in 1998. In 1997 the fish showed significant improvement in the response with repeated exposure ($X^2 = 12.683$, df = 4, Prob > $X^2 = 0.013$). When tested again in 1998, the fish showed relatively low escape latencies from the first run onwards (Figure 5.1). 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 apparent panic when confronted with the moving trawl.

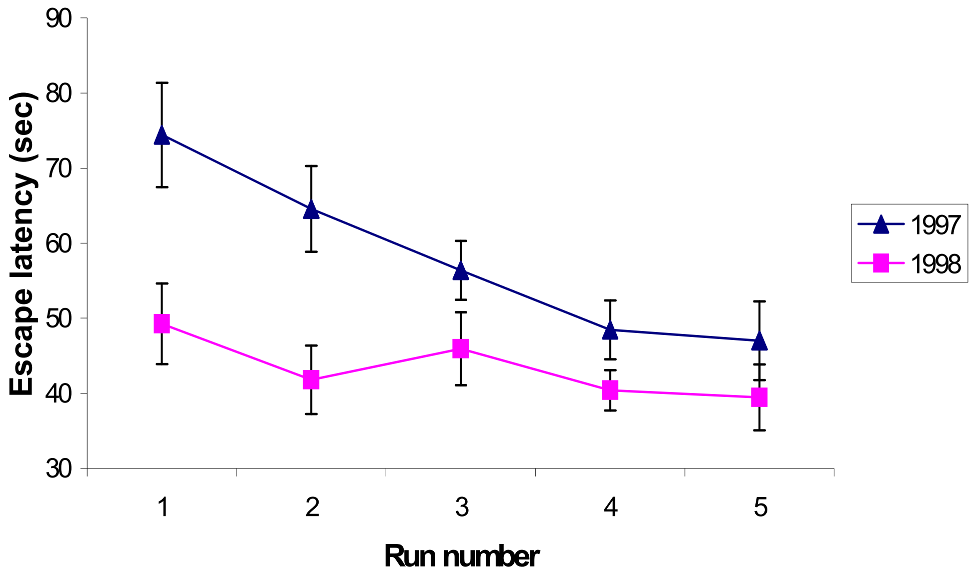

Figure 5.1. The mean escape latencies (± se) of fish escaping through the hole in the model trawl over a series of five runs. Fish were first tested in 1997 and then again 11 months later (1998).

5.4.2 Experiment 2: Environment familiarity

Fish that were familiar with the experimental tank showed a significantly faster escape response than those that were not familiar with the tank (Figure 5.2, Table 5.1). The difference in escape latency between familiar and unfamiliar fish did not vary significantly with run number (Table 5.1). Neither group showed significant improvements in escape latency with repeated exposure to the trawl (Table 5.1). Fish that were unfamiliar with the tank showed a relatively high degree of response variability (Figure 5.2). 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.

Source	DF	F	Pr > F
Familiarity	1	12.68	0.006
Run Number	4	0.72	0.570
Familiarity-Run	4	0.22	0.914

Table 5.1. Results of an ANOVA conducted on the escape latency of familiar and unfamiliar rainbowfish (Experiment 2).

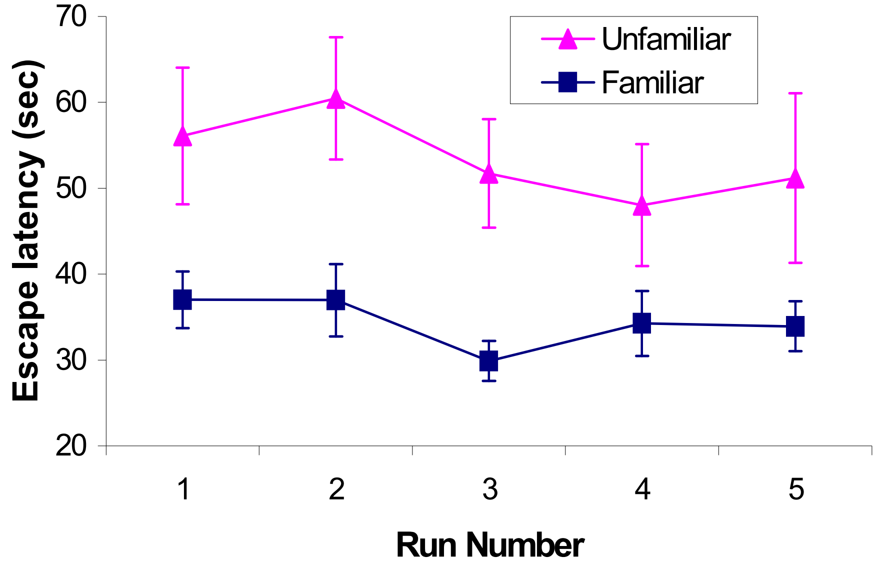

Figure 5.2. The mean escape latencies (± se) of fish familiar and unfamiliar with the test environment. Familiar fish escaped more rapidly than unfamiliar fish from the first run. The difference between the two groups was maintained over the five runs.

5.5 DISCUSSION

Learning has been intensively studied with respect to fish foraging behaviour (Dill 1983), migration, orientation and recognition (see Gleitman and Rozin 1971 and Kieffer and Colgan 1992 for reviews). 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 (*Oncirhynchus nerka*) and largemouth bass are relatively long lived, and although we know little about the longevity of rainbowfish in the wild, in captivity they may live to be over 10 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 there was little more they could do to decrease their escape latency. It is highly unlikely that this response was due to an increase in the average age of the individuals over the 11 month period between the first and second exposure bouts. While it is well known that the learning and responses of fish improve rapidly with ontogeny (Godin 1978, Fuiman 1994), once the fish reach adult size there is no evidence to suggest that further aging improves escape responses. The only exception to this rule results from further experience with the stimuli (i.e. learning) which could not have been the case here.

Animals that are familiar with their surroundings may become aware of danger more readily since they can detect novelty in their surrounding more quickly. The early detection of novelty allows an animal to then devote its attention towards novel stimuli. 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 environment over the three weeks they had spent in the test tank. The only new thing to them was the appearance of the trawl, which they then proceeded to inspect during the 15 min settling period. The attention of the familiar fish appeared to be focussed on the net from the moment they were released into the tank and they located the hole in the net with relative ease. These data 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 rather than through the net. In contrast, familiar fish were aware that there was no escape through the glass walls of the tank, 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 the scouring floods. Under these conditions fish must explore the novel environment in order to increase foraging and predator avoidance success.

During their first exposure to the moving trawl nearly all fish showed signs of panic as signaled by fast and erratic swimming along the edges of the tank. Once they realised that they were not going to be squashed against the end of the tank, they became calmer. As the runs proceeded the fish began to settle down and spent more time looking for a way through the net. Almost a year later the fish showed similarly lower levels of panic when confronted with the moving trawl during the first run.

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. Olla and Davis (1989) suggested that salmon that had had prior experience of predators showed decreased levels of stress and increased survivorship when confronted by an attack. Salmon that were suffering from handling related stress also showed decreased survivorship when attacked by a predator. This effect was lost after 90 minutes, when the fish had recovered from handling. In the current study, rainbowfish that were familiar with the experimental environment 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.

It is difficult to separate out the effects of stress and environmental familiarity since familiarity with the environment may lead to lower levels of stress. Metzgar□s (1967) three explanations 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 their environment 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 such as weed beds.

[References](#)

[Back to Contents](#)