CHAPTER 7 Problems associated with comparative studies of anti-predator behaviour when conducted in the field.

7.1 ABSTRACT

Anti-predator responses are notoriously difficult to observe in the field, especially in fish. The use of predator models to simulate attacks is perhaps one way to get around some of the problems. The results of the experiment outlined in this chapter highlight some of the difficulties faced when attempting to undertake experimental studies in highly uncontrolled circumstances. Considerable problems are caused by varying fish densities between sites and interference from other species, such as turtles and predatory fish. Changes in light conditions, depth of field and uncertainty over independence also represent substantial obstacles. However, in the right locations insight into anti-predator behaviour may be gained by close analysis of video footage. Data from Liverpool Creek suggest that when a model predator was present significantly more rainbowfish entered the field of view. These rainbowfish were also significantly larger than those seen when no model was present, as was mean shoal size. Singletons made up 50-60% of all shoals observed. In the Beatrice River, by contrast, the rainbowfish appeared to avoid the model predator. Site related differences in the size of the fish may explain some of the differences in responses towards the model predator. Rainbowfish from Charappa Creek appeared to completely ignore the bait, the model and the camera. At the Mt Father Clancy Overflow the fish showed extreme levels of curiosity towards any novel object in the water. These observations are discussed by reference to experimental results on population dependent behaviour obtained in controlled laboratory conditions in previous chapters.

7.2 INTRODUCTION

To date the majority of predator avoidance experiments have been conducted in the laboratory where all variables can be tightly controlled or manipulated as desired. Inevitably, one must question the validity of such experiments and their relevance to the real world. While experimental studies have convincingly established the influence that habitat complexity and predators have on prey abundance and behaviour, quantitative field observations are needed to assess whether experimental results are generally comparable to natural systems (Butler 1988).

Observations of predator-prey interactions in the field are few and far between, mostly because predation events are relatively rare. For example, Butler (1988) observed bluegill (*Lepomis macrochirus*) feeding behaviour and shoal size and attempted to make correlations with plant density and the presence of predatory bass (*Micropterus salmoides*). Bass were present in only 5% of the observations made.

A few authors have conducted field experiments by introducing model predators and simulating attacks. Seghers (1981) used a plastic fishing lure resembling a pike (*Esox lucius*) mounted on a glass rod and measured reaction distances of spottail shiners (*Notropis hudsonius*). Magurran and Seghers (1991) introduced a model pike into a stream and recorded the number of inspections made by guppies (*Poecilia reticulata*) and the inspection group size. Helfman (1989) and Helfman and Winkelmand (1997) presented models of Atlantic trumpet fish (*Aulostomus maculatus*) to three spot damselfish (*Pomacentrus partitus*) and juvenile bicolor damselfish (*Pomacentrus patitus*) respectively. Godin and Morgan (1985) used a preserved white perch (*Morone americana*) to frighten killifish (*Fundulus diaphanus*). While many of these experiments yielded interesting results, many of the authors were frustrated by

their inability to discern some of the more subtle differences in behaviour that may have been occurring in large schools (e.g. Seghers 1981).

In previous work, I too have confined most of my efforts to controlled laboratory experiments. The results presented here were collected over a total period of about a month spent in the field in an area around the Atherton Tablelands, 50 km south-west of Cairns in North Queensland. Some of the creeks and rivers in this area have excellent clarity and enable observations to be made either by snorkeling or by underwater video. Snorkeling represent a problem as fish are nearly always distracted by an observer's presence. The natural curiosity of these fish causes more than a few logistical problems.

The aim of these experiments was to validate some of the methods used, and results obtained, in the laboratory. The experiments also represent a pilot study that may enable profitable future research in the field.

7.3 METHODS

An underwater camera was set in place at six locations and baited with dried cat biscuits. Three of these locations (Lake Eacham, Liverpool Creek, and the Beatrice River) contained *M. splendida* populations while the other three (Mt Father Clancy Overflow, Charappa Creek and the Upper South Johnstone River) contained populations of *M. eachamensis*. For exact localities see Figure 7.1. The sites were chosen for water clarity rather than any other specific characteristics. The camera was suspended from a float within a wooden frame and held in place by a brick hanging underneath. A wooden pole was attached to the top of the camera and extended out to a distance of 40cm. At the end of the pole a mesh bag containing the bait hung from the pole in view of the camera. The bait was left for varying lengths of time to allow it to soak and the video recorder was then turned on. Video footage was collected for 15 – 25 min before a realistic model of a mouth almighty, *Glossamia aprion*, (standard length = 17cm) was introduced (this model was slightly larger than those used in the laboratory experiments). The camera remained active for a further 15 – 25 minutes. The model was suspended from a clear plastic bag and attached to the wooden pole by a length of fishing line. When the bag moved in the wind or bobbed up and down the model moved in a similar fashion.

While the camera was recording a habitat description sheet was filled out (see Pusey *et al.* 1995 and Chapter 4 for details of this procedure). Some of the information for the description was gained by snorkeling in the pool within which the camera was placed, after filming had ceased. This enabled me to gain information on the substrate, relative abundance of aquatic vegetation, the other species present in the pool and various other aspects of the habitat. The diversity and abundance of predators present was estimated from snorkeling forays, video analysis and personal communications with Dr Brad Pusey. The results of this survey are displayed in Table 7.1.

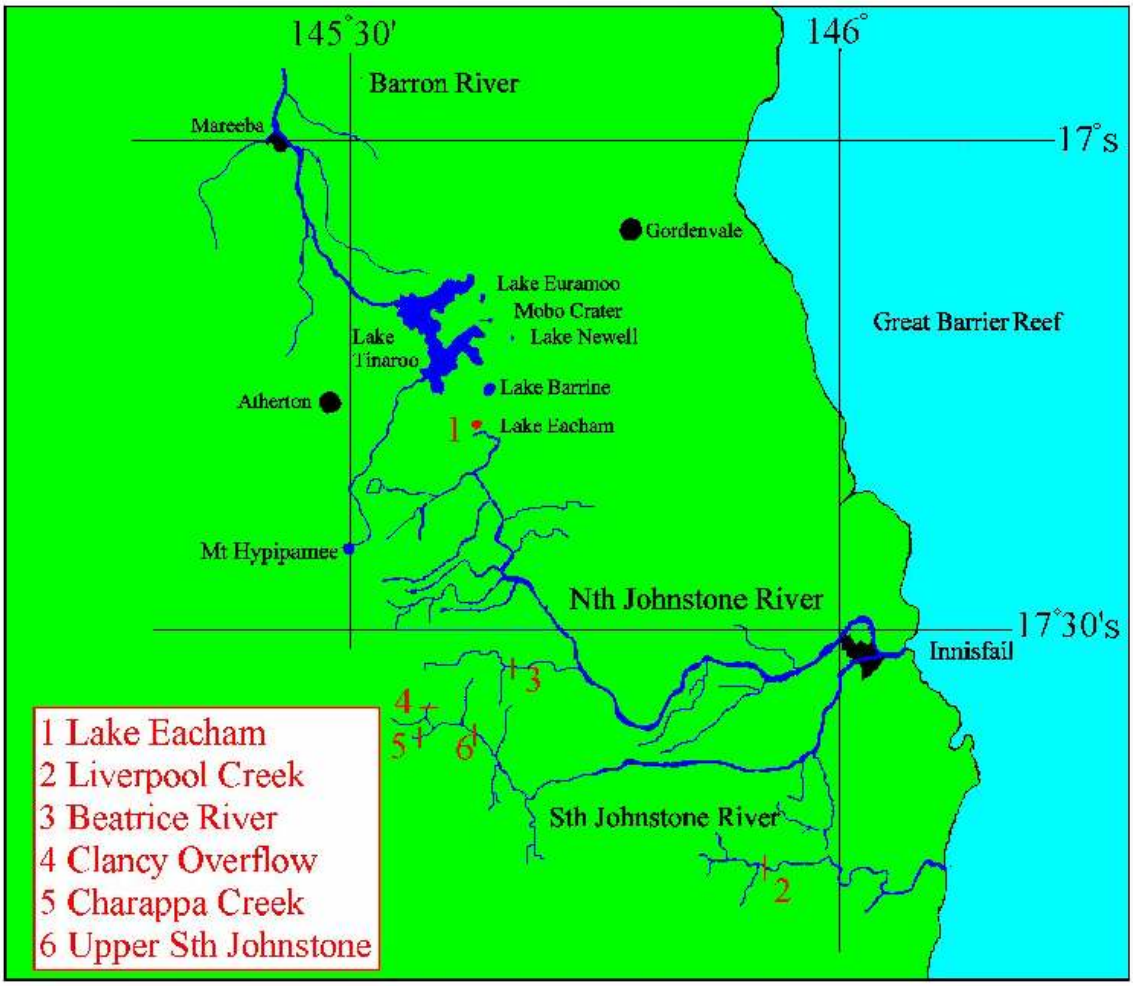

Figure 7.1. The location of the populations examined in this chapter.

Location	Rainbowfish species	Predator density	Habitat complexity	Water Clarity	Rainbowfish density	Relative size of rainbowfish
Lake Eacham	*M. splendida*	High	Moderate	High	Moderate	Large
Liverpool Creek	*M. splendida*	High	Low	Exceptional	Moderate	Very large
Beatrice River	*M. splendida*	Low	Moderate	Moderate	High	Small
Charrappa Creek	*M. each.*	Zero	High	High	High	Small
Mt Father Clancy	*M. each.*	Zero	Moderate	Moderate	High	Small
Upper Sth Johnstone	*M. each.*	Zero	Low	Exceptional	Low	Intermediate

Table 7.1. A summary of the characteristics of each sampling sites.

Upon returning to the University of Queensland, the video footage was transferred to VHS cassette with a timer included onscreen. During the analysis phase, the video was paused every 30 seconds and the number of fish within the field of view was counted. The footage from the upper South Johnstone River was analysed slightly differently owing to the extremely low numbers of fish. At this locations the number of times a fish entered the field of view within each 30 second interval was calculated instead.

The results were analysed in two ways. Firstly, the mean number of fish in view with and without the model was calculated, along with the standard deviation. Each 30 second interval was therefore considered to be independent of the next. While in reality this is probably not the case, it was impossible to determine when new fish arrive on the scene and others leave since the camera was only focused on a small field of view. The periods with and without the models were analysed using a t-test, but these results were treated as indicative only in view of the problem of non-independence. Secondly, the results were graphed over time to determine if there were any obvious patterns in the data set.

The video footage for Liverpool Creek was further analysed to estimate the length of fish during the periods with and without the model. This location was chosen for the excellent quality of the image and the intermediate densities of the fish on screen. The video was stopped at a random point in time and the first 50 fish on the screen were measured. The number of fish measured ensured that many shoals were sampled so one or two large shoals did not heavily influence the data. This was done during both periods. All fish were approximately the same distance from the camera (the bait bag was used as a reference point) and were perpendicular to the field of view. The lengths were compared using t-tests.

Liverpool Creek footage was also used to estimate the frequency of different shoal sizes during the periods with and without the model. A shoal was recorded only if it passed through the field of view without lingering. The distance between individuals had to be three body lengths or less. Any shoal passing within 2 meters of the camera was included in the data set. The shoal frequency data were converted to proportions to allow a comparison between the two periods. The mean shoal size was calculated and a t-test conducted to compare shoal sizes during the two periods.

7.4 RESULTS

The results are very difficult to interpret in general terms and cross population comparisons are very difficult owing to the nature of the data collected. Each location must be considered in turn.

7.4.1 Upper South Johnstone River

At the upper South Johnstone River there was a single increase in fish numbers at the seven min mark during the no model period, and later after approximately 30 min. These pulses are suggestive of schools of fish. The brief occurrence at the 7 minute mark was due to a shoal moving past the camera, while at the 30 minute mark the school stopped to investigate the area (Figure 7.3c). The stretch of river where the camera was placed was about 25 meters across and had a very swift current. Rainbowfish at this site are relatively scarce and were found in eddies and calmer waters behind boulders, or near the banks. The camera was fixed to the bank in some overhanging vegetation. The water depth was around 1.5 meters. *Macrobrachia spp*. were the only other animals observed both on camera and during the snorkeling, trapping and seining activities.

7.4.2 Charrapa Creek

Charrapa Creek fish were more interested in the camera than the bait. Most of the fish entered the view of the camera but appeared to ignore the bait. The bait did attract the attention of several *Macrobrachium*. No other species were observed. Figure 7.2e indicates that there was almost no change in the number of rainbowfish present when the model was introduced, averaging around 2.7 fish at each 30 sec interval (Figure 7.3d). The camera was located in a relatively shallow eddy (40 cm deep) with a cobble substrate.

7.4.3 Lake Eacham

The recently introduced population of *M. splendida* in Lake Eacham showed no change in the number of fish at the bait when the model was introduced ($t = -0.496$, $P = 0.625$, Figure 7.1a). Unfortunately the number of 30 sec observations was low ($n = 15$, $m = 9$) due to technical difficulties. Mouth almighty were abundant within the lake as were archer fish (*Toxotes chatareus*), both of which have been known to predate on rainbowfish (White 1991 and Pusey pers. comm.). During the taping period a great many archer fish visited the bait and scared the rainbowfish off. Most of the rainbowfish had left the bait before the archer fish came into view. Small banded grunter (*Amniataba percoides*) also frequented the bait but represented no threat to the large rainbowfish. A turtle disturbed the bait bag for some time causing a slight increase in the number of rainbowfish present. The camera was located in Turtle Bay, an area closed to swimmers. It was placed amongst fallen timber, which contributed the greatest amount of structure in the water. The bank fell away very quickly and although the camera was 3 meters from the bank, the depth of water was well over 4 meters. SCUBA forays revealed that large mouth almighty seemed to occupy the deeper waters (> five m) during the day but probably moved to the surface to hunt during the night. No mouth almighty visited the bait during the filming period.

7.4.4 Beatrice River

At the Beatrice River site there was a decrease in the number of fish around the bait with the introduction of the predator model ($P = 0.010$, $n = 31$, $t = 2.694$). The only other species observed in the pool within which the camera was located or in adjoining pools was the hardyhead (*Cratercephalus stercusmuscarum*). The density of rainbowfish in the pool was very high (Figure 7.2c). Figure 7.3b indicates a gradual increase in the number of fish at the bait from around 10 fish to over 20 at $t = 15$ min. The introduction of the model was associated with an instant decrease in the number of fish. The level remained relatively low (around 14 fish) for the remainder of the trial. The camera was located in an eddy in a stretch of water about 70 cm deep. The bank was dominated by grass and overhanging vegetation and the substrate comprised of leaf litter and gravel.

7.4.5 Mt Father Clancy Overflow

The Mt Father Clancy Overflow had numerous large boulders and a gravel substrate dominated the area where the filming took place. The water was fast-moving in the deeper channels, but the camera was placed far from the current amongst some large boulders next to a grassy bank. The depth was a little over a meter. As with the other *M. eachamensis* sites no other fish species were observed, although a turtle visited the bait at this site. Of all the sites the results from this site were the most confusing. The rainbowfish in this area were very small with a standard length a little over four cm. Any new objects in the water fascinated them. During the snorkeling surveys, I was constantly followed by very large schools of fish. Figure 3e indicates the number of fish in view over the recording period. Initially the number of fish was relatively low, around 4 fish. At the 24.5 minute mark, the number of fish rose suddenly to well over 20 individuals and remained that way for the rest of the trial. The sudden change coincided with a

brief change in the light regime. The clarity increased slightly for a minute before returning to normal once more. The number of fish, however, remained high. Many fish pecked at the lens of the camera, the bait bag and the model once it was introduced.

Interestingly, once the model was introduced, the fish moved out of view for one min and 45 sec, then moved in and investigated the model. This continued for three min and 15 sec. The fish appeared to be attracted by shining objects such as small bubbles and the plastic fins on the model. After the investigation ceased the number of fish decreased for around a minute before recovering and the attention of the fish was fully redirected back to the bait bag.

7.4.6 Liverpool Creek

The Liverpool Creek produced the most interesting result of all. During the period when no model was present swarms of blue eyes (*Psuedomugil signifer*) surrounded the bait bag and the number of rainbowfish stayed relatively low. Once the model was presented, however, the blue eyes disappeared and the number of rainbowfish at the bait increased (paired t-test; t = -2.536, n = 41, P = 0.0014, Figure 7.1b). Liverpool Creek had the largest rainbowfish of any of the sites. Mean standard length was estimated at well over 12 cm and fish over 15 cm were not uncommon. Some of the rainbows at this site were bigger than the predator model. The bait was also visited by several small jungle perch (*Kuhlia rupestris*) (standard length around 12 cm) and at least one larger jungle perch (standard length over 20 cm). Some of the small jungle perch appeared to school with the larger rainbowfish. While large sooty grunter (*Hephaestus fuliginosus*) were common in the deeper water as evidenced from the snorkeling forays, they never visited the bait. The appearance of the larger jungle perch coincided with a period when no rainbows were in view, so that no reaction towards this predator was observed. Figure 7.3a shows the number of fish in view over the test period. During the period with no model, the numbers of rainbowfish were generally very low. A period of relatively high numbers of fish occurred approximately five min after the introduction of the model was introduced and lasted about seven min. Interest in the model seemed to return to background levels. The camera was located in a shallow area (40 cm deep) two meters from fast flowing, deeper water. The substrate was mostly gravel and cobbles.

The body length data for Liverpool Creek revealed that the length of fish on screen when the predator model was present was significantly greater than during the period when no model was present (t = -6.093, n = 50, $P < 0.001$) (Figure 7.4).

The school size frequency distributions for both periods were similar and singletons dominated during both periods (Figure 7.5). Singletons represented 62% of the shoals observed when the predator was absent and 49% when the predator was present. Shoal sizes of three and over were more common when the predator model was present. The mean (± se) shoal sizes for the periods without and with the predators were 1.772 (±0.177) and 2.424 (±0.196) respectively. The average shoal size was significantly larger when the predator was present (t = -2.44, n1 = 79, n2 = 149, $P = 0.015$).

Figures 7.2 a-e. The mean (± SE) number of fish at the bait before and after the introduction of the model for five sites. *P*-values are the results from t-tests. *M. splendida* populations from Liverpool Creek (2b) and the Beatrice River (2c) showed significant but opposite changes in mean fish number with the introduction of the model.

Figure 7.3 a-e The number of rainbowfish with in the field of view with and without the predator model over the entire length of time for five sites.

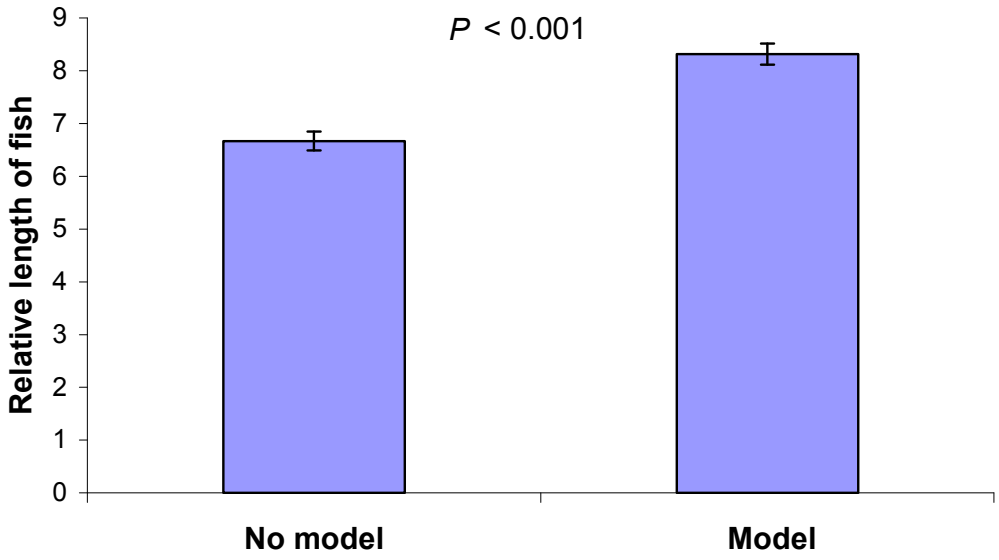

Figure 7.4. The average length of rainbowfish when no model was present and when the model was introduced into Liverpool Creek

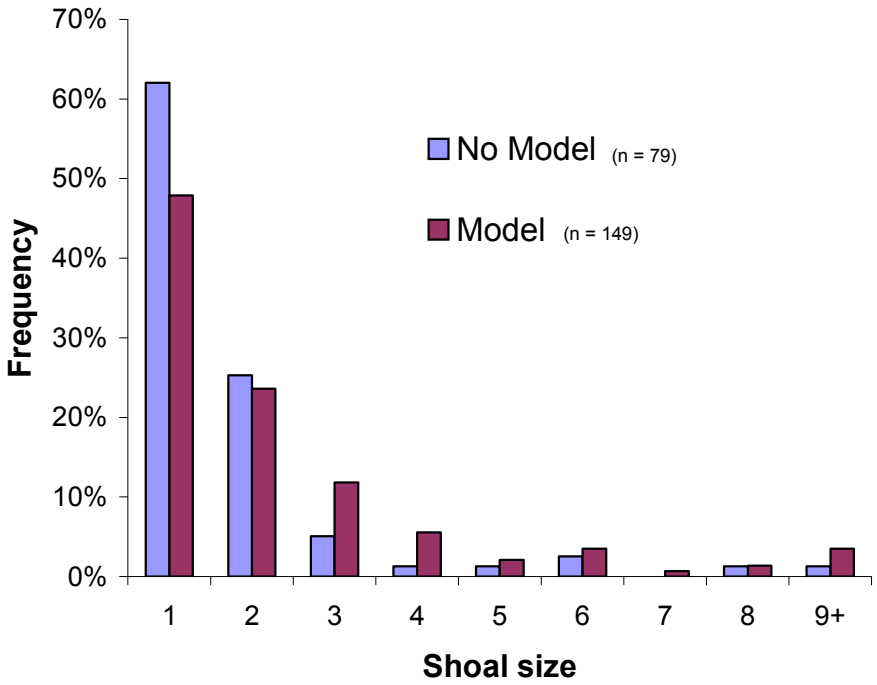

Figure 7.5. The frequency distributions of shoal sizes when the model was present and absent at Liverpool Creek.

7.5 DISCUSSION

These experiments highlight the difficulty of carrying out field experiments to look at anti-predator responses in rainbowfish and probably all fish in general. However, despite the obvious logistical and statistical problems, some valuable insights into how fish behave in the wild can be obtained. This is especially highlighted by the Liverpool Creek results.

7.5.1 Liverpool Creek

Following the introduction of the predator model at Liverpool Creek, the number of rainbowfish in the vicinity of the bait increased significantly. The rainbowfish in Liverpool Creek are exceptionally large and may well have been further attracted to the area by the model perhaps by forage area copying (Pitcher and House 1987) or some other social attraction mechanism. Most of the fish in view during the model period appeared to be large males which where perhaps least afraid of the model. When the model was presented the average relative length of the rainbows increased significantly. This interesting information, combined with a significant increase in the number of rainbows, suggests that the larger rainbows were recruited into the area while the smaller individuals remained, thus boosting the number of fish and the mean size.

Prior to the model being introduced at Liverpool Creek, hundreds of blue eyes (*Psuedomugil signifer*) swarmed around the bait and rainbowfish numbers were relatively low. The location of the camera may offer some explanation for this observation as it was situated in relatively shallow, slow flowing water amongst cobbles, which appeared to be the main microhabitat for blue eyes. The current in the deeper portions of the creek would be far too great for their swimming capabilities and large predators such as jungle perch and sooty grunters occupied these areas. The blues eyes, far smaller fish than rainbowfish, appeared to show a strong avoidance response to the predator model. Unfortunately they were too small to count accurately so quantitative data could not be collected to verify this observation.

7.5.2 Between site variation in curiosity

At no stage did any of the fish at any location show predator inspection behaviour or prolonged avoidance of the model (with the exception perhaps of the Beatrice fish). It may well be the case that inspection behaviour occurred outside the field of view at a much safer distance or that the model wasn't realistic enough to invoke it. Earlier work suggests that movement is an important cue used in the assessment of threat (Brown and Warburton 1997, 1998, Chapter 2). The bobbing and swinging motion of the predator model may not have been realistic enough to cause a threat response in the rainbowfish. Fear of the model may even be context dependent (see Chapter 4). However, it seems likely that two of the *M. splendida* sites (Lake Eacham and Liverpool Creek) contained mostly large fish which probably excluded them from attack by a predator of the size of the model used in this experiment. The lower regions of the Beatrice River certainly contain predators (Pusey pers. comm.), but at the up-stream location where the filming took place, no predators were sighted either on snorkel or on video. At this location the fish appeared to be much smaller than at the other two *spendida* sites. The extremely high density of rainbows in the area suggests that there were few piscivorous fish species.

The data from the Beatrice River site showed a significant decrease in the number of fish once the predator was introduced. The most plausible explanation is that some fish avoided the bait once the predator was present, indicating an underlying ability to recognise predators in this population. It may well be the case that the smaller fish avoided the model at this site, but the high densities of fish around the bait made length 'guestimates' virtually impossible. In future work it would be interesting to estimate the size of the fish visiting the bait during the periods with and without the model at all sites. This type of information may be gained by taking a scoop of the fish at the bait with a dip net at a random point in time.

At the Mt Father Clancy Overflow an inexplicable increase in the number of fish shortly before the introduction of the model made analysis impossible. However, closer inspection of the footage around the time the model was presented provided an interesting insight into the high level of curiosity and boldness of these fish. As the model was introduced, the disturbance caused the fish to flee but only for a matter of seconds. After 1 minute and 45 seconds they

returned to their initial abundance and began to investigate the model. Investigation continued for a few minutes before their attention was fully redirected to the bait bag. Investigation was marked by very large numbers of fish gathering around the model and repeated nipping at the surface (presumably to remove the small shiny bubbles or to determine if it was edible). This type of response (i.e. attraction) is typical of most of the predator naïve populations I have tested in the laboratory.

The Upper South Johnstone site by contrast had so few fish that the results were overwhelmed by the appearance of a couple of shoals at the 6.5 and 30 minute marks. At face value these results seem to have remarkable similarities with the data from Liverpool Creek. However, the densities are exaggerated in the former owing to slight differences in the tallying of the data. Charrappa Creek fish ignored both the bait bag and the model. The model was never inspected by any of the rainbowfish at this location. Therefore, the number of fish onscreen probably reflected natural densities.

7.5.3 Trouble shooting with field experiments

It is abundantly clear that field based experiments are difficult to interpret. Luyten and Liley (1985) found it was difficult to compare field collected data because many activities are likely to be affected by local environmental conditions. One of the most important confounding factors in these experiments was the lack of control over the numbers of fish that visited the baiting station and the inability to distinguish between individuals. It is not known, for example, from how far away the fish were recruiting, making replication within a pool impossible due to a lack of independence. Similarly it is unknown if fish were recruited to the bait and quickly left, or if they stayed in the immediate area for some time and entered and exited the field of view several times. In some cases the density of fish around the bait was simply too high to calculate visitation rates, while at other sites visitation was very low and the data were dominated by the appearance of one or two schools. Variations in light regime resulted in changes in the depth of field and the number of visible fish on the screen. Large predatory fish species visiting the bait also impacted heavily on the number of fish observed. While predator visitation data may provide useful insights into the reactions of rainbowfish towards natural predators, the frequency and circumstances of visits were unpredictable.

For the future it may be necessary to estimate the number of fish with a pool and determine how far the fish are recruiting from. An estimate of the size distribution in the pool would also be very useful to determine which sub-set of fish visit the bait station. A white board could be placed in front of the camera at a set distance to control for depth of field and to increase contrast. All the equipment should be left to stand for a substantial length of time prior to the commencement of the trial. This would enable fish to become accustomed to the strange objects in the water. Each location would have to be repeatedly surveyed, perhaps by focussing on neighbouring pools, to increase the power of the results.

7.5.4 School size frequency in the field

Most of the species observed in previous field studies were commonly found in schools consisting of five or less individuals, with singletons being by far the most common group size (Seghers 1981, Godin and Morgan 1985, Butler 1988, Magurran and Seghers 1991, Magurran and Seghers 1994.). The data from Liverpool Creek and from casual observations suggests that this was also the case in this study. This contradicts the dominant paradigm that suggests that schooling provides multiple benefits associated with foraging and predator avoidance. Why then do single fish occur so commonly? Eggers (1976) suggested that schooling could be disadvantagous for small planktivorous fish because the visual range of each individual overlaps with that of their neighbors, resulting in foraging competition. Evidence from field

data highlights the fact that many small fish species are probably facultative schoolers and form large schools only when a threat presents itself (Seghers 1981, Butler 1988).

7.5.5 Comparing natural and experiment school sizes

Assuming the estimate of mean school size is accurate, the Liverpool Creek data suggest that the fish entered the area in larger groups when the model is present. This is consistent with the hypothesis that group sizes increase with increasing threat (the threat sensitivity hypothesis, Helfman 1989). Mean school sizes were consistent with the average shoal sizes observed in the laboratory, which typically range from 1.5 to 3 depending on the population tested and the context (Brown and Warburton 1997). However, some situations encourage relatively high average group sizes (e.g. 4-5, see Chapter 2). When placed in the laboratory, fish find themselves in a restrictive, totally artificial environment with little of interest to distract them other than their shoal mates. Such an environment is lacking in stimuli such as foraging cues, and may result in unusually high group sizes. Similarly, elevated stress levels resulting from the unfamiliar surroundings in the test tank may also have a significant effect on schooling tendencies. The small size of test enclosures may also artificially raise mean group size by constricting the space in which the fish can move. Luyten and Liley (1985) found differences between field and laboratory results and were unsure as to whether these differences arose due to experimental factors or differences between the observation tank and the natural environment. Nevertheless the Liverpool Creek data do add credence to the use of relatively small groups of fish (4 or 5) during laboratory experiments.

7.5.6 Conclusions

1) Rainbowfish populations appear to differ in terms of curiosity and boldness. This may be related to the predator regime at each locality and/or the relative size of the fish with respect to the predator model.
2) Avoidance appeared to be size dependent as highlighted by the response of the blue eyes in comparison to the larger rainbowfish at Liverpool Creek.
3) Shoal sizes of less than 5 fish are extremely common in the field and singletons comprise around 50-60% of all observations.
4) Clear avoidance of the model could only be demonstrated at the site in the Beatrice River. At the other sites (in particular at Mt Father Clancy Overflow) rainbowfish showed initial fear of the model as it was introduced to the water. Soon afterwards the fear response dissipated.
5) Clear inspection behaviour towards the model occurred only at the Mt Father Clancy Overflow site.
6) Behavioural responses appear to be independent of the rainbowfish species, although it is difficult to draw clear conclusions about species-predator pressure interactions since *M. eachamensis* has been found to occur with predators at only a single locality.

References

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