CHAPTER 2
Differences in timidity and escape responses between predator-naïve and predator-sympatric rainbowfish populations.

2.1 ABSTRACT

Responses of rainbowfish (*Melanotaenia duboulayi*) from two populations towards an active and a passive predatory fish or a novel trawl apparatus were compared. The two populations differed in the level of predation pressure experienced in the wild. Predator-sympatric fish avoided the fish predators and showed stronger avoidance behaviour in response to the active predator. These fish used predator inspection excursions to rapidly assess the potential risk and their escape responses were consistently effective. In contrast, the predator naïve fish ignored the passive predator but were continually drawn towards the active predator, possibly due to generalised curiosity and the absence of significant negative feedback from the predator, which was restrained by a perspex partition. Despite this attraction, the predator-naïve fish did not display typical predator inspection behaviour and showed very poor escape performance when initially confronted by the trawl apparatus. Many of these fish, however, showed rapid improvement in their escape performance through learning. These results suggest that predator-sympatric rainbowfish have the capacity to assess the level of threat posed by a predator and to learn to implement appropriate escape strategies when forced to evade a threat.

2.2 INTRODUCTION

Predation is one of the greatest selective pressures operating on prey animals and can lead to substantial differences in morphology, physiology and behaviour of individuals from different geographic locations (Magurran 1990, Endler 1995). Animals which live sympatrically with predators should budget their time and alter their behaviour according to the perceived level of threat. For example, guppies alter their courtship behaviour from full display to sneak mating depending on the level of predator threat (Magurran and Seghers 1990). Scanning for predators and responding to them are costly in terms of time, which could be otherwise spent feeding or courting. Hence predator-sympatric populations should utilize mechanisms which allow them to minimize the amount of time spent on anti-predator behaviours in order to maximise fitness.

A number of studies have compared the behaviour of predator-sympatric populations of fish with "predator-naïve" populations and have uncovered several startling differences. Fish from areas of high predation are better able to recognise predators, detect predators earlier, inspect more readily, show attack cone avoidance, form tighter cohesive groups and display more appropriate escape responses compared with predator-naïve fish (Seghers 1974, Liley and Seghers 1975, Magurran 1986, Licht 1989, Magurran and Seghers 1990, Gelowitz *et al*. 1993). Predator sympatric fish are also much better able to determine the level of threat a particular predator represents. When faced with a predator, fish from predator-sympatric populations increase their vigilance but forage for longer periods of time before fleeing (Fraser and Gilliam 1987) and are quicker to return to pre-exposure behaviour (Magurran and Pitcher 1987). Fish from predator-sympatric and naïve populations also show variation in their ability to learn about predators (Magurran 1990, Mathis and Smith 1993)

Guppies are capable of determining the difference between satiated and hungry predators, presumably by responding to the movements of the predator (Licht 1989). This suggests that the behaviour of the predator may not only allow prey to determine what species the predator is (Coates 1980), but also something of its motivational state (Pitcher *et al*. 1986, Pitcher 1992, Huntingford *et al*. 1994). Hence, predator behaviour may be an important cue picked up by prey partaking in predator inspection and may determine the anti-predator responses displayed by prey species following inspection visits (Magurran and Pitcher 1987).

Owing to ethical considerations many investigators have used models in place of live predators to study anti-predator responses. However, even the best predator models are likely to lack subtle behaviours displayed by particular predatory species, thus altering the prey animal's perception of threat. The use of models has another drawback in that it is much more difficult to assess the effects of predator experience over repeated trials because habituation rapidly sets in if little or no physical harm occurs (Csanyi 1986).

Nets and other fishing gear evoke responses in fish resembling that of predator avoidance (Francis and Williams 1995) and enable an experimenter to explore the learning and memory capabilities of fish, allowing the measurement of more realistic learning responses to repeated exposure to threat than may be displayed to predator models. This is because predator models often result in rapid habituation in the prey species.

It is often difficult to distinguish the effects of predation pressure alone since a number of other habitat characteristics often co-vary with the presence of predators (Endler 1995). The rainbowfish populations in the present study varied in the level of predation experienced in the wild, while most other characteristics of their native streams (habitat complexity, light conditions, flow rate, depth etc.) were similar. The study comprises two complementary experiments. The first compared the responses of members of two populations of rainbowfish (*Melanotaenia duboulayi*) to predatory fish that differed only in their level of activity. The live predator was placed behind clear perspex so no negative feedback was forthcoming. The second experiment explored the abilities of fish from the two populations to solve a novel anti-predator problem (escape from a trawl). In this experiment negative feedback was applied and allowed the learning rates of each population to be compared through repeated exposure.

2.3 METHODS

Two populations of fish were sampled from two tributaries of the Mary River in South East Queensland using standard bait traps. The first population was from Amamoor Creek (26° 21'S, 152°40'E), a relatively small stream dominated by large still pools and occasional riffles. A number of large predatory fish species were present including spangled perch (*Liopotherapon unicolor*), saratoga (*Scleropages leichardti*) and mouth almighty (*Glossamia aprion*) (pers. obs.). The second population was derived from the upper part of Obi Obi Creek (26°45'S 152° 55'E) near Maleny. The headwaters of Obi Obi Creek were characterised by fast flowing waters with a number of waterfalls, deep pools and rapids. The fish community differed profoundly from that of Amamoor Creek. Obi Obi Creek contained very few large fish species with the exception of the eel-tailed catfish (*Tandanus tandanus*) and the long-finned eel (*Anguilla reinhardtii*) (pers. obs.). Both sites were characterised by submerged woody debris, submerged aquatic vegetation and a mean water depth of about 1.5 m. Flow rate and light regimes were also very similar. Rainbowfish were the most abundant fish species at both sites. Random sampling established that the mean (± SE) length of the fish was 51.9 ± 1.4 mm at Amamoor and 53.8 ± 0.8 mm at Obi Obi (n = 24 in each case).

2.3.1 Experiment 1: Active and passive predators

Rainbowfish were collected on four separate occasions during April 1987. They were transported to the University of Queensland and placed in large holding tanks for two days prior to experimentation. The water temperature in the holding tanks and the experimental arena was 22°C. The fish were not fed while held in captivity prior to experimentation.

The experimental arena consisted of a 1 m x 1.6 m tank divided along its length by opaque perspex. A trap door was located at one end and could be raised to reveal a predator (spangled perch) behind clear perspex (Figure 2.1). Lighting consisted of two overhead flourescent tubes and another mounted on the wall over the predator end of the arena. All glass walls were covered with blue plastic to prevent fish shoaling with their reflection. The floor of the tank was white. The tank was surrounded by a curtain and a video camera was suspended overhead.

The two spangled perch used in this experiment both had a standard length of 22 cm and were of similar appearance. Spangled perch are a bland silver-grey colour with a light speckling of small, dark grey spots and lack any obvious bands or large spots. The two individuals used in this experiment differed greatly in their general behaviour. Both in the wild and in captivity, variation exists in the level of activity and aggression displayed by individuals of similar size. I was able to exploit this difference in behaviour by choosing animals that varied markedly in these characteristics. The aggressive perch's behaviour was characterised by constant patrolling of the holding tank and, when exposed to rainbowfish, it repeatedly charged at the perspex divider. The other perch sat motionless in the water column and its fins moved only to maintain position. Throughout the period of the experiment both predators were permanently housed in the compartments at end of the experimental arena. They were left undisturbed so that moving them between tanks did not disrupt their normal behaviour. The results of a pilot study indicated that in the absence of predators, the distribution of rainbowfish shoals in the experimental tank did not differ significantly between the left and right sides (e.g. proportion of time spent in inspection range, ANOVA; $F = 0.015$, $N=7$, $P = 0.910$, following transformation).

Fifty fish from each population (Amamoor and Obi Obi) were divided randomly into ten groups of five fish. Two groups from the same population were simultaneously placed on either side of the experimental arena and allowed to settle for 15 min. Following this adjustment period, the rainbowfish were exposed to a predator. Every minute for five min the location of all fish was recorded on video. From these data the following indices were calculated: the mean distance of the shoal from the predator, and the proportion of time spent in the front (inspection range), middle (mid range) and back (refuge range) thirds of the arena (Figure 2.1). Only the distribution data are reported here.

Since the mean distance to the predator tells us little about how the fish were distributed throughout the experimental arena only the proportion of time in the inspection range were compared using a Wilcoxon/Mann-Whitney U-test (SAS Institute Inc., 1991). Since I was interested in interaction effects, each population was analysed separately to examine the effect of predator activity within populations. Similarly, I was interested in examining the difference the level of predator activity had on my ability to distinguish between the behaviours displayed by the two populations, the data for active and passive predators were also examined separately.

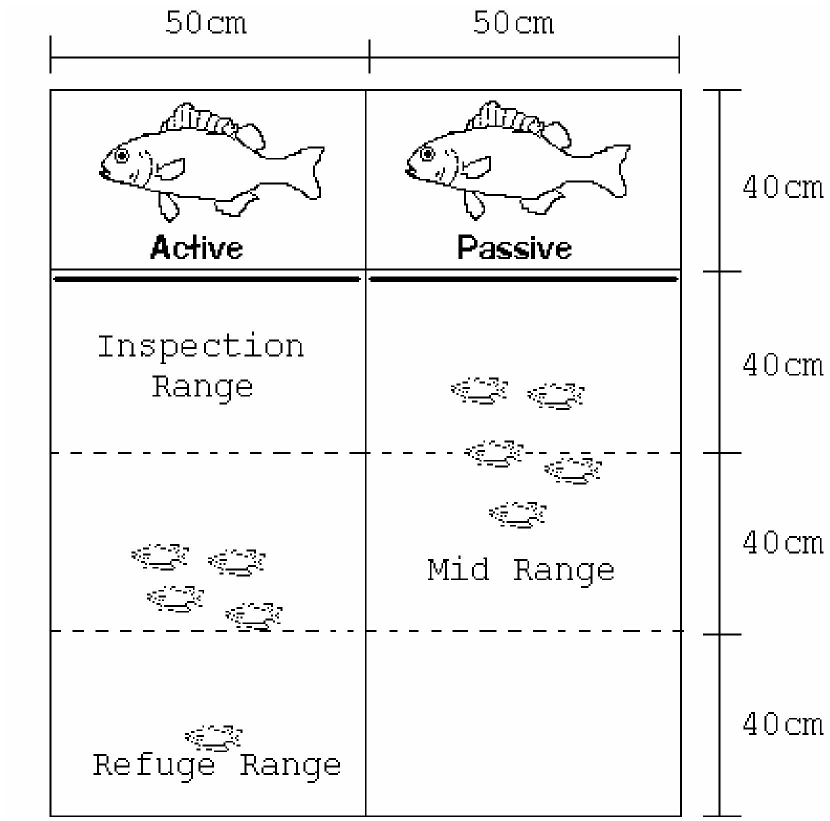

Figure 2.1. Diagrammatic view of the experimental tank used in experiment one showing the divisions into the three ranges (Inspection, Mid and Refuge). A clear perspex divide and an opaque division, which could be drawn up by a pulley system to reveal the predators to the prey, separated the predators from the rainbowfish.

2.3.2 Experiment 2: Trawl

An experimental tank measuring200 x 30 x 30 cm was equipped with a pulley system which allowed a vertical net to be pulled along the long axis of the tank. The depth of the water in the tank was maintained at 20 cm and the temperature at 22□C. The net had a mesh size of 1cm and completely blocked the tank with the exception of a small hole. The hole (2 x 2 cm) was placed directly in the centre of the net about 10cm from the bottom of the tank and 14 cm from each side. The fish could use the hole to avoid being trapped as the net was dragged from one end to the other (Figure 2.2).

Fish from the two populations were housed separately in large holding tanks each containing 50 fish each. A group of five fish was randomly taken from either of the holding tanks, placed in the experimental tank and allowed to adjust to the new surroundings for 15 min. The net was drawn along the tank until it was three cm from the end, at which point it was held in position for 60 sec. The time taken for the net to move from one end to the other was 30 sec. Any fish that did not avoid the net became trapped. Fish which 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 at two min intervals in order to investigate the effect of negative experience (i.e. being trapped) on the learning ability of fish from each population. For each run, the time taken for each fish to escape through the hole and the number of fish that successfully escaped were recorded. From these data the mean escape time for each shoal was calculated for each run. Each group was exposed to a total of five runs. Six replicates from each population were tested. Owing to the nature of the data, non-parametric statistical analysis were conducted. Wilcoxon tests were used to determine the effect of population origin and Kruskal-Wallis test analysis (SAS Institute Inc.1996) was performed to determine the effect of run number on escape latency and the percentage of fish escaping.

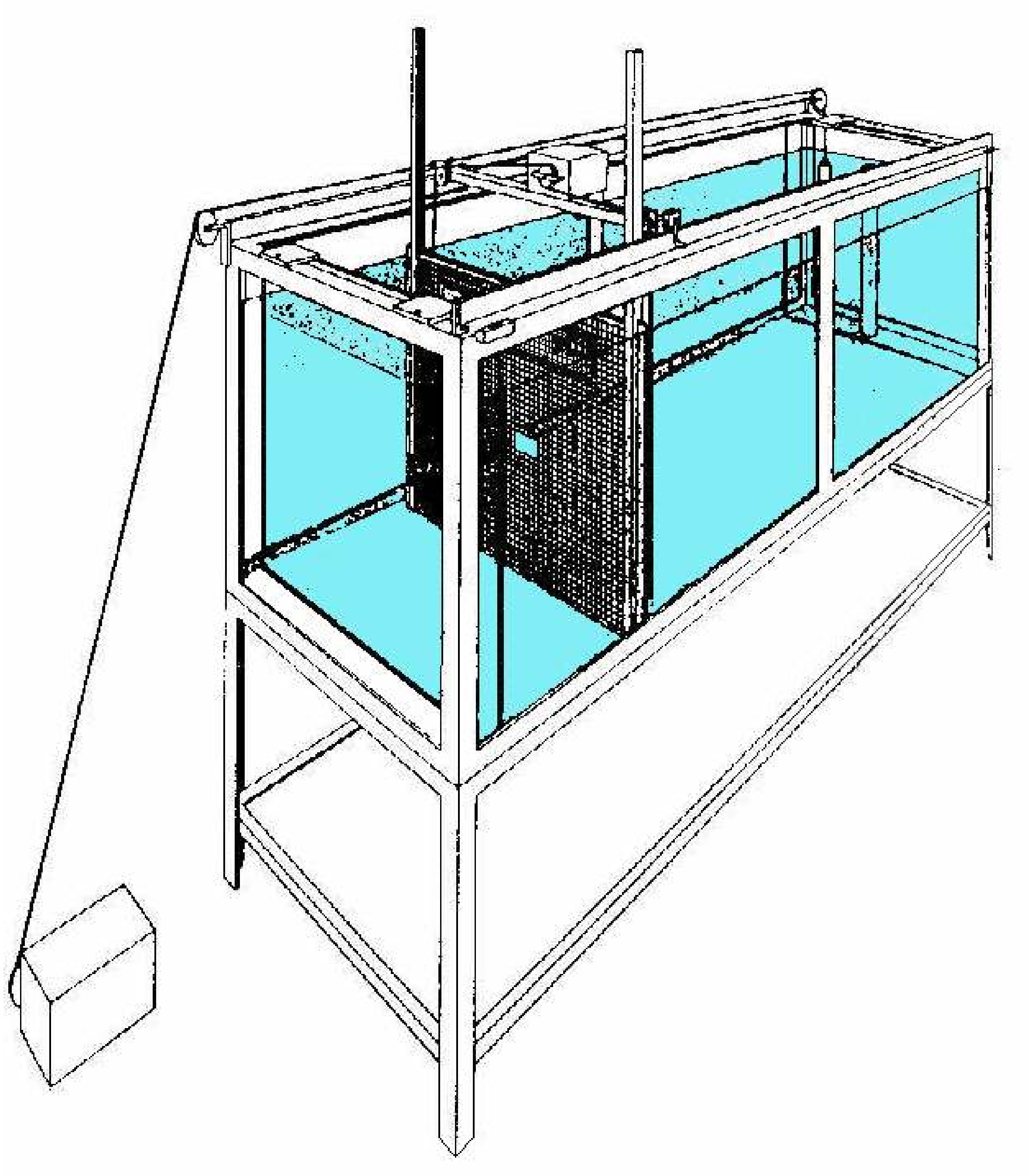

Figure 2.2. A diagram of the trawl setup used in experiment 2 (modified from Hunter and Wisby 1964).

2.4 RESULTS

2.4.1 Experiment 1: Active and passive predators

When exposed to a passive predator the Amamoor Creek fish spent a large proportion (80%) of their time in the mid region. When exposed to an active predator this declined to 48%, and the remaining time was spent in the refuge end of the tank. Therefore, high predator activity appeared to increase the wariness of the Amamoor Creek rainbowfish as displayed by movement away from the predator end of the tank. This change in distribution was reflected in the refuge range data (Figure 2.3) where the predator-experienced population (Amamoor Creek) showed a marginal difference in their response towards the two predators (Wilcoxon test; S= 12.5, n = 6, $P = 0.084$).

In contrast, when exposed to a passive predator, the Obi Obi Creek fish were distributed randomly throughout the tank, spending 24%, 36% and 40% of their time in the refuge, mid and inspection ranges respectively. When exposed to the active predator, however, they spent 92% of their time in the inspection range (Figure 2.4; statistical results are not reported here, because the distributions of fish between the inspection and refuge ranges were not completely independent of each other). The change in response to the level of predator activity as displayed by the proportion of time spent in the refuge range (Figure 2.3), suggests that the Obi Obi Creek fish significantly altered their response depending on the level of predator activity (Wilcoxon test; S =15, Z = -2.683 and $P = 0.007$).

The data for the active and passive predators were analysed separately to compare the differences between the two populations when faced with an active or passive predator. The analysis of the refuge range data for the passive predator revealed no significant differences between the two populations (Wilcoxon test; S = 13, Z = -1.626, $P = 0.104$). The analysis for the active predator, however, showed a significant outcome (Wilcoxon test; S = 15, Z= -2.777, $P = 0.006$) (Figure 2.3).

When exposed to the active predator the Amamoor fish initially made several inspection visits. They approached the predator typically as singletons or in pairs and then returned quickly to their shoal mates. Later they moved to the far end of the tank, relaxed their schooling activities and resumed social activities including courtship and dominance displays. The Obi Obi Creek fish gathered in a tight shoal and spent almost the entire time within 20cm of the active predator.

On many occasions the Obi Obi fish were repeatedly charged and harassed by the perch. The general response of the Obi Obi fish to this aggression was to wheel away from the predator for one or two seconds and then return to the perspex divide. Several individuals elicited fast start responses to the activities of the active predator, but regardless of how many times the predator attacked, the Obi Obi rainbowfish always returned.

2.4.2 Experiment 2: Trawl

The Amamoor Creek fish escaped from the trawl more frequently than the Obi Obi Creek fish (Wilcoxon test; S= 668.5, Z= -3.66, $P < 0.001$)(Figure 2.5). Escape latency times were significantly lower for the Amamoor fish (Wilcoxon test; S = 1176.5, Z= 4.024, $P < 0.001$)(Figure 2.6). Both populations showed improvement in their performance through successive exposure, but not significantly so (Kruskal-Wallis test; X^2= 4.541, df= 4, $P = 0.338$ for Amamoor and X^2 = 9.258, DF = 4, $P = 0.055$ for Obi Obi fish). The improvement was most marked for the Obi Obi Creek fish, which did not escape at all during the first trial (Figure 2.5) Escape latencies decreased with experience and were lower for the Amamoor fish than for the Obi Obi Creek fish (Kruskal-Wallis test X^2= 3.663, DF= 4, $P = 0.454$ for Amamoor and X^2 = 8.749, DF = 4, $P = 0.067$ for Obi Obi fish)(Figure 2.6).

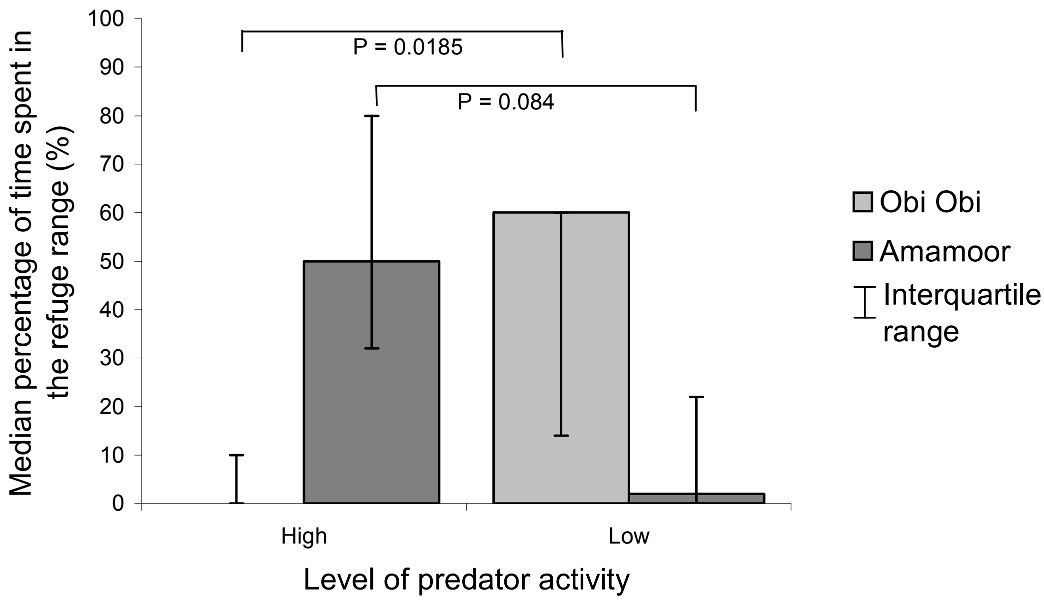

Figure 2.3. The median percentage of time spent in the refuge range by both populations̩ the interquartile ranges. Predator-experienced fish from Amamoor Creek spent more time in the refuge range in the presence of an active predator. A significant and opposite trend for the predator-naive fish from Obi Obi creek is evident.

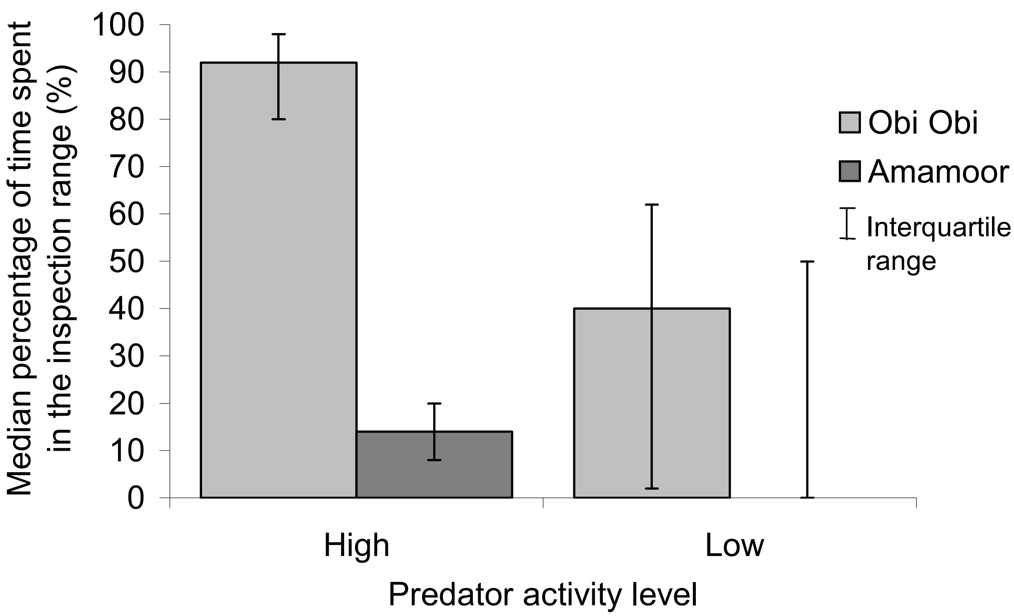

Figure 2.4. The median percentage of time spent in the inspection rangę the interquartile ranges. Obi Obi creek fish spent more time in the inspection range when an active predator was present compared to when the passive predator was present. Amamoor Creek fish rarely entered the inspection range.

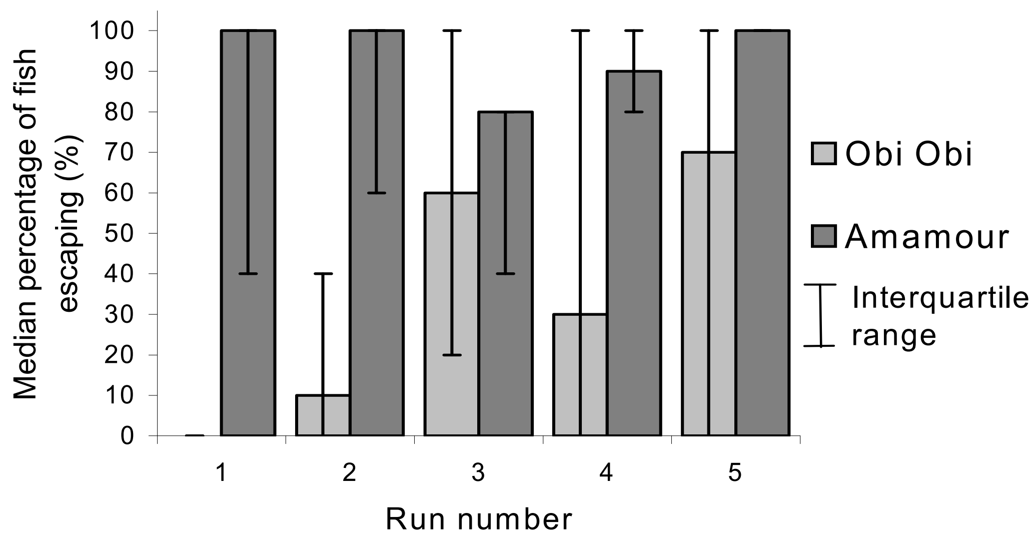

Figure 2.5. The median percentage of fish escaping from the trawl (interquartile range) for each population of rainbowfish and each of the five runs.

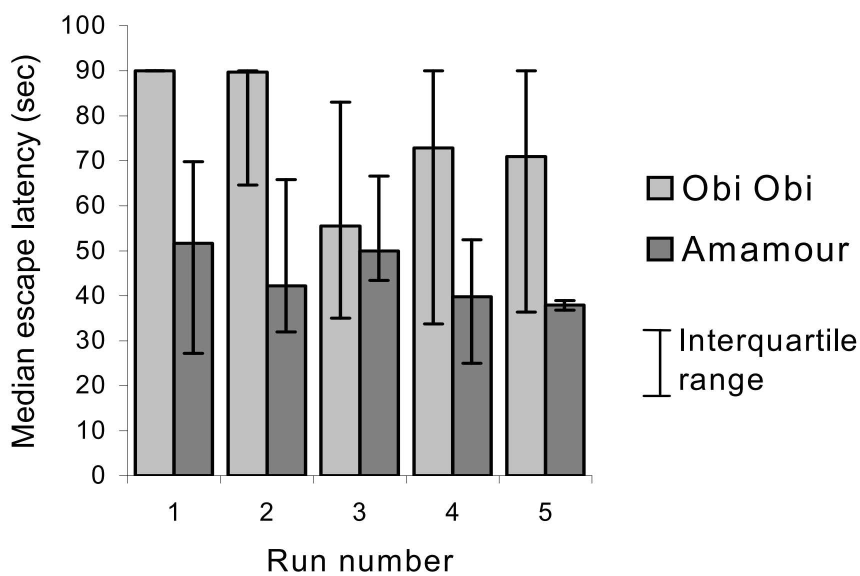

Figure 2.6. The median (interquartile range) time taken for shoals to make an escape from the trawl. Predator experienced fish from Amamoor Creek escaped significantly faster than fish from Obi Obi Creek.

A high level of variability was evident in the Obi Obi Creek data. Half of the Obi Obi Creek fish groups performed equally as well as the Amamoor fish while the other half failed to escape from the trawl at all.

Throughout and between the runs, Amamoor fish were often observed approaching and inspecting the net in pairs or as singletons. However, fish rarely swam through the escape hole while the net was moving; rather they swam just in front of it and waited until it had stopped before turning and escaping. In the early runs the Obi Obi Creek fish often showed nervous, skittish behaviour toward the approaching net. In the final few runs, however, their behaviour was similar to that
displayed by the Amamoor fish.

2.5 DISCUSSION

The data from the first experiment indicated that the level of activity displayed by predatory fish can have a significant effect on the behaviour of prey individuals. The behaviour observed in the Amamoor (predator-experienced) fish in response to the active predator would, under natural circumstances, allow them to maintain activities such as foraging and courtship (Godin and Crossman 1994). Indeed, a large number of social interactions were observed among the Amamoor fish while they were at the refuge end of the tank far from the predator. Male fish showed clear hierarchical behaviour and continued to court females. Presumably, if food had been available the fish would have also foraged. When presented with the passive predator, the Amamoor fish primarily utilised the middle and inspection areas of the tank, indicating that the perceived threat was low. Amamoor fish probably utilised information gained during early inspection visits to assess that the passive predator did not pose an immediate threat and continued with normal activities. Pitcher *et al.* (1986) suggested that minnows are able to predict when a predatory pike is likely to make a strike by observing the behaviour of the pike during inspection visits. This observation is indicative of the potential importance of subtle behavioural cues displayed by predators to inspecting prey.

In the second experiment, Amamoor fish spent the time between runs, as well as during the early stages of the trawl, inspecting the net. These findings indicate that in the wild, Amamoor fish may be able to quickly assess the level of threat a particular predator represents and respond appropriately, enabling them to exploit variations in predator pressure and thus lower their time and fitness costs. This type of behavioural flexibility may be selected for in regions of high predation pressure. In order to examine this possibility further, fish from a range of sites differing only in terms of predator presence would have to be tested. Such an experiment would address the problem of pseudoreplication present in this experiment caused by the limited number of populations represented, and permit generalisations about differences in behaviours displayed by populations living sympatrically with predators and those that are predator naïve.

An unexpected result was the intense interest the Obi Obi Creek (predator-naïve) fish showed towards the active predator in Experiment 1. This is contrary to the widely accepted observation that predator-sympatric fish inspect predators more frequently than predator-naïve fish (Magurran 1986, Magurran and Seghers 1990, Huntingford *et al.* 1992, Pitcher 1992). In the presence of a passive predator the naive Obi Obi Creek fish ignored the predator and moved randomly throughout the tank. The increased level of "predator inspection" observed in these fish during their exposure to the active predator would have probably led to the death of most school members were it not for the perspex separating them from the predator. Huntingford and Coulter (1989) have also observed that fish from low-risk sites spent more time inspecting a goldfish than did fish from high risks sites. Is this a case of predator inspection or fatal attraction?

Predator inspection is generally carried out by small groups (typically pairs) of fish that approach and then stop at a relatively safe distance from the predator. The trip is often exceptionally short in duration with only a few seconds spent fixating on the predator. Obi Obi Creek fish approached as a tight shoal of five fish and were only a few millimeters from the predator on many occasions. Rather than quickly fleeing to a safer distance after inspection, they tended to stay within a few body lengths of the predator most of the time.

The tendency to inspect and the minimum approach distance appear to vary greatly between different populations of fish (Magurran and Pitcher 1987, Magurran and Seghers 1994, Dugatikin and Alfieri 1992, Huntingford *et al*. 1994). Magurran and Seghers (1994) found that minnows from areas of low predation pressure showed high levels of predator inspection behaviour. They argued that this may be a remnant behaviour left over from the juvenile stage when fry have to avoid cannibalistic attacks. This may also be the case with rainbowfish. Similarly, Curio (1993) reported the retention of a minimal capacity to assess and evade predators in localities that no longer contain high numbers of predators. The behaviour of the Obi Obi Creek fish sometimes resembled classical predator inspection behaviour, but it was by no means well developed. Indeed it appeared that occasionally the Obi Obi fish were attempting to school with the predator rather than inspect it.

Obi Obi Creek fish may have initially been investigating the active predator, but rather than fleeing, they rapidly habituated because they suffered no physical harm. Habituation to models and non-threatening live fish has been shown to occur in many fish species but is often fastest in populations lacking high levels of predation (Huntingford and Coulter 1989). When confronted by the trawl apparatus, on the other hand, the Obi Obi Creek fish were forced to respond owing to the negative impacts of pursuit and physical confinement and showed marked improvements in their escape performance with repeated experimental exposure.

The results of this study highlight the importance and difficulty of distinguishing between general curiosity and predator inspection behaviour. It is widely accepted that predator inspection behaviour has a large curiosity component (Godin and Crossman 1994). It is not unusual for novel objects to attract the attention of rainbowfish in the wild. In many locations rainbowfish will inspect virtually any disturbance in their vicinity, even the body of a floating human observer (pers obs.). This high level of curiosity is probably related to the generalised feeding habits of rainbowfish and the variable environment in which they live. In habitats such as small creeks, which are dynamic in terms of food availability and habitat structure, it pays to sample novel objects in the hope of uncovering and exploiting new resources.

Magurran and Seghers (1994) stated that a general tendency to investigate novel objects in the environment is a widespread and adaptive behaviour regardless of the level of predation risk, since such risk may fluctuate over the medium to long term. However, I believe the introduction of predators to an environment such as Obi Obi Creek would almost certainly be disastrous. Such a scenario seems to have already occurred in Lake Eacham in Northern Queensland, where the disappearance of the Lake Eacham rainbowfish (*M. eachamensis*) from its type locality followed the introduction of predators (Barlow *et al*. 1987). Translocation of large predatory fish species in Australia for recreational angling is extremely common and represents a real threat to many smaller prey species. The trawl data suggest that with repeated exposure to predators, rainbowfish may be trained to avoid predators under controlled conditions, as is possible with hatchery-reared salmonids (Jarvi and Uglem 1993). This could certainly be a useful tool for future conservation reintroductions should they ever be deemed necessary.

References

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