

# Murray-Darling Basin Authority Native Fish Strategy

## Strategies to improve post release survival of hatchery-reared threatened fish species



**Michael Hutchison, Danielle Stewart, Keith Chilcott,  
Adam Butcher, Angela Henderson, Mark McLennan and Philip Smith**

Bribie Island Research Centre  
Department of Employment, Economic Development and Innovation.

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## Executive Summary

Fish stocking is one tool that can be used in conservation programs to help restore threatened fish stocks. Unfortunately hatchery reared fish can have some behavioural deficits related to domestication that can hinder their survival in the wild. Pond reared fingerlings seem to retain live food foraging skills and some bird avoidance behaviours, but they are naïve in avoiding predatory fish. Fish reared to larger sizes (*i.e.* to adult or sub-adult stage) in grow out facilities tend to be fed on artificial pellet diets and are protected from birds and other predators. These fish are likely to be inexperienced in foraging for live foods and poor at avoiding predatory birds like cormorants and pelicans if stocked into the wild. Pre-release training of hatchery reared and grow-out facility reared fish is one strategy available to improve survival after stocking into the wild. The value of pre-release training was evaluated in this study.

Tank-based training exposed fingerlings of Murray cod, Silver perch and Freshwater catfish *en masse* to predatory fish and chemical alarm signals from fish skin extract. Tank-based validation experiments confirmed that this training significantly improved the predator response behaviour of all three species compared to untrained fish. At least 72 hours training was required for Murray cod and Silver perch fingerlings and 48 hours training for catfish fingerlings to significantly change predator avoidance behaviour.

Sub-adult Murray cod and sub-adult Silver perch from grow-out facilities (where they were reared on pellet diets and protected from bird exposure) were trained to avoid simulated cormorant attacks. Training used a combination of bird models to harass and chase fish, cormorant odour and alarm signals from fish skin extract. Trained sub-adult Silver perch showed significant behavioural changes in response to simulated cormorant attack compared to untrained groups. However sub-adult Murray cod showed no significant change in behaviour.

Sub-adult Murray cod and adult Silver perch from grow-out facilities were also trained to take live food. To assist this process a wild Murray cod or silver perch was introduced into each training tank to help cue the behaviours of the fish from the grow-out facilities. Silver perch readily adapted to taking live shrimp in the training tank, but pellet reared cod refused to take live shrimp over a one month training period.

Sub-adult and adult Silver perch seem to be highly trainable, but sub-adult Murray cod are not. Silver perch are a social schooling species and this may enhance training. In contrast Murray cod tend to be territorial and solitary. Therefore we recommend avoiding use of long term pellet reared sub-adult Murray cod in conservation restocking programs. If large fish are required for conservation stocking, we suggest translocation of wild caught sub-adults or adults may be a better option.

Stocking trials at three sites in the northern Murray-Darling Basin were used to test if pre-release training improved survival of stocked fingerlings of Silver perch and Murray cod. Predator free release cages were also tested as a stocking method to improve survival. Prior to stocking, fingerlings were marked with visual implant elastomer tags to indicate if stocked fish were trained or untrained and whether they were released directly into the wild or into predator free release cages. Half of the

trained fish and half of the untrained fish were stocked into predator free cages at each of the stocking sites. Fish were given 90 minutes to adjust to local waters in the cages, before being released into the wild. Trained and untrained fish were stocked at least 1 km apart.

Pre-release training led to a significant improvement in survival of trained Murray cod, compared to untrained control fish. At locations where predators were more abundant, the survival of trained Murray cod was up to four times higher than untrained Murray cod. Across all locations the average survival rate of trained Murray cod was twice that of untrained Murray cod. We recommend pre-release training of Murray cod fingerlings that are to be used in conservation stocking programs. This training may also be of benefit to recreational fish stocking programs.

Predator release cages seemed to disadvantage the survival of stocked Murray cod fingerlings. The reason for this is uncertain, but it is possible that the behaviour of cod within the cages may have attracted predators and these predators then preyed on cod when they were released from the cage. We suggest that stocking cod fingerlings directly into a dam or river is the best option.

In contrast to the tank based validation results, there were no significant differences detected between trained and untrained Silver perch stocked into the wild. One possible explanation is that Silver perch are a schooling fish. Rapid dispersal from the stocking sites and amalgamation into mixed schools of trained and untrained fish may have led to rapid social learning of the untrained fish from the trained fish. Based on observations of improved predator avoidance behaviour in tanks and the likelihood that social interactions confounded the field results, we recommend that pre-release training still be used when stocking Silver perch fingerlings for conservation purposes.

Predator free cages neither advantaged nor disadvantage stocked Silver perch. We conclude it is acceptable to release Silver perch directly into river or dam waters.

Predator abundance had a significant impact on survival outcomes for both Murray cod and Silver perch. Survival was lowest in locations with high predator abundance. The patchiness of predator distributions within a site means it is best to use several release points at a site. This will spread the risk. Large batches should be stocked at each release point to ensure some swamping of predators.

**Key recommendations**

- Pre-release training for predatory fish avoidance should be used when stocking Murray cod fingerlings for conservation purposes.
- Pre-release training should be used when stocking Silver perch fingerlings for conservation purposes.
- To reduce predation risk several release points should be used at any given site. Fingerlings should be stocked in large batches at each release point.
- Until alternative predator exclusion designs with proven results are developed, stocking of Murray cod fingerlings should be done directly into the receiving waters.
- It is acceptable to release Silver perch directly into the receiving waters.
- Further research should be conducted into the influence of social learning on Silver perch predator avoidance, to determine what proportion of stock need training to benefit untrained fish released at the same time and location.

## Background

### **The role of stocking for threatened fish recovery in the Murray-Darling Basin**

Several native fish species in the Murray-Darling Basin, south-eastern Australia, have declined significantly and are listed as vulnerable or endangered in part of, or across all of their former range within the Basin (Lintermans, 2007). These species include large bodied species such as Murray cod *Maccullochella peelii peelii*, Trout cod *Maccullochella macquariensis*, Macquarie perch *Macquaria australasica*, Silver perch *Bidyanus bidyanus* and Freshwater catfish *Tandanus tandanus*, as well as small bodied species like the Southern purple-spotted gudgeon *Mogurnda adspersa* and the Olive perchlet *Ambassis agassizii* (Murray-Darling Basin Commission, 2004).

Actions such as rehabilitating fish habitat, protecting fish habitat, managing riverine structures (including barriers to migration), controlling alien fish species, protecting threatened fish species and managing fish translocation and stocking are all likely to contribute to the recovery of fish stocks (Murray-Darling Basin Commission, 2004), including threatened fish species. Unfortunately there are catchments in the Murray-Darling Basin where some native fish species have already become locally extinct. For example Murray cod and Freshwater catfish are presumed extinct in the Paroo River system. If not extinct, then they are in extremely low numbers. In such situations a carefully managed reintroduction program may be required. Such a program may involve the use of hatchery-reared fish as part of a conservation restocking program. Within Australia hatchery reared Mary River cod *Macullochella peelii mariensis* and Trout cod *M. macquariensis* have already been stocked as part of the recovery programs for these species (Simpson and Jackson, 1996; Lintermans and Ebner, 2006).

Combined with actions such as removal of migration barriers, habitat restoration and pest fish management, reintroduction programs have a greater chance of success. However stocking of hatchery reared fish does not always lead to the dramatic improvements in fish stocks that might be expected (Blaxter, 2000; Hutchison *et al.*, 2006 and Larscheid, 1995). Fisheries scientists have known of poor post-release survival rates of hatchery reared fishes for over a century (Brown and Day, 2002).

### **Hatchery domestication effects**

Svåsand *et al.* (2000) noted that more than a century of cod *Gadus morhua* stocking in the Atlantic had not led to any significant increases in cod production or catches. A review paper by Brown and Laland (2001) provided evidence that hatchery reared fish have lower survival rates and provide lower returns to anglers than wild fish. There is evidence that hatchery rearing of fish can produce behavioural deficits that can impact on their post-release survival (Olla *et al.*, 1994, Stickney, 1994). Brown and Laland (2001) also noted the difference in mortality rates between hatchery-reared and wild fish is especially large when size and age are taken into account.

Many of the life-skills considered as instinctive or inherited traits such as foraging, predator avoidance and reproductive behaviour are now considered to have a significant learned aspect. This includes social learning from other fish (Jonsson, 1997; Brown & Laland, 2001; 2003). Absence of natural conditions and experienced conspecifics in a hatchery environment can therefore impact on natural and social learning in hatchery reared fishes.

A review of hatcheries (supplying fingerlings of threatened Murray-Darling Basin fish species) and grow out facilities (that were potential providers of adult and sub-adult threatened fish) was carried out by some of the authors of this current report (Hutchison *et al.*, in press). The most commonly reared threatened species were Murray cod, Silver perch and Freshwater catfish. A common trend across all species was that hatchery-reared fish tend to be produced in ponds and exposed to live foods. There was also some exposure to predation by birds, but hatchery owners tried to limit this. Excluding cannibalism in cod, there was virtually no exposure to predation by fish.

All grow-out facility reared Silver perch and Murray cod were pellet fed. The bulk of grow-out facility reared Murray cod were tank reared and not exposed to predation by birds or fish. In contrast Silver perch were pond reared and just over half were exposed to birds.

One key deficit in hatchery-reared fish is their failure to recognise or respond appropriately to predators. Various studies have confirmed this in a variety of marine and freshwater fish species (Alvarez and Nieceza, 2003; Malavasi *et al.*, 2004; Stunz and Minello, 2001; Ebner *et al.*, 2006). This deficit most likely arises because under most hatchery rearing conditions fish are reared under predator free conditions, and are therefore naïve to predators when stocked. Another deficit in hatchery reared fish (particularly those reared on artificial diets) is that stocked fish fail to recognise natural or wild foods or may have less efficient foraging behaviour (Brown *et al.*, 2003; Ersbak and Haase, 1983). Norris (2002) observed physiological changes in the taste receptors of whiting *Sillago maculata* reared on pellets. However Olla *et al.* (1994) state that many pellet reared fish readily switch to live prey food under laboratory conditions. Masee *et al.* (2007) found that juvenile sockeye salmon *Oncorhynchus nerka* reared either on pellets, *Artemia* or a combination of pellets and *Artemia* showed no significant difference in their ability to capture pellet, *Artemia* or mosquito larva prey.

Other deficits have also been reported in hatchery reared fish released into the wild. They include different migration and dispersion patterns (Ebner and Thiem, 2006; Bettinger and Bettoli, 2002) compared to wild fish, differences in degree of aggression (Pettersson and Jaervi, 1999) and poorer mating success (Pettersson and Jaervi, 1999; Heggenes *et al.*, 2006). Butler and Rowland (2009) speculate that the complex parenting skills essential for Eastern freshwater cod *M. ikei*, to successfully reproduce may involve learned behaviour. They suggest that strategies such as planting of experienced parents to act as surrogate trainers may be required to ensure the success of future remediation programs.

#### **Factors affecting post-stocking survival.**

Other than hatchery related behavioural deficits, factors that can contribute to poor stocking related outcomes include transport stress (Portz *et al.*, 2006) and timing of stocking. Hutchison *et al.* (2006) recommend stocking fingerlings as early as possible in order to take advantage of the spring and summer growing season. Other researchers have also suggested stocking early in the season improves chances of survival (Sutton *et al.* 2000, Leber *et al.*, 1996, Leber *et al.*, 1997).

Most mortalities occur immediately after stocking, *i.e.* in the first few days, rather than first few weeks (Sparrevohn and Stoettrup, 2007, Brown and Laland, 2001, Olla, *et al.*, 1994). One of the major causes of mortality is predation (Olla *et al.*, 1994). Buckmeier *et al.* (2005) estimated 27.5% of stocked Largemouth bass *Micropterus salmoides* fingerlings were taken by predators within 12 hours of stocking into a Texas Lake. In contrast mortality in predator-free enclosures was only 3.5% after 84 hours, indicating mortality from transport and other variables was low. Hutchison *et al.* (2006) sampled predatory fishes 4 hours after releasing micro-tagged hatchery reared Barramundi *Lates calcarifer* fingerlings into an impoundment. Hutchison *et al.* (2006) found that variation between predation levels on different batches of fingerlings released on the same day, but into different parts of the same water body were reflected in recapture rates of the stocked fish more than 12 months later, suggesting that initial predation on release had the biggest influence on overall survival patterns. This suggests that if fingerlings are able to survive the early stages of stocking, they have a much better chance of surviving to adult size.

### **Stocking size and post-stocking survival**

One strategy that has been used to try to combat predation of stocked fish has been to increase release size. Hutchison *et al.* (2006) showed that fingerlings of Barramundi *L. calcarifer*, Australian bass *M. novemaculeata*, Golden perch *M. ambigua* or Silver perch *B. bidyanus* had significantly better survival when stocked at 50-65 mm TL, compared to 20-30 mm TL and 35-45 mm TL. However the degree of improvement obtained by stocking larger sized fish varied according to the predator composition of the stocked water body. Similar conclusions have been reached for stocking experiments conducted with other species including Red drum *Sciaenops ocellatus* (Willis *et al.*, 1995), Whitefish *Coregonus lavaretus* (Jokikokko *et al.*, 2002), Largemouth bass *M. salmoides* (Miranda and Hubbard, 1994), Sea mullet *Mugil cephalus* (Leber and Arce, 1996), Lake trout *Salvelinus namaycush*, (Hoff and Newman, 1995), Rainbow trout *O. mykiss* (Yule *et al.*, 2000) and Muskellunge *E. masquinongy* (McKeown *et al.*, 1999).

Stocking fish at a size beyond which they are likely to be taken by most predatory fish has often given the best results. For example stocking Rainbow trout larger than 208 mm (Yule *et al.*, 2000) and Red drum at mean length of 201.7 mm (Willis *et al.*, 1995) have resulted in higher survival compared to smaller fish. Recent work on Barramundi *L. calcarifer* in predator dominated north Queensland rivers and impoundments suggests that stocking barramundi at sizes greater than 300 mm TL gives better survival outcomes than stocking fingerlings and is also more cost effective. (Russell, pers comm.<sup>1</sup>; Pearce, pers comm.<sup>2</sup>). However the experience of Ebner and Thiem (2006) and Ebner *et al.* (2006) with poor survival of large hatchery reared Trout cod *M. macquariensis* suggests that hatchery domestication can have the potential to remove the advantages of large size-at-release in some species. Similarly Koike *et al.* (2000) had better returns for Masu salmon *O. masou* stocked in spring as 0+ fry, compared to larger 0+ parr stocked in autumn and 1+ smolts stocked in spring. Stocking of fertilized eggs had the poorest success rate.

### **Learning in fish**

Recent research supports the concept that fish can learn. Social learning of predator avoidance is reported to be widespread among fishes. A review by Brown and Laland (2001) provided ample evidence of predator naïve fish being able to rapidly acquire

predator avoidance skills with training. Kelley and Magurran (2003) state that visual predator recognition skills are largely built on unlearned predispositions, but olfactory recognition typically involves experience with conspecific alarm cues. According to Brown (2003) many prey species do not show innate recognition of potential predators. Rather they acquire this knowledge based on the association of alarm cues with the visual and /or chemical cues of the predator. Brown *et al.* (1997) demonstrated that a population of 80,000 fathead minnows in a 4 ha pond, learned to recognise the chemical cues of northern Pike within 2 to 4 days.

Fish not only learn predator avoidance skills but other skills as well, including foraging behaviour. A review by Hughes *et al.* (1992) provided evidence that fishes can optimise foraging behaviour through learning. Warburton (2003) presented further evidence for learning of foraging skills by fish.

### **Reducing domestication effects prior to stocking**

Conservation biologists have long recognised the importance of conditioning captive bred mammals and birds prior to release and using soft release strategies to improve post-release survival (Brown and Day, 2002). There are numerous examples where this approach has been used or trialled (Beck, *et al.*, 1994; Biggins and Thorne, 1994; Box, 1991; Carpenter *et al.*, 1991; Kleiman, 1989; McLean *et al.*, 1996; Miller and Vargas, 1994 and Soderquist and Serena, 1994). There has also been increasing interest in pre-release training and conditioning of hatchery reared fishes to overcome domestication effects. Most of the experiments involving fish have been lab-based, with some expanding to pond-based experiments. But there have been few field based experiments to confirm the tank and pond-based experimental results to date.

There have been quite a number of lab-based evaluations of training hatchery reared predator naïve fish to recognise and respond to predators. Training has involved a number of different approaches, including non-contact training where hatchery fish are exposed to a predator through transparent netting (Jarvi and Uglem 1993) and contact training where hatchery fish are exposed to a free roaming predator (Jarvi and Uglem 1993). Contact training had better results than non-contact training, but non-contact trained fish still responded better to predators than unexposed control fish.

Odours can be used to enhance training. Vilhunen (2006) exposed hatchery reared arctic charr *Salvelinus alpinus* to odours of Arctic charr fed Pike-perch *Sander lucioperca*. Ferrari and Chivers (2006) used alarm cue odours derived from skin extract of Fathead minnows *Pimephales promelas*, to condition Fathead minnows to exposure to Brook charr *S. fontinalis*. Conditioning to alarm cues appears to work well (Ferrari and Chivers, 2006; Leduc *et al.*, 2007) and fish seem to be highly sensitive to predator and alarm odours. The predator avoidance response varies according to the intensity of these odours (Brown *et al.*, 2006; Ferrari *et al.*, 2006a, Ferrari *et al.*, 2006b). Odour cues could be important in turbid environments like the Murray-Darling River system.

Visual and vibration stimuli are also important for predator recognition. Mikheev *et al.* (2006) found that visual cues were important for perch *Perca fluviatilis* to avoid predatory pike and olfactory cues enhanced the visual cues. Berejikian (1995) visually exposed hatchery reared Steelhead fry *Oncorhynchus mykiss* to predation of

sacrificial steelhead fry by Sculpin *Cottus asper*. The visually trained fry performed better than naïve fry in subsequent direct exposure to Sculpin.

Some predator avoidance training strategies have used predator models combined with a negative stimulus such as simulated capture with an aquarium net (Mesquite and Young, 2007) or electric shock (Fraser, 1974). The latter experiment used an electrified model of a bird. Although trained fish learned to avoid the model, it did not translate into better survival when fish were release into a lake. Fraser (1974) supposed this was because the fish hadn't been exposed to a real predator that would turn and chase its prey. They had merely learned to maintain a distance where they could avoid being shocked.

Other researchers have investigated training hatchery reared fish in foraging for wild feeds. Norris (2002) found that after 30 days on a live food diet, whiting fed live prey were significantly faster at locating live prey than pellet fed fish. Brown *et al.* (2003) found a combination of habitat enrichment in a tank with exposure to live food prior to release, enhanced the ability of Atlantic salmon parr to generalize from one wild prey type to another. According to Brown and Laland (2001) there is ample evidence for both individual and social learning of foraging behaviour by fish, but the potential to train hatchery fish en-masse remains largely untested.

Another strategy to reduce domestication effects is to use semi-natural rearing methods. For example pond rearing of fingerlings on a diet of zooplankton could be considered semi-natural rearing, as compared to tank rearing on artificial diets (Olsen *et al.*, 2000). After accounting for stocking size, it has been demonstrated that pond reared fish survive better than tank reared fish after stocking (McKeown *et al.* (1999). Fingerlings of threatened Murray-Darling Basin fish species are already pond reared on zooplankton, so this is a positive situation. It is only the larger fish (>60 mm) that are reared on artificial diets or in tanks (Hutchison *et al.*, in press).

### **Reducing transport and post-release stress**

Although pre-release conditioning of hatchery reared fish may be beneficial to post-stocking survival, the benefits of training could potentially be undone if fish arrive at a release site in a stressed condition. Stress of handling can impair the ability of fish to avoid predators. Olla and Davis (1989) found that it required 90 minutes for coho salmon to overcome this effect. Therefore strategies to reduce transport stress and to protect fish from predators when first released until they have had time to recover from transport stress could be beneficial.

Transport stress can have detrimental impacts on the overall health and wellbeing of fish (Portz *et al.* 2006) and can therefore impact on stocking success. Transport stress can be reduced by minimising temperature fluctuations during transport, making sure transport water is adequately oxygenated and fish are not overcrowded. (Simpson *et al.* 2002). Adding 0.5 to 1 kg of sodium chloride (salt) to 1000 L transport freshwater can also help reduce stress and minimise infection (Simpson *et al.*, 2002, see also Carneiro and Urbinati, 2001). Cowx (1994) recommended that fish be starved 24 hr before transportation, to reduce oxygen demand and ammonia build up during transport (Cowx, 1994). However withholding feed longer than this could lead to risky feeding behaviour that increases the probability of predation (Miyazaki *et al.*, 2000). Lowering the temperature and pH during transport can also reduce the toxicity

of un-ionized ammonia (Cowx 1994). On arrival at the stocking site Simpson, *et al.* (2002) recommend gradually mixing water from the receiving environment to equilibrate temperatures and water chemistry to avoid shocking the fish.

Although the above steps will minimise stress, it is likely that the journey to the stocking site and the handling involved in stocking (even if minimal) will result in some level of stress to the stocked fish. Schlechte *et al.* (2005) found that habituating Florida largemouth bass *Micropterus salmoides floridanus* fingerlings (30-64 mm TL) in predator free enclosures for at least 15 minutes improved post-release survival from 26% to 46% after 2 hours of exposure to predators.

Schlechte and Buckmeier (2006) conducted habituation experiments in 20 m x 100 m ponds containing high densities of predators. Fish were released into either open water or structurally complex dense habitat made from fir tree branches and bamboo with no habituation, or into these two habitats after a 60 minute habituation period in a predator exclusion cage. Exclusion cages were constructed from 3 mm nylon mesh and consisted of a floating ring at the top and a leaded line at the bottom that could follow the bottom contours. Non-habituated fish from open water had significantly poorer survival than all other treatment groups. Survival for open water released fish was improved by habituation and was not significantly different to that of habituated and non-habituated fish released into complex cover, which also afforded protection from predators.

Brennan *et al.* (2006) found that Common snook *Centropomus undecimalis* acclimated to the release habitat in predator free enclosures for three days had recapture rates 1.92 times higher than unacclimated fish released at the same time. A review by Brown and Day (2002) provides further examples of the benefits of habituation or acclimatization at release.

### **Objectives of this study**

The broad objective of this study was to develop techniques to improve survival of stocked hatchery reared fish used for conservation stockings, as part of recovery actions for threatened Murray-Darling Basin fish species.

The specific objectives were

1. To determine if hatchery reared threatened fish species native to the Murray-Darling Basin can be trained to reduce hatchery domestication effects and test if this leads to improved survival in the wild.
2. To determine if release in predator exclusion cages (soft release strategy) to overcome transport stress, leads to improved post stocking survival.

## Methods

### General approach

Test fish used in these experiments were sourced from commercial hatcheries (fingerlings) or grow-out facilities (sub-adult and adult fish). Murray-cod *M. peelii peelii*, Silver perch *B. bidyanus* and Freshwater catfish *T. tandanus* were selected for testing of training techniques and release strategies in this study. All three species are currently produced in hatcheries in Queensland and south-eastern Australia and all of these species formerly had Basin-wide distributions, so are of importance to all jurisdictions in the Basin. These species also represent each of the key large-bodied fish families with threatened species in the Basin, suggesting results may be transferable to other species in each family.

Based on a review of hatcheries and grow-out facilities supplying these three species (Hutchison *et al.*, in press) it was thought that fingerlings of these species would benefit from training to recognise and avoid predatory fish. As many hatcheries reported some exposure to birds in ponds, it was concluded that bird training might be of less benefit, but as exposure was generally limited, it was decided to at least test bird training in the laboratory before deciding whether or not to apply this to field released fish. As all hatchery reared fingerlings were reared in ponds, where they fed on live zooplankton and aquatic insects it was decided that foraging training would be unnecessary for hatchery reared fingerlings.

Only Silver perch and Murray cod were available from grow-out facilities. Catfish were unavailable. All adult or sub-adult fish sourced from grow-out facilities were reared long term on pelletised diets. It was therefore planned to provide live food foraging training to these fish. These fish were also not exposed to birds or fish predators at the grow-out facilities. As most fish were already too large to be taken by predatory fish (excluding very large cod) it was decided to focus predator training on bird recognition and avoidance. Large cod would not be abundant at most release sites and it would have been impractical to conduct replicated experiments using large (800 mm+) cod as predators.

Experiments were planned to follow a staged approach. Tank based training followed by tank based validation; then if any training technique was validated in the laboratory tanks, it would be followed by field based validation.

### Tank-based validation experiments for fingerlings

#### *a. Predatory fish training*

Fish predator awareness training for the treatment groups was run in a 5000L tank provided with cover areas and a mesh screen permeable to fingerlings, but not to predators. The permeable screen was fitted with a solid removable PVC screen. The solid screen could be inserted at any time to reduce predation rates or hide predators from view (Figure 1). Predatory fish (Murray cod, Golden perch and Spangled perch) were kept on one side of the screen. Predatory fish were kept in the tank for at least two weeks prior to introduction of fingerlings to ensure that they were behaving and feeding normally. Predators were provided with sections of PVC pipe to use as shelters and cover. The predators were all sourced from the wild by electrofishing

several months before the predatory fish training experiments. Use of wild fish was essential to ensure that these fish would recognise and react to potential prey. Wild fish were maintained on a diet of dead prawns and small fish purchased from commercial suppliers of frozen bait and seafood products.

When predators were settled in the tank, 500 fingerlings (of either Silver perch, Murray cod or Freshwater catfish) were introduced to the predator free side of the training tank at 08:00, and then the solid screen was removed. Fingerlings were free to swim to the predator side of the tank and predators could chase, or prey on fingerlings that strayed to their side of the tank. Mean sizes of Silver perch, Murray cod and Freshwater catfish fingerlings were  $54\pm 8$  mm,  $75\pm 5$  mm and  $61\pm 7$  mm respectively. Shortly after introduction of fingerlings to the tank 40 ml of skin extract from the test species (Silver perch, Murray cod or Freshwater catfish) was added to the water on the predator side of the tank. This was repeated at midday and 15:00 on day one, and at 09:00, midday and 15:00 over the following two days. Skin extract contains alarm pheromones and was used to enhance training and minimise actual predation. The extract was prepared following the procedures of Ferrari and Chivers. (2006).

After 24 hours exposure to predators a sub-sample of fingerlings ( $n=80$ ) was removed from the training tank by trapping and rapid dip-netting. Further sub-samples were removed after 48 hours and 72 hours exposure to predators. Approximately 3-4% of fingerlings were lost to predation over the three day training period. Removed fish were used in validation experiments (see below). A control group of fish were kept under the same conditions as the trained fish, but in a predator free environment.



**Figure 1:** Predator recognition and avoidance training tank. The mesh screen is permeable to fingerlings but not predators in place in a 5000L tank. Note the removable solid PVC screen. Solid screens were removed to initiate predator exposure. (Photo M. Hutchison)

### ***b. piscivorous bird training***

Ideally we would have liked to use a live bird (cormorant) for the training, using methods similar to those used in traditional Chinese cormorant fishing. However, several potential suppliers of tame cormorants expressed concerns about transport stress and possible stress to the bird in an unfamiliar environment of a large tank.

The alternative was to obtain dead cormorants from fish hatcheries (killed under EPA permits) and to simulate chasing behaviour. We obtained a freshly deceased cormorant that was then frozen for use in all bird-avoidance training and evaluation. Training of fish took place in a 5000 L tank. The tanks contained four 30 x 30 cm patches of artificial weed that fingerlings could use as cover. Groups of 500 fingerlings (of Silver perch, Murray cod or Freshwater catfish) were introduced to the training tank at 08:00 on the first training day and allowed to settle. At 09:00 the frozen dead cormorant was introduced into the tank in a net to provide cormorant odour. Immediately after introduction of the cormorant 40 ml of skin extract from the training target species was released into the tank to provide an alarm cue that would be associated with the cormorant odour. Over a 15 minute period the cormorant in the net was moved around the tank to harass fish in open areas. Fish that bolted for cover were left alone. A wooden cormorant silhouette was also moved about the tank alternately with the dead cormorant to harass fish that remained in the open.

The training combination of dead cormorant, skin extract and wooden cormorant silhouette was repeated at midday and 15:00 on day one and at 09:00, midday and 15:00 over the following two days. At 08:00 (following 24 hr, 48 hr or 72 hr training) a subsample of fingerlings was removed for use in aquarium-based evaluation experiments. It was expected that real cormorant odour, and simulated chasing could overcome some of the deficiencies of Fraser's (1974) model bird training methods.

### ***Aquarium based validation of trained fingerlings' responses***

Fish from each tank based treatment were tested for predatory fish and predatory bird behavioural responses. All experiments were recorded by video camera to enable accurate counts. Four replicates were recorded simultaneously using Ness security cameras and a DVR multi-channel recorder. At the conclusion of each set of four replicates two copies were burned to DVD.

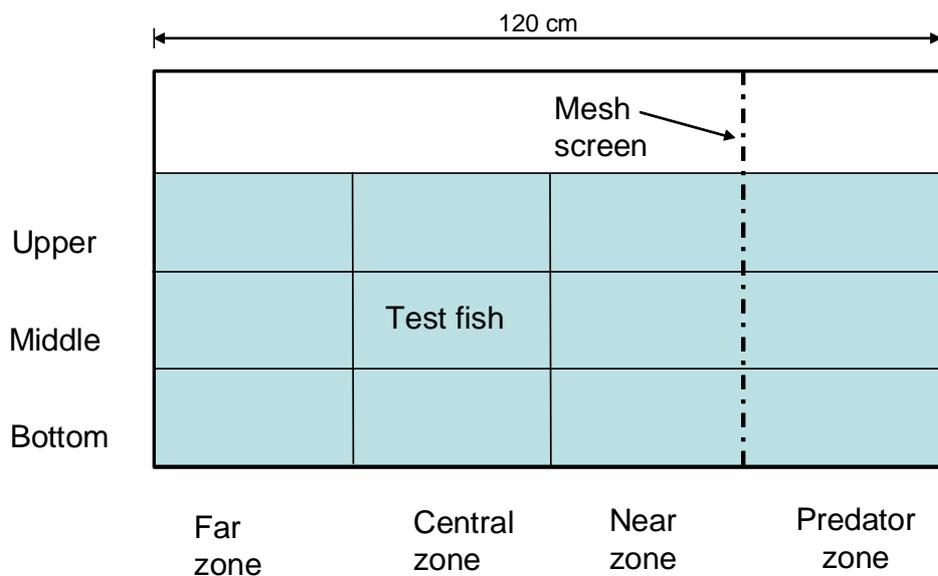
#### ***a. Response to predatory fish***

Groups of eight fingerlings of the test species were released into screened aquaria (60 cm x 60 cm x 120 cm) and permitted to settle for 30 minutes before recording commenced. Each experiment for each treatment (control, 24 hr, 48 hr and 72 hr trained) was replicated eight times (Table 1). Four batches were recorded simultaneously in four identical aquaria. After 15 minutes of recording a predator was released into a screened quarter of the aquarium. This was done as quickly as possible (in a few seconds) from behind a black plastic screen to minimise external disturbances to the test fish in the aquarium. The predator could not pass through the mesh screen in the aquarium, but the fingerlings could. Recording continued for a further 15 minutes after introduction of the predator. All aquaria were drained and refilled between replicates to remove predator odours and alarm odours.

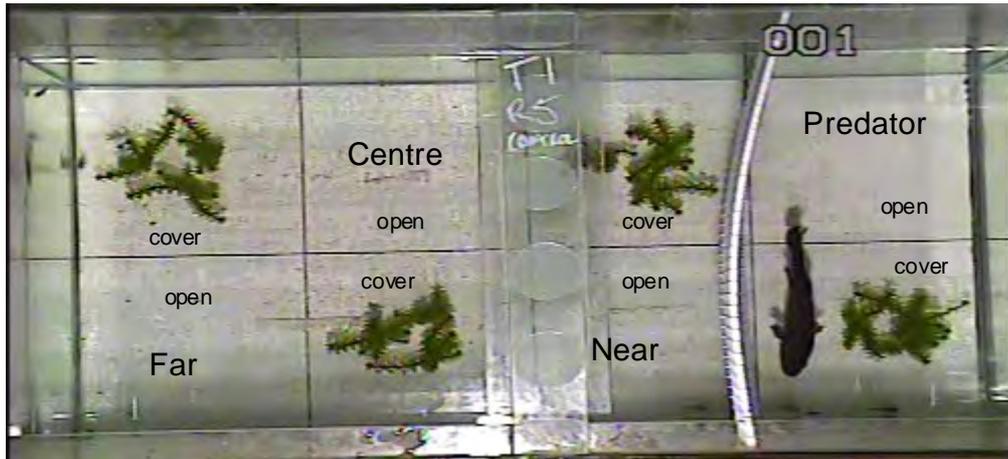
A slightly different setup was used for Silver perch, compared to that used for Murray cod and Freshwater catfish. Silver perch are more of a pelagic and shoaling species than catfish or Murray cod, and were tested in a bare aquarium. The front of the aquarium was marked off into 4 horizontal sections (the predator zone, a near zone, a central zone and a far zone) and into 3 vertical sections (a bottom zone, a mid-water zone and a top zone). The aquarium set up is shown in Figure 2 and is similar to that used by Malavasi *et al.* (2004). This set up was recorded from the front of the aquarium.

Evaluation experiments involving Murray cod and Freshwater catfish fingerlings used an aquarium set-up as shown in Figure 3. The aquarium was of the same dimensions as that used in the Silver perch experiments. The setup consisted of a screened predator compartment, half of which contained artificial weed. The remainder of the tank was divided up into near, central and distal cells. Each cell was marked into two areas, one containing cover and the other open water. Catfish and cod experiments were recorded from above. This was because both species are essentially benthic and it was easier to observe use of cover from above.

For cod fingerlings, a Golden perch was used as a predator. For catfish and Silver perch fingerlings a Murray cod was used as a predator. Cod and Golden perch used in the validation trials were sourced from grow-out facilities and were between 200 and 250 mm TL. As these were captive reared it was thought that they should be less stressed in the confines of the predator compartment. If the training was successful, then the trained fingerlings should recognise the odour and shape of the predator. All experiments were recorded for 15 minutes prior to and 15 minutes after introduction of the predator. At the conclusion of the experiments the videos were analysed. The position of all test fish in the tank was recorded every 15 seconds. For catfish and cod fingerlings, the number of fish moving was also recorded for each fifteen second period.



**Figure 2:** Tank set up for testing Silver perch fingerlings' predatory fish response. The mesh screen is permeable to Silver perch fingerlings but not the predator.



**Figure 3:** Tank set up used to test response of Freshwater catfish and Murray cod to predatory fish. Note semi-permeable screen to contain the predator and use of open water and cover areas in the predator, near centre and far cells. The experiment pictured shows a control group of catfish after introduction of a predator (Murray cod) to the tank. (Freeze frame from video monitor).

**Table 1:** Evaluation experiments for fingerlings

Species trained	Treatment	Evaluation type	Number of replicates
Silver perch	Control	school of 8	8
Silver perch	Pred fish training 24 hr	school of 8	8
Silver perch	Pred fish training 48 hr	school of 8	8
Silver perch	Pred fish training 72 hr	school of 8	8
Silver perch	Control (bird training)	school of 8	12
Silver perch	Pred bird training 24 hr	school of 8	12
Silver perch	Pred bird training 48 hr	school of 8	12
Silver perch	Pred bird training 72 hr	school of 8	12
Murray cod	Control	school of 8	8
Murray cod	Pred fish training 24 hr	school of 8	8
Murray cod	Pred fish training 48 hr	school of 8	8
Murray cod	Pred fish training 72 hr	school of 8	8
Murray cod	Control (bird training)	school of 8	12
Murray cod	Pred bird training 24 hr	school of 8	12
Murray cod	Pred bird training 48 hr	school of 8	12
Murray cod	Pred bird training 72 hr	school of 8	12
Freshwater catfish	Control	school of 8	8
Freshwater catfish	Pred fish training 24 hr	school of 8	8
Freshwater catfish	Pred fish training 48 hr	school of 8	8
Freshwater catfish	Pred fish training 72 hr	school of 8	8
Freshwater catfish	Control (bird training)	school of 8	8
Freshwater catfish	Pred bird training 24 hr	school of 8	8
Freshwater catfish	Pred bird training 48 hr	school of 8	8
Freshwater catfish	Pred bird training 72 hr	school of 8	8

*b. Response to simulated bird*

The response of fingerlings of Silver perch, Murray cod and Freshwater catfish to a simulated bird predator was tested using a similar tank set up to that shown in Figure 5. The only difference being that the predator screening was removed. As for the predatory fish experiments fingerlings, were introduced to the tank and allowed to settle for 30 minutes. Recording then commenced and after 15 minutes a simulated bird predator was introduced for 1 minute, and then withdrawn. It was introduced again after 4 minutes for a further minute and withdrawn again. After another four minutes the simulated bird predator was introduced for a final minute. Filming continued for fifteen minutes after the first introduction of the simulated predator.

The simulated predator was made from plywood and cut into the shape of a cormorant. Underneath it was painted to mimic cormorant markings. A bunch of cormorant feathers was attached by fishing line to the base of the wooden bird (Figure 4). When simulating presence of a bird predator the wooden silhouette was moved back and forth over the predator cells of the test tank. The silhouette was fixed to the end of a PVC pole and was manoeuvred over the tank by a person hidden behind a black plastic screen. The cormorant feathers were permitted to dangle into the water to introduce cormorant odour.

Predatory bird response evaluation experiments tested groups of eight fingerlings, with 12 replicates for each treatment. However, the numbers of replicates for catfish were reduced to eight from twelve (refer to Table 1), as there was a shortage of suitable catfish fingerlings. At the conclusion of all experiments videos of the simulated bird attacks were viewed and the position of fingerlings in each test tank was recorded every fifteen seconds, for fifteen minutes before and after introduction of the simulated predator. Movements of cod and catfish were also recorded for each 15 second segment.



**Figure 4:** Cormorant silhouette. Note the feathers attached by fishing line to provide cormorant odour. (Photo M. Hutchison)

### ***Statistical analyses of fingerling validation experiments.***

Aquaria were designated as the replicate experimental units, as these were independent. The individual fish and sampling times were assumed as sub-sampling, and pooled into overall % groups for each replicate (e.g. % predator, % near, % centre, % far) for each aquarium, separated into before and after datasets. These data were subjected to two separate parametric analyses for each lateral aquarium zone, and for 'before' vs 'after' behaviour.

Initially, we ran Bartlett's test of homogeneity of variances to test for significant ( $P < 0.05$ ) differences between the treatment variances. If significant differences were detected then this result invalidated any use of analysis of variance, because ANOVA assumes poolable variances. In these cases, differences between the treatment means were tested via unpaired t-tests between the successive pairs of treatments. This uses the individual variances appropriate to each t-test. However, where Bartlett's test was insignificant, the homogeneity of variances permitted us to use ANOVA to examine variances. If an ANOVA produced a significant result then a post-Hoc pairwise comparison of treatments was run in Genstat<sup>TM</sup> using an LSD procedure.

The same procedures were followed for statistical evaluation of use of the water column (*i.e.* top, middle and bottom) by Silver perch from each treatment group and for use of cover cells and open cells by Murray cod and Freshwater catfish from each treatment group.

Mean abundances of fingerlings in validation tank zones were calculated across 15 second intervals and plotted as line graphs for all treatment groups to provide a visual representation of how use of cells changed over time after introduction of a predatory fish.

### **Tank-based experiments for sub-adult fish**

#### ***Piscivorous bird training***

Sub-adult Silver perch and Murray cod purchased from grow-out facilities were trained to avoid cormorant predation in the same way as the hatchery fingerlings. Twenty sub-adult Silver perch or Murray cod (around 30 cm TL) were trained in 5000 L tanks. For a 15 minute period, three times per day, over three days, fish were exposed to cormorant odour, whilst being chased by a wooden cormorant model on a plastic pole and exposed simultaneously to skin extract of conspecifics. The extract was prepared following the procedures of Ferrari and Chivers (2006). Control fish were housed in identical tanks and were not exposed to simulated cormorant attack. Trained fish had at least one wild conspecific in the tank with them to enhance social learning. Untrained fish had no wild conspecifics present.

#### ***Live food foraging training***

Twenty adult Silver perch (around 30-35 cm TL) housed in a 7000 L tank were trained to take live food over a period of two weeks. Twenty sub-adult Murray cod (35-45 cm TL) were also housed in a 7000 L tank for live food training. Initially training was meant to be for two weeks, but this was extended to one month, as it was

apparent that cod were not taking live food in the initial training period. One wild Murray cod and one wild Silver perch were housed in the training tanks for each of the respective species. The training tank contained habitat enrichment including PVC pipes and artificial clumps of weed constructed from strips of shade cloth. During the training period pellet feeds were withdrawn and the fish were offered live shrimp and live freshwater prawns (captured from local creeks and purchased from a commercial supplier) twice daily. In the month prior to live feed introduction, the training group were offered a mixture of pellet feeds and dead (frozen) fish and prawns

Control fish were housed in identical tanks (also with habitat enrichment), but were not exposed to live or dead food. Instead they were maintained solely on a diet of commercial pellets as in a grow-out facility. Untrained control fish had no wild conspecifics present in their tanks.

### ***Validation of sub-adult fish responses***

#### ***a. Response to simulated bird***

The responses of sub-adult Silver perch and Murray cod were tested using tanks set up as shown in Figure 5. The tank dimensions were 180 cm long, by 60 cm wide by 60 cm deep. Each tank was divided into cells, two at the predator end of the tank, two in the centre and two at the far end of the tank. One of each pair of cells contained artificial cover made from shade cloth. A sub-adult fish would be introduced to the tank and allowed to settle for 30 minutes. Recording then commenced and after 15 minutes a simulated bird predator was introduced for one minute, and then withdrawn. It was introduced again after four minutes for a further minute and withdrawn again. After another four minutes the simulated bird predator was introduced for a final minute. Filming continued for 15 minutes from the first introduction of the simulated predator. 16 replicate experiments were run for the control and trained fish from each test species.



**Figure 5:** Bird training evaluation tank. Note wooden bird, marked cells, cover and sub-adult Murray cod (Freeze frame from video monitor)

The simulated predator was made from plywood and cut into the shape of a cormorant. Underneath it was painted to mimic cormorant markings. A bunch of cormorant feathers was attached by fishing line to the base of the wooden bird. When simulating presence of a bird predator the wooden silhouette was moved back and forth over the predator cells of the test tank. The cormorant feathers were permitted to dangle into the water to introduce cormorant odour. The cell occupied by a sub-adult fish in the tank was recorded every 15 seconds for 15 minutes before and after

introduction of the simulated predator. Statistical analyses followed the same methodology as for the fingerlings above.

*b. Response to live food.*

After training Silver perch were moved in pairs into eight 1000 L tanks (Figure 6). Pairs rather than individual fish were used, as Silver perch appear to be a social fish and feed better in groups. Fish were allowed to settle into their tanks over a period of a week and continued to be offered live shrimp (trained group).

After the settling in period both control fish and trained fish were offered live shrimp at the normal feeding time. Time taken for the first shrimp to be consumed after introduction into a tank was recorded. If nothing was consumed after 15 minutes observations ceased.

The same procedures were planned to be followed with the control Silver perch but following results with the trained fish this was abandoned (see results section)



**Figure 6:** 1000L tanks used for evaluation of live food foraging trials (Photo M. Hutchison)

It was planned to do a similar validation trial with Murray cod. However after one month of training hatchery reared sub-adult Murray cod refused to take any live food in the training tank (see results for further details). Therefore it was decided that there was no point proceeding with validation trials if the trained fish were not feeding. *i.e.* it was obvious that the training was having no effect.

**Field-based trials and tagging**

Based on encouraging results from the fingerling tank trials (see results section), it was decided to proceed with field trials where trained hatchery-reared fish were stocked into the wild and their survival could be compared with untrained control fish. It was also decided to test the effectiveness of predator free enclosures (soft releases) to enhance post-stocking survival.

### *Selection of stocking sites*

Three stocking sites were selected in the Condamine catchment. Cotswold Dam (a 250 ha dam on a tributary 800 m above its junction with the Condamine River), Reilly's Weir (on the Condamine River) and Caliguel Lagoon (a large permanent waterhole on an anabranch of the Condamine River). A fourth site was added to the study in the upper Dumaresq catchment at Storm King Dam near Stanthorpe. This site was added because several flood events caused sampling difficulties at Reilly's Weir. Predatory fish were known to be present at all sites, and other predators including piscivorous birds and turtles were also present. The location of all sites is shown in Figure 7. Figure 8 shows the habitat characteristics of each site.



**Figure 7:** Map showing location of study sites (red dots).

1. Cotswold Dam 2. Reilly's Weir 3. Caliguel Lagoon 4. Storm King Dam



**Figure 8:** Habitat characteristics of stocking sites: A. Cotswold Dam B. Reilly’s Weir pool (during rising flow) C. Caliguel Lagoon D. Storm King Dam (Photos by M. Hutchison)

### ***Tagging***

A total of 24,000 fingerlings of Murray cod and Silver perch were ordered from hatcheries for use in the stocking experiments. Unfortunately no hatcheries were able to supply sufficient numbers of Condamine strain Freshwater catfish for stocking experiments. Fingerlings were tagged with fluorescent visible implant elastomer (VIE) tags at the base of the anal fin in both species (Figure 9). Past work by Gallagher and Hutchison (2004) and Simpson (unpublished) have demonstrated that the base of the anal fin provides good tag visibility and tag retention in Silver perch and *Maccullochella* spp. VIE tags also appear to have no significant effect on predation rates of fingerlings (Haines and Modde, 1996; Malone *et al.*, 1999; Roberts and Kilpatrick, 2004; Reeves and Buckmeier 2009; Bouska and Paukert 2010).

Tagging was done using two Northwest Marine Technology (NMT) air driven tagging machines. Up to 3000 fish were tagged in a day. Four colours were used to represent the different treatments (Table 2). The numbers of fish from each treatment planned to be stocked at each site are shown in Table 3.

**Table 2:** Release treatment and VIE batch tag colours.

<b>Treatment</b>	<b>VIE tag colour</b>
Trained soft release	Green
Trained standard release	Red
Untrained soft release	Orange
Untrained standard release.	Pink

**Table 3:** Planned number of fish to be stocked by treatment and release method at selected sites in the Murray-Darling Basin

	Predator free cage prior to release (soft release)	Standard release
<b>Fish predator trained</b>	1350/species /site	1350/species/site
<b>Predator naïve</b>	1350/species/site	1350/species/site



**Figure 9:** VIE tagged Murray cod. Note green tag at base of anal fin. (Photo M. Hutchison)

*VIE tag retention trial*

As it was originally intended to conduct stocking trials with Freshwater catfish a VIE tag retention trial was also run with this species. Sixty anaesthetised Freshwater catfish with a mean size of 58.98 mm ( $\pm 7.12$  mm) total length were VIE tagged in translucent tissue near the lower jaw (Figure 10) with a NMT hand tagger. The lower jaw appears to be the only suitable location to VIE tag small catfish where the tags can be observed without sacrificing the fish. Other body locations have opaque skin tissue that obscures the tag, even under UV light. After tagging, the catfish were maintained in a 1000 L tank for 5 months. Catfish were fed a mix of frozen (bloodworm and pieces of prawn) and pellet foods. Tanks were cleaned daily and water changed regularly.



**Figure 10:** VIE tagged catfish fingerling. Note red tag near lower jaw.

#### *PIT tag retention trial*

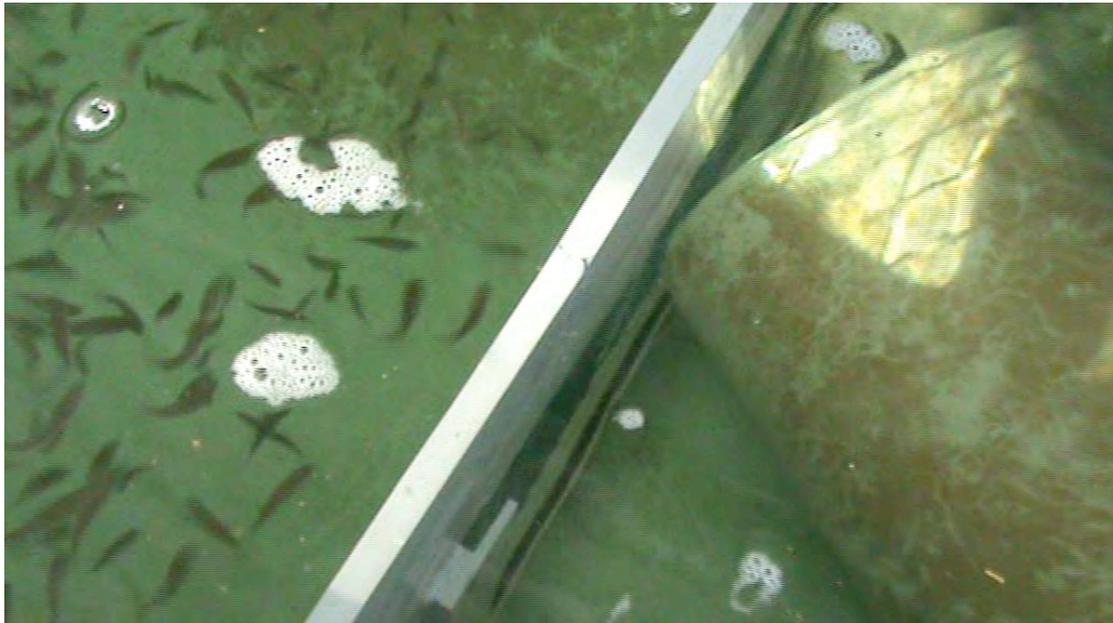
After fish were at large for 12 months and large enough to individually tag, it was planned to use passive integrated transponder (PIT) tags for a mark recapture experiment. As fish could be targeted by anglers in the future and eaten, it was decided to tag these fish in the gut cavity rather than in the dorsal muscle. This would minimise the chance of people accidentally ingesting a PIT tag. To determine if this was a suitable tag location 40 anaesthetised Silver perch of a mean size of 172.4 mm ( $\pm 28.3$  mm) FL were tagged with Trovan® PIT tags by inserting the PIT tag needle at a shallow angle into the upper rear gut cavity. Just as the needle penetrated the muscle wall into the gut cavity, the tag was injected.

Following tagging the Silver perch were maintained in a 7000L tank for six weeks and fed a diet of commercial pellet, and prawns. Water quality was maintained with a biofiltration system. Tagged fish were monitored for post tagging mortalities or complications. After six weeks tag retention was determined by scanning each fish with a Trovan® PIT tag reader. As the mark recapture population estimate experiments were not intended to last for more than a month, six weeks was considered an adequate monitoring period.

#### *Pre-stocking training*

Fish were trained in groups of approximately 1400 (enough to stock one site) in 5000 L tanks provided with cover areas for fingerlings and a mesh screen permeable to fingerlings, but not to predators (Figures 11 & 12). One tank was used to train fish bound for soft release, and another identical tank was used to train fish bound for standard release. Predatory fish (one each of Murray cod, Golden perch and Spangled perch) were kept on one side of the screen. Predatory fish were kept in the tank for at least two weeks prior to introduction of fingerlings to ensure that they were behaving and feeding normally. Predators were provided with sections of PVC pipe to use as shelter and cover (Figure 12).

Training followed the same procedures as for the tank based validation studies except that all fingerlings remained in the training tank for three days. Untrained control fish were maintained in predator free 5000 L tanks. Densities of fingerlings in training tanks (pre-stocking) were almost three times higher than for the tank based validation training. For training to be a viable stocking tool, it must be feasible to train fish *en-masse*. A total of 2-3% of fingerlings was lost to predation over the three day training period.



**Figure 11:** Silver perch fingerlings in training tank (day3). Note large numbers on the left or predator free side of the barrier. (Frame from video by M. Hutchison)



**Figure 12:** Predator side of the training tank. Note mesh barrier, low number of fingerlings on the predator side and Murray cod sub-adult in shelter. (Frame from video by M. Hutchison)

### ***Transport***

After three days training, fish were removed from the tank, and counted into labelled (by treatment) oxygenated bags for transport to a stocking site. Densities were around 140 fish per bag. The two untrained groups were also counted into labelled oxygenated bags. Fish were transported to the release sites in an air conditioned vehicle.

Transport in bags only occurred for fish stocked at Cotswold Dam and Reilly's Weir. A vehicle breakdown led to bagged cod being transported to Reilly's Weir being exposed to high temperatures. Until this incident bagged fish had travelled well with transport related mortalities being well below 1%. However fish mortalities resulting from the vehicle breakdown were unacceptably high. After this incident fish stocked in Caliguel Lagoon and Storm King Dam, were transported in two large insulated fibreglass fish carriers. Each fish carrier contained two 400 L compartments supplied with bubbled oxygen and air. In the event of any future vehicle breakdowns it was felt that fish in the fish transporters would be unaffected. All fish transported in this manner had transport related mortalities well below 1%.

### ***Release***

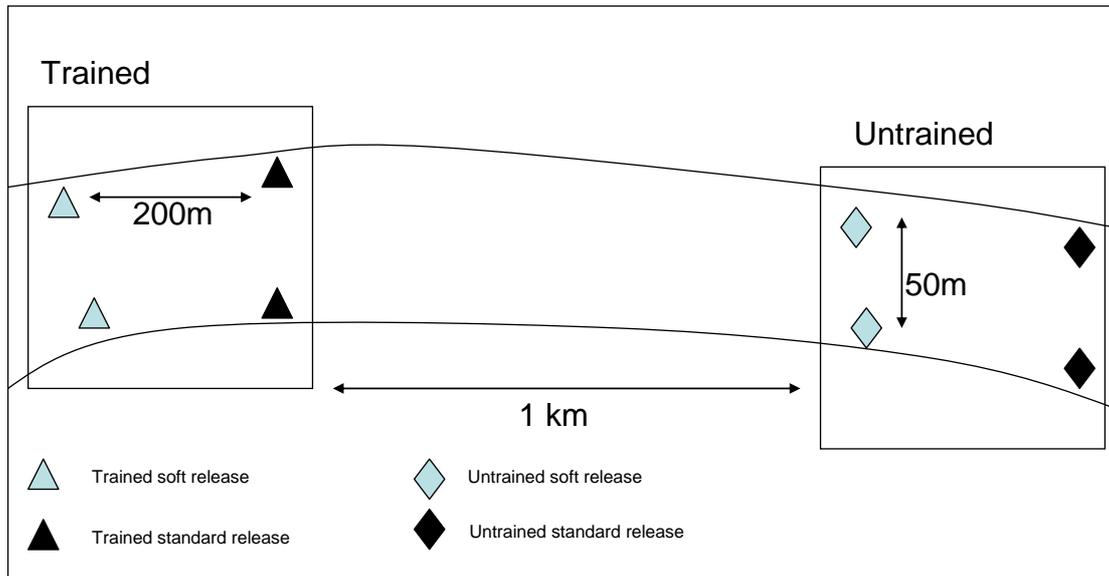
On arrival at the release site, predator free socks were installed, and then each batch of fish was loaded into plastic cages in the live well of a large electrofishing vessel. River or dam water was pumped through the live well to allow fish to adjust to the local water chemistry while the boat motored to a predefined release points. The adjustment period was approximately ten minutes.

Trained fish were released at least 1 km apart from untrained fish (Figure 13). This was to help prevent trained fish from influencing the behaviour of untrained fish. This was expanded to 2 km for fish stocked into Storm King Dam, as it became apparent that Silver perch were rapidly forming mixed schools of trained and untrained fish (see results). Two predator proof socks were installed in each of the trained fish and untrained fish release zones. Socks were 1.8 m in diameter, with a 1.6 m drop to an open ended lead lined skirt. Socks were set at a depth of 1.2 m. The lead skirt followed the bottom contours and prevented escape by fingerlings contained within, and also prevented access by predators.

Each sock had a floating aerator pump installed and a floating plastic cover to provide protection from birds (Figure 14). Socks were set at least 50 m apart to reduce the risk of all fish being released next to a high concentration of predators, or alternatively into an area devoid of predators. Standard (hard) release fish (Figure 15) were stocked at least 200 m from fish released into socks. This was to prevent satiation of predators in the vicinity of the release socks. If predators were already satiated on hard released fish before fish were liberated from the socks it could potentially produce misleading results. Standard release fish were also released at least 50 m apart from each other to reduce the risk of chance stocking of all fish into a predator free zone or directly on top of predators.

Fish stocked into release socks were left in the socks for 90 minutes. After this period socks were lifted by two people vertically onto a boat, enabling fish to swim out the now open bottom of the sock. The mean size of Silver perch stocked into Cotswold Dam and Reilly's Weir was 44.8. mm ( $\pm 3.7$  mm), 49.5 mm ( $\pm 7.0$  mm) for Caliguel

Lagoon and 58.28 mm ( $\pm 8.0$  mm) for Storm King Dam. The mean size of cod stocked into Caliguel Lagoon, Cotswold Dam and Reilly's Weir was 66.1 mm ( $\pm 12.5$  mm) and 63.8 mm ( $\pm 3.2$  mm) for those fish stocked into Storm King Dam.



**Figure 13:** Schematic diagram of release points. Trained fish were always released at least 1 km from untrained fish. Standard release fish were always released at least 200 m from soft release fish. Fish of the same release strategy were stocked at least 50 m apart.



**Figure 14:** Soft release of fingerlings into a predator free sock. Plastic cover has been folded back for release. Note floating aerator at rear. (Photo A. Butcher)



**Figure 15:** Standard release of fingerlings. (Photo A. Butcher)

When fish were released any transport mortalities were tallied and the total number released in each batch recorded. Transport mortalities were generally only a small fraction of one percent, with the exception of one occasion.

#### ***Post-release surveys***

Post stocking surveys were initially carried out just over 24 hours after each stocking event. The first post stocking survey at each site was run from dusk to well past midnight and was done by electrofishing boat using two dip netters (Figure 16). Past experience has shown that fingerlings tend to move into shallow margins at night where they are more susceptible to electrofishing. Surveys commenced with sampling within 50 m of each release point. Both potential predators of fingerlings and recaptured fingerlings were recorded in the vicinity of each release point. Any recaptured fingerlings were held temporarily in an on-board 300 L live-well with flow through water. At the end of a shot fingerlings were anaesthetised, measured to FL (for Silver perch) or TL (for Murray cod) then checked for a VIE tag with a blue light torch. Tag colour was confirmed by a minimum of two observers and compared to reference tags on a plastic fish provided by NMT. The colour of the tag was then recorded onto a data sheet. After recovery from anaesthesia recaptured fish were released.

Numbers and species of predators from the vicinity of each release point were used to calculate a predation index for the release points of each release strategy. Only predatory species that had reached a large enough size to potentially prey on the fingerlings were considered. Most predators had to be at least 150 mm FL. Carp and Goldfish had to be at least 200 mm FL. Carp and Goldfish were given a weighting of

one, as they are only occasional piscivores, whereas Spangled perch, Golden perch, Murray cod and Freshwater catfish were given a weighting of three. The number of each potential predatory fish species was multiplied by its weighting. The scores for each species were summed to give a total score for the release point. One was added to the total to prevent any zero scores. The predation index was used as a parameter in the statistical analyses of recapture results.

Based on a review of the literature it was assumed that the bulk of predation would occur on the first day after release (Sparrevohn and Stoettrup 2007; Hutchison *et al.*, 2006; Buckmeier *et al.*, 2005; Brown and Laland, 2001; Olla, *et al.*, 1994). Therefore predators within the vicinity of release points were expected to have the greatest influence on survival outcomes.

Predator numbers at release points were not assessed at the time of stocking, as it was thought that electrofishing might interfere with predatory behaviour and confound results. Predator scoring was therefore confined to 24 hours after stocking. Some degree of short term site fidelity by the predators was assumed, which is reasonable based on results of radiotelemetry studies for Spangled perch (Hutchison *et al.*, 2008), Golden perch (Crook, 2004), Carp (Jones and Stuart, 2007; Crook, 2004) and Murray cod (Jones and Stuart, 2007).

An assessment of predator numbers outside of the general release point areas was not done. Even though these predators might prey on fish dispersing away from release areas, it would be difficult to quantify any impacts of predators in the broader site area as dispersal directions and patterns could be variable. The assumption was that predators near release points would have an impact, and their impact would be greater than predators away from the release points.



**Figure 16:** Night electrofishing. (Photo M. Hutchison)

Following the 24 hour post stocking survey, further post stocking surveys were carried out seasonally. Follow-up surveys generally consisted of two nights sampling per site. The electrofishing boat would cover as much ground as possible to maximise recaptures of tagged fish. One night of effort was equivalent across all sites. Total sampling effort varied slightly across all sites due to access difficulties post flooding at some sites. Effort was recorded as total sampling nights and entered as a covariate in the analyses of data (see below). Ratios of the different treatment groups recaptured were assumed to represent relative survival. After processing, recaptured fish were released near to the areas where they were captured. The electrofishing boat would then move on to sample new ground, to minimise the chance of recapturing the same batch marked individuals. Some supplementary sampling was conducted by backpack electrofishing and fyke netting, to sample areas less accessible by electrofishing boat, and to hunt for fish that may have been displaced downstream below weirs that had overtopped. However these methods were less efficient and could not cover as much ground as the electrofishing boat and accounted for less than 1% of the total catch.

### ***Mark recapture***

Although recapture ratios of tagged fish could be used to indicate relative survival, it was planned to estimate absolute survival after one year at one of the more closed sites (Caliguel Lagoon) using mark recapture methods. After stocked fish had been at large for 12 months they were sufficiently large to be marked with individual passive integrated transponder (PIT) tags. PIT tagging commenced in March 2010. Unfortunately a flood event in March connected the lagoon to the river for several days, and a second rainfall event caused the lagoon to overflow into the river for a second time two weeks later. This resulted in large losses of stocked fish from the lagoon and made it impossible to complete the mark recapture survey as the assumption of no emigration was invalidated.

### ***Statistical analyses of recapture data***

Data on the relative recapture rates of the different batches of fish were analysed in Genstat 9.2 ® using a generalised linear model (GLM) of binomial proportions with a logit link function. This model used actual recaptures as a proportion of the number of fish stocked in each category at each site. The maximal model was set with the following parameters (factors and covariates), site, predator index, training status, release method and sampling effort. All factors in the GLM were fixed effects and of specific interest. The significance of each parameter in the model (including interaction effects of interest) was assessed by a forward stepwise procedure. Significant parameters were kept in the model. Non-significant parameters were rejected unless they were the specific treatments of interest to this study (*i.e.* release method and training status). Adjusted mean recapture rates were calculated for each stocking treatment using the predict function. Means and standard errors calculated using this function were adjusted for the effects of other covariates and factors in the model. The dispersion parameter was fixed at 1 (McCullagh and Nelder, 1989).

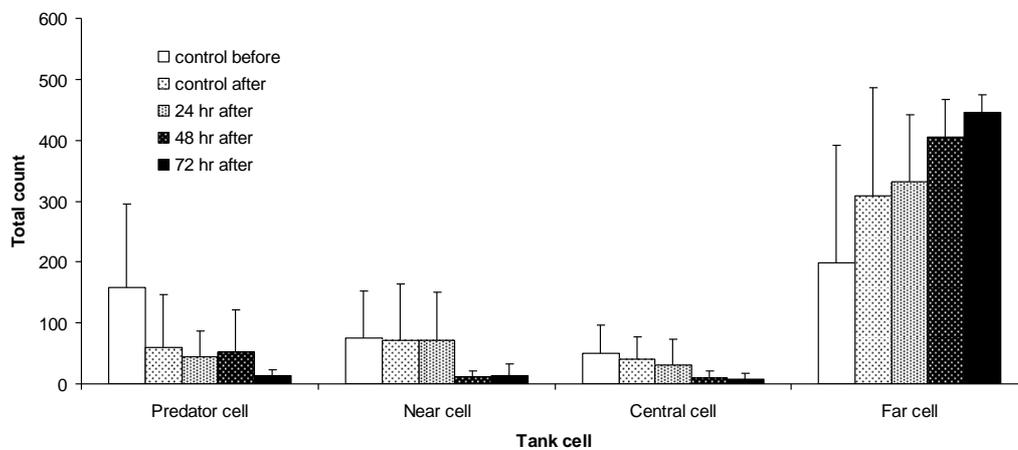
# Results

## Tank-based validation trials

### Responses to predatory fish

#### *Silver perch*

Figure 17 summarises the use of aquarium cells by groups of Silver perch from different treatments for the fifteen minutes before (control only) and fifteen minutes after introduction of a predator. Before values were similar between treatment groups, so only before control data is shown in the graph for simplicity. The figure shows a tendency for increased use of the far cell and decreased use of the predator cell after introduction of the predator. Use of the far cell trends upwards with training, with mean use highest in the far cell in 72 hour trained fish and least in the predator cell for 72 hour trained fish. Use of near and central cells decreased in 48 hour and 72 hour trained fish.



**Figure 17:** Use of tank cells by groups of eight Silver perch before (control only) and after (all treatment groups) introduction of a predator (Murray cod) to the predator cell. The maximum possible count in any cell is 480. Number of replicates is eight. Bars show mean values. Error bars show one standard deviation

Bartlett's test of homogeneity of variances showed significant differences between the variances of treatment groups in the predator cell ( $p < 0.001$ ), near cell ( $p < 0.001$ ), central cell ( $p < 0.001$ ) and far cell ( $p < 0.001$ ).

Before introduction of a predator, control fish showed a tendency to favour ends of the tank (i.e. the predator cells or the far cells). A similar pattern occurred in the before samples of all other treatment groups. Favouring of either of two ends led to quite large variances in the before samples, in both the far and predator cells (note standard deviations in Figure 17). Introduction of a predator led to a shift towards the far cell and away from the predator cell in the naïve control fish. However the response was variable, with some fish remaining in the predator cell. The response was more consistent in trained fish, leading to reduced variances. Note the very low standard deviation values in the 72 hour trained Silver perch (Figure 17). These fish showed a strong preference for occupying the far cell when a predator was present. Figure 18 shows the typical response of 72 hour trained Silver perch compared to the inconsistent response of control fish (Figure 19).

The mean values between treatments (compared by two sample t-test) were generally not significantly different due to unequal variances. The 24 hour trained fish in the far cell were significantly different to 72 hour trained fish in the far cell ( $p = 0.022$ ) and

control fish were significantly different to 48 hour trained fish ( $p = 0.045$ ) and 72 hour trained fish ( $p = 0.031$ ) in the centre cell. However the large variances in the control fish group caused mean values to be not significantly different to those of 72 hour trained fish in the predator and far cells, although only marginally so in the far cell ( $p = 0.066$ ). F-tests run with the two sample t-tests showed that variances between 72 hour trained fish and control fish were unequal in all cases ( $p < 0.001$ ).

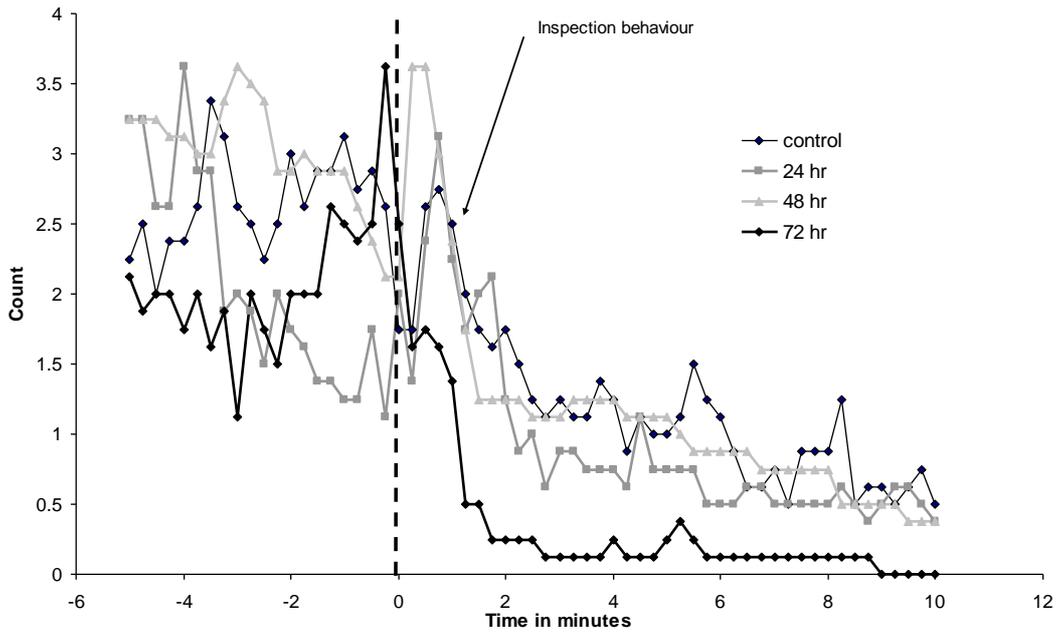


**Figure 18:** Typical response of 72 hour trained Silver perch. Note tight school of Silver perch in lower left of the far cell. A Murray cod is present in the lower right cell.

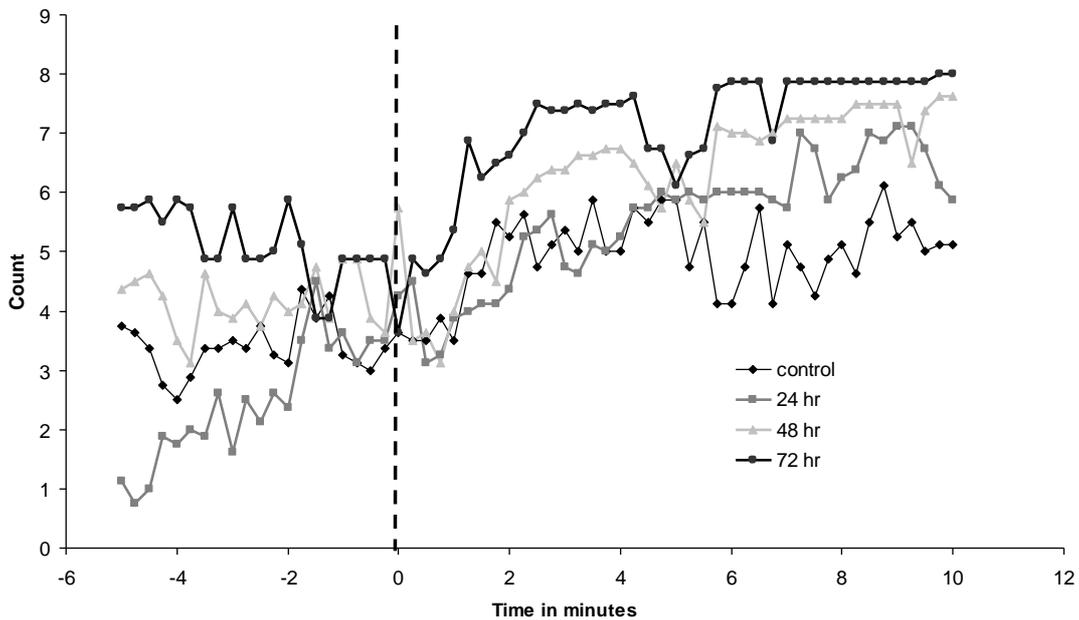


**Figure 19:** Control group of Silver perch after introduction of Murray cod to the predator cell. Note some Silver perch are occupying the predator cell. Although control Silver perch showed a tendency to move away from the predator cell, this response was inconsistent compared to 72 hour trained fish.

Figures 20 and 21 show the varying mean responses over time of the different treatment groups in the predator and far tank cells respectively. Mean responses are plotted at 15 second intervals for five minutes before and ten minutes after the introduction of a predator. Post predator introduction responses are similar across all treatment groups except in the 72 hour trained group. The trend is for a peak in fish numbers in the predator cell immediately after introduction of the predator before a gradual decline that flattens 6 minutes after the introduction of the predator. The peak in Silver perch numbers in the predator cell just after introduction of the predator reflects predator inspection behaviour. Predator inspection behaviour tended to involve fewer fish and the inspection period was shorter in 72 hour trained fish. In the 72 hour trained group numbers of fish in the predator cell were consistently lower than all the other treatments after introduction of the predator. The number of 72 hour



**Figure 20:** Mean numbers of Silver perch (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the predator cell for 5 minutes before introduction of a predator and for 10 minutes after introduction of a predator. The predator was introduced at time 0 denoted by the dashed line. Counts of Silver perch were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates is 8 per treatment. Error bars have been excluded for clarity of reading the graph.



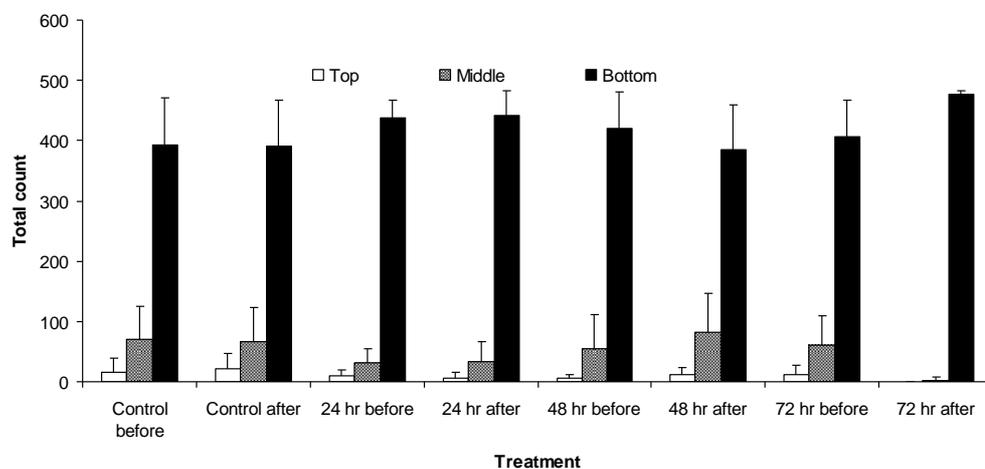
**Figure 21:** Mean numbers of Silver perch (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the far cell for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of Silver perch were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph.

trained fish in the predator cell bottoms out to near zero by two minutes and by 9 minutes is averaging zero, with a variance of zero.

In the far cell, mean counts of Silver perch climbed most rapidly in the 72 hour trained group and generally remain higher compared to other groups. The 72 hour trained fish mean counts are near the maximum possible count of eight after two minutes and reach eight after 9.5 minutes, with a variance of zero. The response of the control fish is much more irregular.

The use of the water column by Silver perch from the different treatment groups is shown in Figure 22. Across all groups the majority of time was spent near the bottom of the water column. There was also some use of the middle of the water column and the top level in all groups before introduction of a predator, and in most groups after introduction of a predator. In the 72 hour trained group, use of the top of the water column was totally eliminated (mean=0 standard deviation =0) and use of the middle water column declined after introduction of a predator.

Variances were not homogenous between treatment groups using the middle ( $p < 0.001$ ) and bottom ( $p < 0.001$ ) sections of the water column. Note standard deviations (and hence variances) are smaller in the 72 hour treatment groups after introduction of a predator, compared to other groups (Figure 11). Bartlett's test uses log functions for estimating homogeneity of variances. As the mean value for the 72 hour trained group using the top of the water column was zero, Bartlett's test could not be used on this group.



**Figure 22:** Mean total counts of groups of eight Silver perch in the water column zones (top, middle or bottom) across treatment groups before and after introduction of a predator. Counts are based on spot measures at 15 second intervals for 15 minutes before and 15 minutes after introduction of a predator (Murray cod). Number of replicates for each group is 8. Maximum possible total count for a zone is 480. Error bars show one standard deviation.

Results from the t-test analyses indicate that mean use of the bottom of the water column was significantly different between the 72 hr treatment group after introduction of a predator compared to the either the control group ( $p = 0.016$ ), 24 hour trained group ( $p = 0.042$ ) or 48 hour trained group ( $p=0.009$ ).  $F$  probabilities were also significant within each of these pairs ( $p < 0.001$ ), indicating unequal sample

variances. This is indicative of more consistent behaviour in the 72 hour trained group compared to the other groups. The 72 hour trained group (after introduction of a predator) also showed significant differences in use of the water column compared to groups before the introduction of a predator. For example, use was significantly different to the control group prior to introduction of a predator ( $p = 0.019$ ).

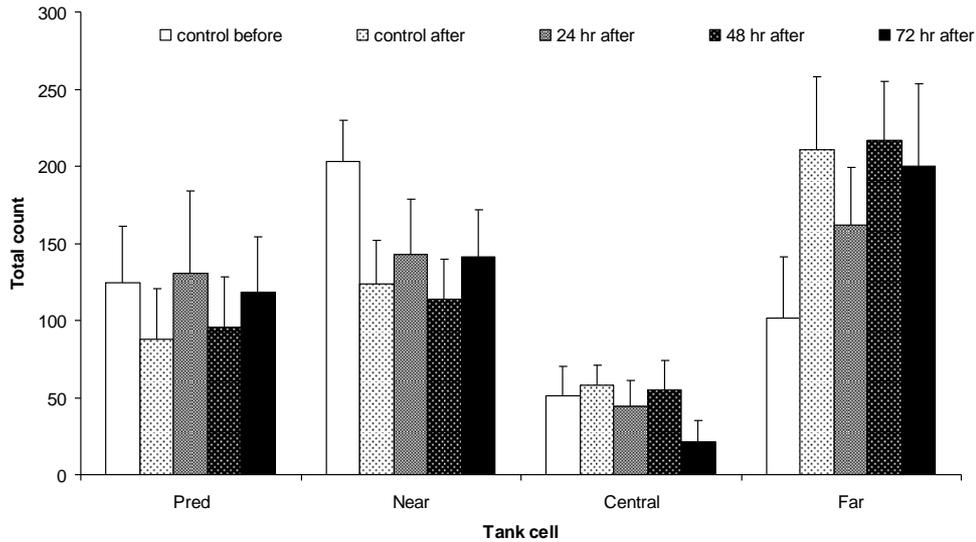
After introduction of a predator, use of the middle zone was significantly different between 72 hour trained Silver perch and 24 hour trained fish ( $p = 0.029$ ), 48 hour trained fish ( $p = 0.009$ ) or control fish ( $p = 0.012$ ). Once again variances were unequal between each pair as indicated by an  $F$  test ( $p < 0.001$ ). Mean use of the middle water column by control fish before introduction of a predator was also significantly greater than that of 72 hour trained fish ( $p = 0.011$ ).

Mean use of the top zone after introduction of a predator (Murray cod) was significantly different between 72 hour trained Silver perch and 48 hour trained Silver perch ( $p = 0.014$ ) and between 72 hour trained fish and control fish ( $p = 0.027$ ). Other pairings were not significantly different and this is related to the relatively large variances for the other groups of fish.

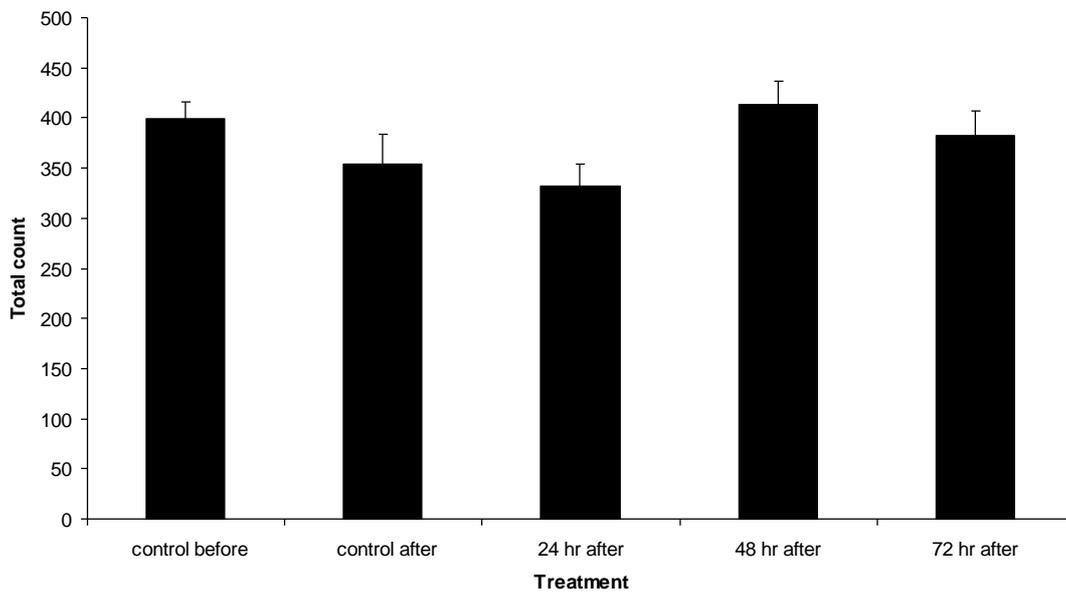
In all water column zones, after introduction of a predator 72 hour trained fish were consistently different to other treatments after introduction of a predator. Prior to introduction of a predator water column usage was similar between all groups. Only the 72 hour trained fish changed their use of the water column after introduction of a predatory fish. Other pair combinations showed no significant statistical differences. After 72 hours training the typical position of Silver perch in the test aquarium was the far bottom cell (Figure 18).

#### *Murray cod*

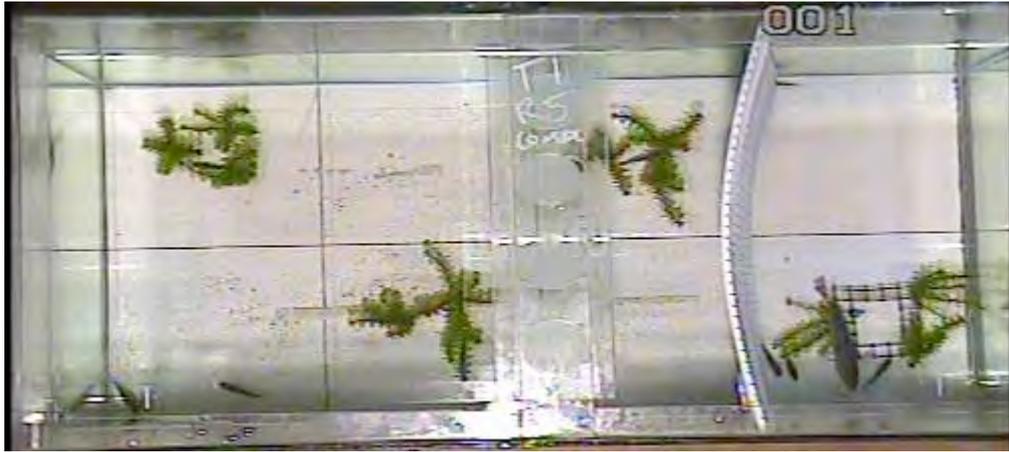
Variances were homogenous between treatment groups in each of the aquarium zones (predator, near centre and far) and there were no significant differences between groups in the mean use of each of the zones after the introduction of a predator. Before introduction of the predatory fish, cod tended to favour cells with corners, including the far cell, predator cell and near cell adjacent to the dividing screen. There was a tendency for all treatment groups to increase use of the far cell after introduction of a predator (Figure 23), although this was not significant at the  $p$  level of 0.05. All groups post introduction of a predator used the near zone less than the pre-introduction control group ( $p < 0.05$ ). There was also a tendency to use cover cells regardless of whether a predator was present or not. All variances relating to use of cover were homogenous between groups and no significant differences were detected by general ANOVA in mean use of cover between treatment groups (Figure 24) after introduction of a predator. The tendency across all groups post-introduction of a predator was to use either cover cells or cells distal from the predator (Figure 25).



**Figure 23:** Use of tank cells by groups of eight Murray cod fingerlings before (control only) and after (all treatment groups) introduction of a predator (Golden perch) to the predator cell. The maximum possible count in any cell is 480. Number of replicates is eight. Bars show mean values. Error bars show one standard error of the mean.

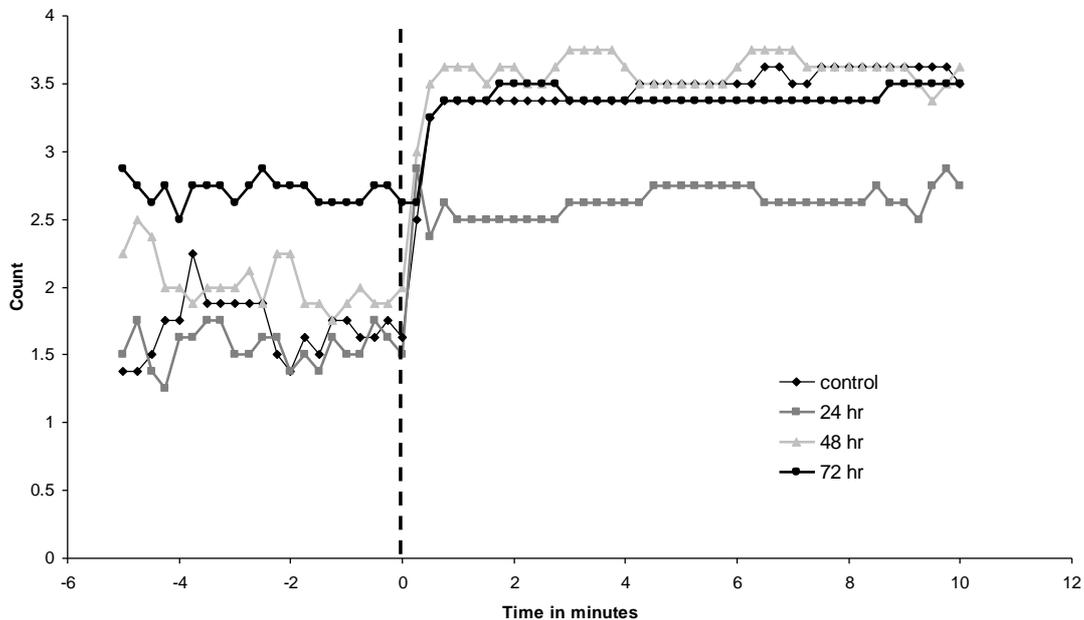


**Figure 24:** Use of cover by groups of eight Murray cod fingerlings before (control only) and after (all treatment groups) introduction of a predator (Golden perch) to the predator cell. Maximum possible count in cover is 480. Number of replicates is eight. Bars show mean values. Error bars show one standard error of the mean.

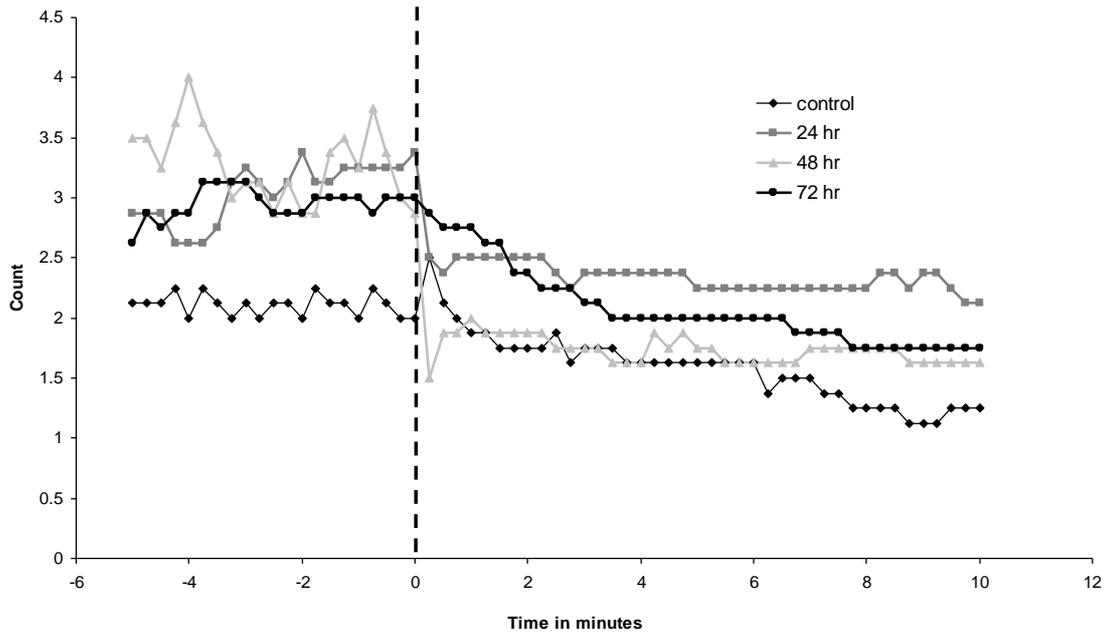


**Figure 25:** Cod from all treatment groups showed a tendency to use cover cells (including the cover cell in the predator compartment) and cells distal from an introduced predator. The image above shows a control group of Murray cod fingerlings. The predator is a Golden perch.

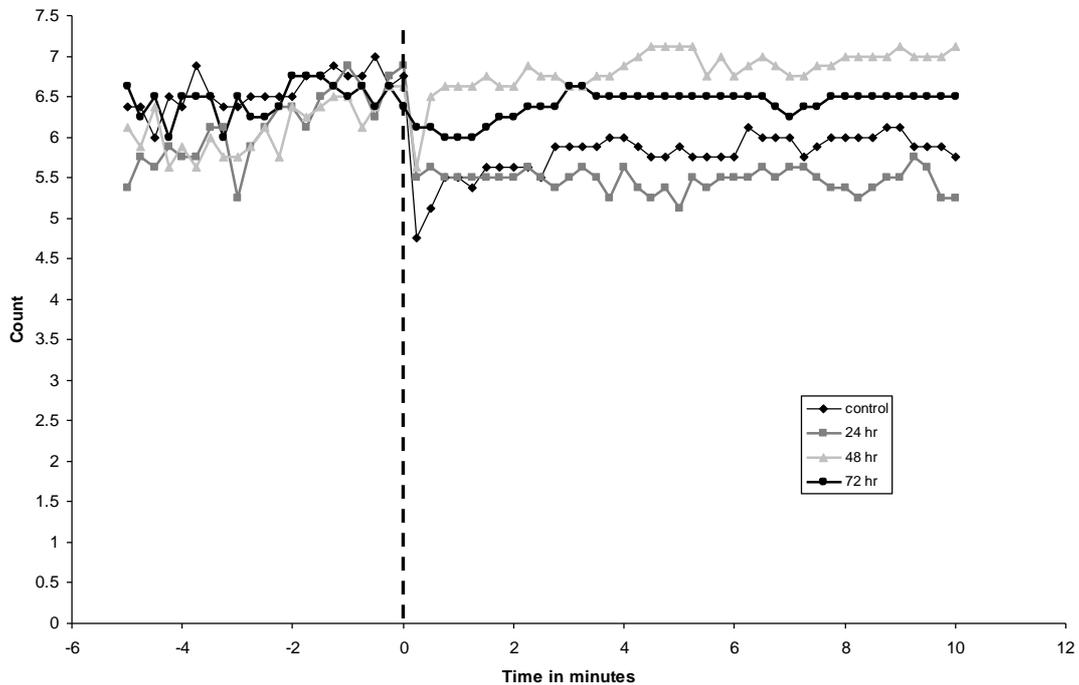
The movement of increased numbers of cod fingerlings to the far cell after introduction of the predator is clearly seen in Figure 26, but none of the trained groups showed greater use of the far cell compared to control fish. A minor drop in use of the predator cell can be seen across all groups in Figure 27 following introduction of the predatory fish. In Figure 28 it can be seen that use of cover cells changed little before and after introduction of the predator. A minor drop can be seen immediately after introduction of the predator across all groups. This was related to initial startle responses and scattering when the predator was released into the predator cell.



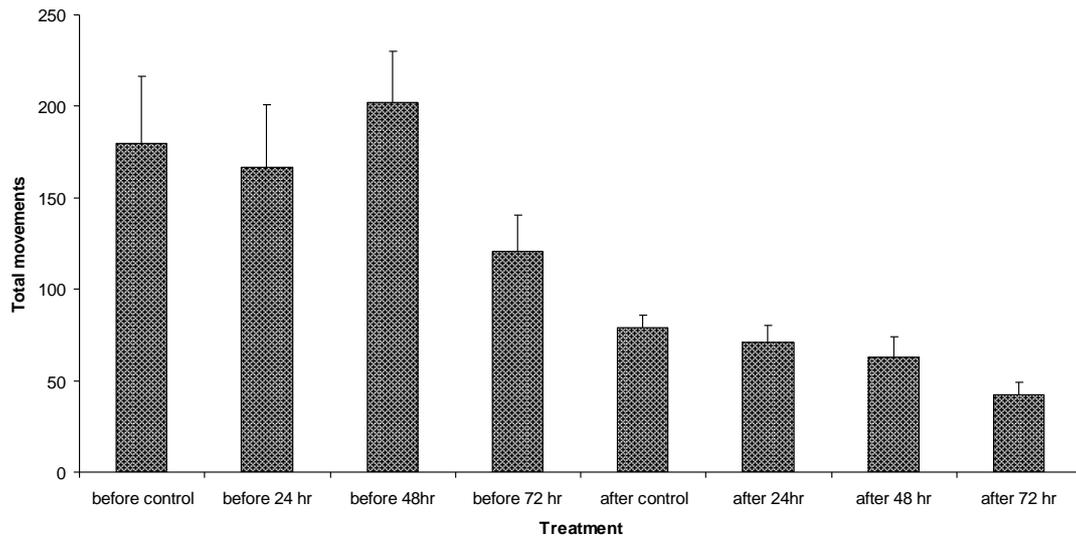
**Figure 26:** Mean numbers of Murray cod (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the far cell for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of Murray cod were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph.



**Figure 27:** Mean numbers of Murray cod (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the predator cell for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of Murray cod were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph.



**Figure 28:** Mean numbers of Murray cod (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in cover cells for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of Murray cod were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph.



**Figure 29:** Total number of movements recorded for trained (24 hr, 48 hr & 72 h) and untrained control groups of Murray cod for 15 minutes before and after introduction of a predator (Golden perch). Movements were recorded every 15 seconds. Number of replicates in each treatment group=8. Error bars show one standard error of the mean.

Before the introduction of a predator, movements were frequent across all treatment groups. Post-introduction of a predator, movements were reduced across all groups (Figure 29). Movements were least in the 72 hour trained fish and were almost half those of control fish exposed to a predator (Figure 29). Variances were homogenous between treatment groups after exposure to a predator and general ANOVA showed a significant difference between the means of treatment groups ( $p = 0.039$ ). The 72 hour trained fish suppressed their territorial behaviours and related movements more than both the control fish and 24 hour trained fish. The other trained groups were not significantly different to the control group (Table 4).

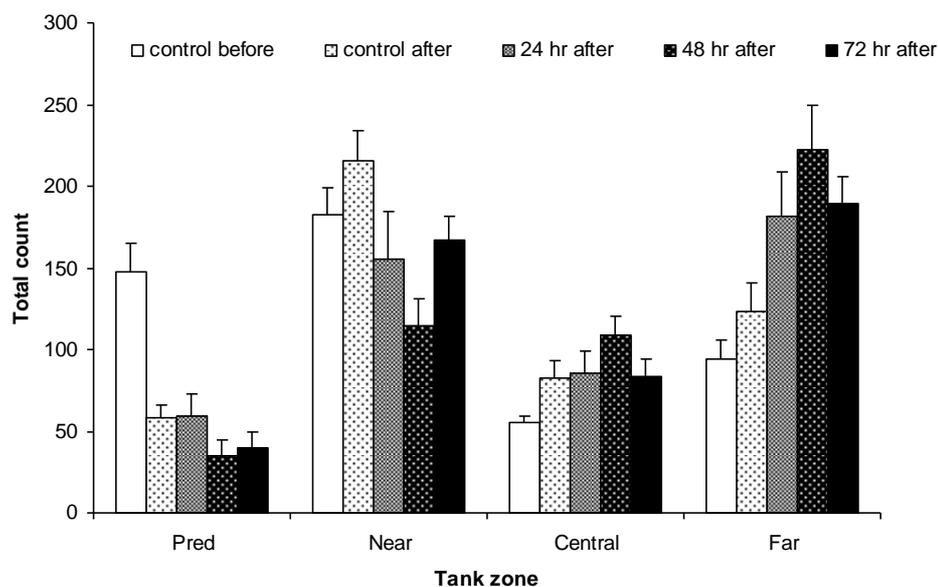
**Table 4:** Pairwise differences in movements of Murray cod fingerlings post introduction of a predator. Means with the same subscript are not significantly different at the  $P=0.05$  level

Treatment	Replicates	Mean
Control	8	78.75 a
24 hour trained	8	71.12 a
48 hour trained	8	62.62 ab
72 hour trained	8	42.25 b

#### *Freshwater catfish*

Variances were homogenous between all treatment groups in each of the tank zones (predator, near, centre and far). There was a tendency for increased use of the far zone of the aquarium by 24 hour 48 hour and 72 hour trained catfish (compared to controls) after introduction of a predator (Murray cod) to the aquarium (Figure 30). ANOVA showed a significant difference between treatments in the far cell ( $p=0.05$ ). Pairwise tests confirmed that 48 hour trained fish used the far cell significantly more than controls (Table 5) after introduction of a predator. Figure 31 shows typical use of cells by 72 hour trained fish after introduction of a predator. ANOVA also showed significant differences in use of the near cell after introduction of a predator ( $p=0.028$ ). The trend was for reduced use of the near cell by trained fish compared to

control fish. Table 6 shows that 48 hour trained fish used this cell significantly less than control fish after introduction of a predatory fish.



**Figure 30:** Use of tank cells by groups of eight Freshwater catfish fingerlings before (control only) and after (all treatment groups) introduction of a predator (Murray cod) to the predator cell. The maximum possible count in any cell is 480. Number of replicates is eight. Bars show mean values. Error bars show one standard error of the mean.

Figure 30 suggests a trend for fewer 48 and 72 hour trained fish in the predator cell compared to control fish after introduction of a predator, but this was not statistically significant at the five per cent level. There were no significant differences between treatments in the central cell after introduction of a predator.

**Table 5:** Pairwise differences in per-centage use of the far cell by Freshwater catfish fingerlings post introduction of a predator. Means with the same subscript are not significantly different at the  $P=0.05$  level

Treatment	Replicates	Mean
Control	8	24.48 a
24 hour trained	8	37.73 ab
48 hour trained	8	46.25 b
72 hour trained	8	39.50 ab

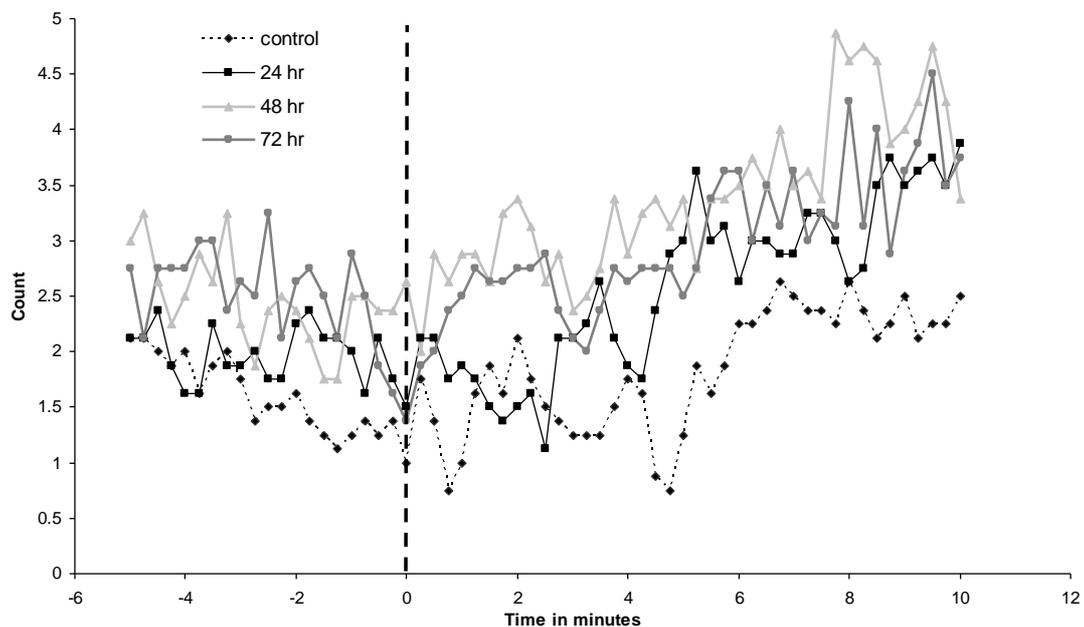
**Table 6:** Pairwise differences in per-centage use of the near cell by Freshwater catfish fingerlings post introduction of a predator. Means with the same subscript are not significantly different at the  $P=0.05$  level

Treatment	Replicates	Mean
Control	8	42.21 a
24 hour trained	8	32.27 ab
48 hour trained	8	23.83 b
72 hour trained	8	34.74 ab

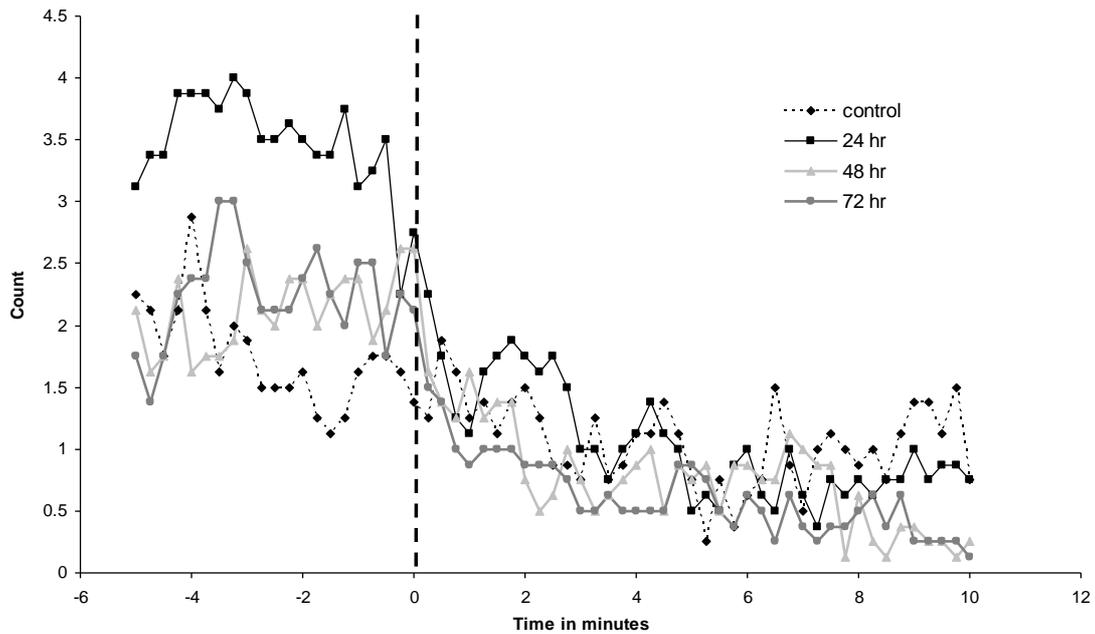


**Figure 31:** Catfish trained for 72 hours showed a tendency to use far cells.

Figures 32 and 33 show changes in use of the far and predator cells respectively by catfish fingerlings before and after the introduction of a predator. A general increase in use of the far cell with time after introduction of the predator can be seen across all treatment groups. The upward trend is steeper in the trained fish compared to the control fish (Figure 32). The use of the predator cell declines across all groups after introduction of the predator. Mean use of the predator cell declines to close to zero in 48 hour and 72 hour trained fingerlings within 10 minutes of introduction of a predator (Figure 33).

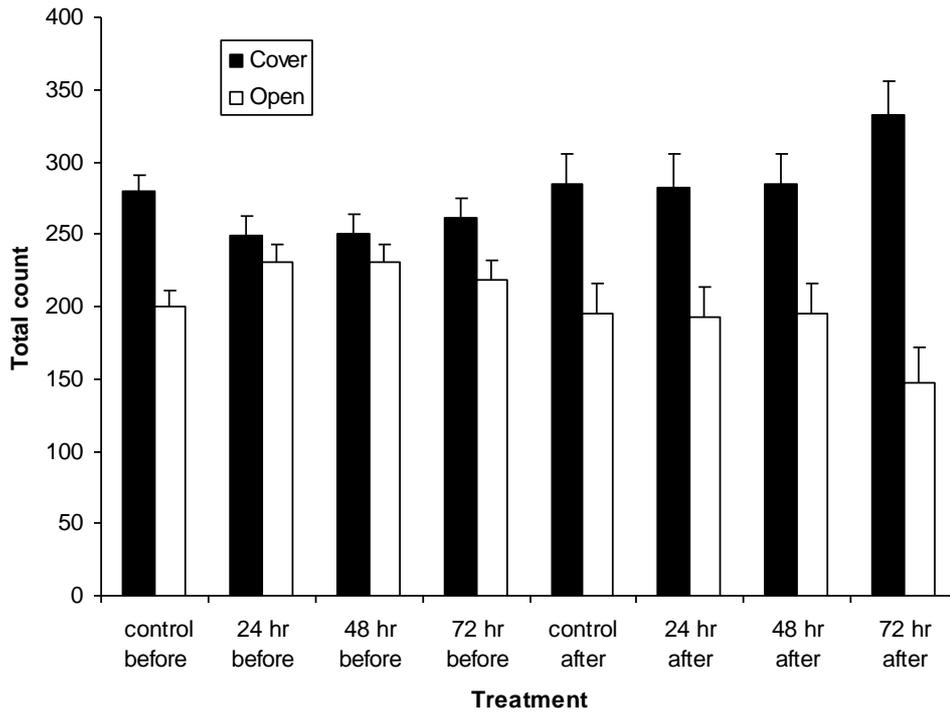


**Figure 32:** Mean numbers of Freshwater catfish (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the far cell for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of catfish were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph

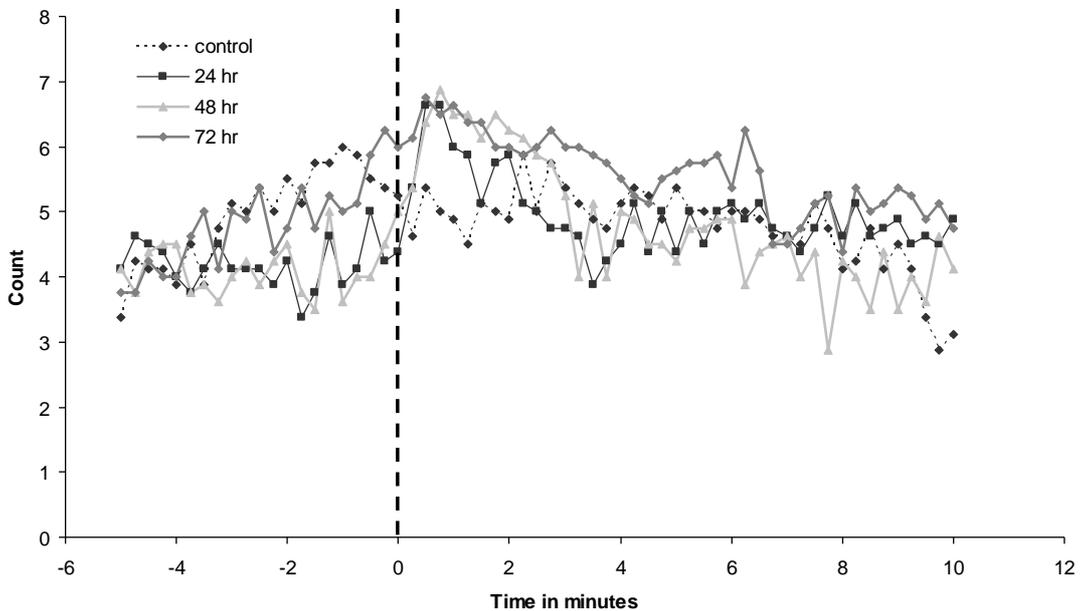


**Figure 33:** Mean numbers of Freshwater catfish (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in the predator cell for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of catfish were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph.

Variances relating to use of cover and open areas were homogenous between groups and no significant differences were detected between treatment groups by general ANOVA in mean use of cover (Figure 34) after introduction of a predator. Use of cover was similar both before and after introduction of a predator across most treatments, with the tendency being for a greater use of cover than open areas. Figure 34 suggests a trend for increased use of cover and a corresponding decrease in use of open areas by 72 hour trained catfish, but this was not significant at the five per cent level. Figure 35 suggests an initial upward trajectory in use of cover after introduction of the predator, followed by a gradual decline towards pre-introduction levels. Mean use of cover by 72 hours trained fish is consistently higher than that of control fish (Figure 35) in the first 10 minutes after introduction of a predator.



**Figure 34:** Use of cover and open water cells by groups of eight Freshwater catfish fingerlings before and after introduction of a predator (Murray cod) to the predator cell. Maximum possible count in cover or open areas is 480. Number of replicates is eight. Bars show mean values. Error bars show one standard error of the mean.



**Figure 35:** Mean numbers of Freshwater catfish (control, 24 hr trained, 48 hr trained and 72 hr trained) recorded in cover cells for 5 minutes before introduction of a predator and for 10 minutes after. The predator was introduced at time 0 denoted by the dashed line. Counts of catfish were recorded every 15 seconds. The maximum possible count at any one time is 8 fish. Number of replicates for each treatment is 8. Error bars have been excluded for clarity of reading the graph

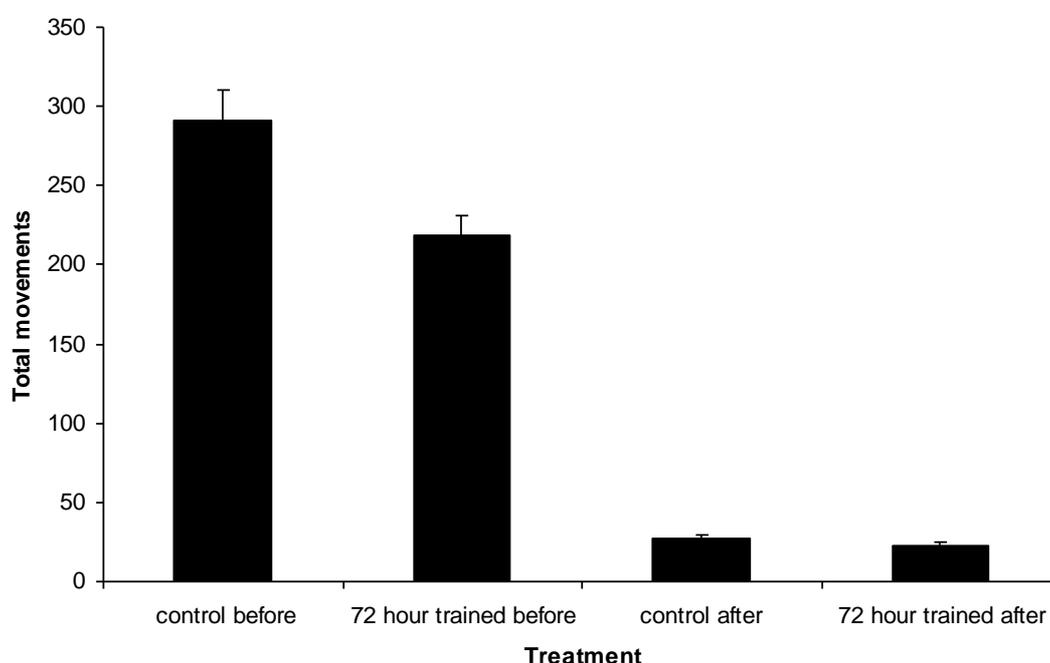
### *Movements*

Movements of catfish decreased after the introduction of a predator, but there were no significant differences between any of the treatment groups. Movement behaviour by catfish consisted of roaming and loose schooling behaviour with no evidence for territorial interactions. After introduction of the predator, most movements occurred in cells external to the predator cell.

### ***Responses to simulated bird predation***

#### *Fingerlings*

Bartlett's test of homogeneity of variances showed no significant differences between treatment variances in each of catfish, Silver perch and Murray cod fingerling groups. Analyses of fingerling data by ANOVA shows that although the fingerlings in each species group responded to the presence of a simulated bird, the response of trained fish was not significantly different at the 5% level to that of control fish in all cases. *i.e.*, both trained and untrained fish responded in the same way.



**Figure 36:** Movements by 72 hour trained and untrained cod before and after introduction of a simulated predatory bird attack. Observations were made every 15 seconds for 15 minutes before 15 minutes after the first simulated attack. Number of cod in each test tank=8. Number of replicates=12. Error bars show one standard error of the mean.

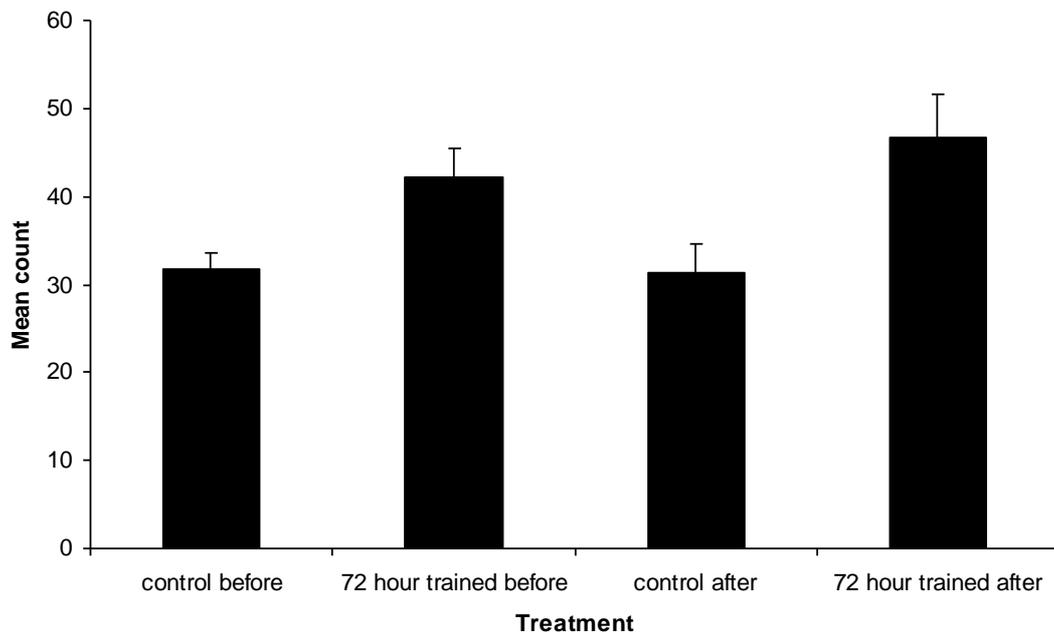
All species showed a tendency to increase use of the far cells after exposure to simulated bird attack, but differences were not significantly different between trained and untrained fish.

Both trained and untrained catfish increased use of cover after simulated bird exposure but there was no significant difference between treatment groups. Trained and untrained Silver perch also increased use of cover after bird exposure. However cod showed no increase in use of cover after simulated bird exposure. Cod innately used cover before and after introduction of a simulated bird attack. Initial exposure to

the “bird” led to decreased cover use as cod fled, but cod movements then became remarkably reduced compared to the period prior to simulated bird attack in both trained and untrained fish, but with no significant differences between groups (Figure 36).

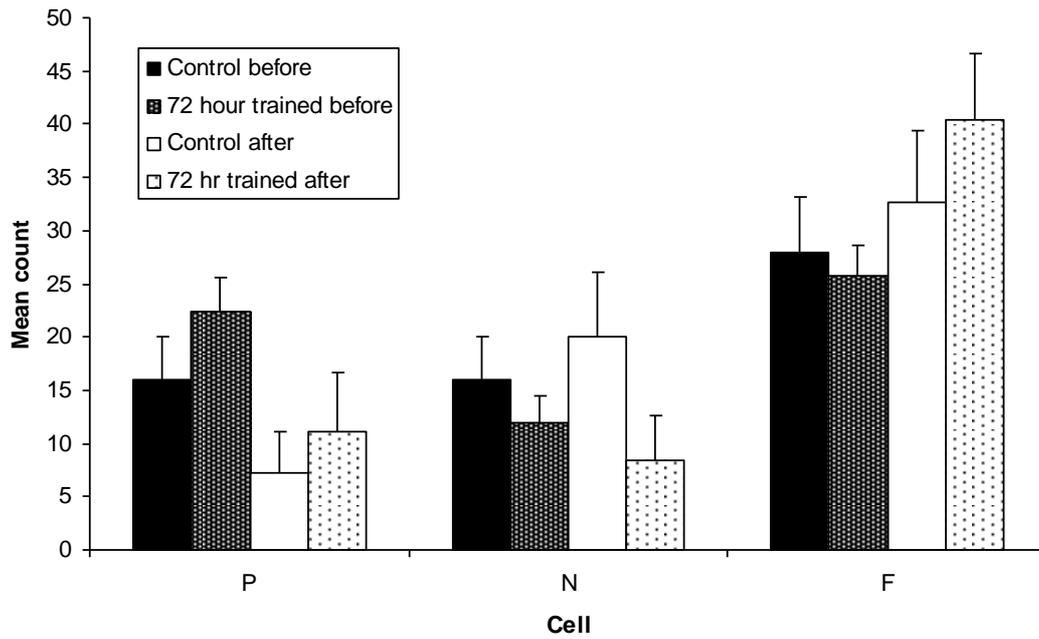
#### *Sub-adult fish*

Trained and untrained sub-adult Silver perch showed no significant difference in the use of predator, near or far cells ( $p > 0.1$ ). However there was a significant difference in the use of cover between trained and untrained Silver perch both before ( $p < 0.01$ ) and after ( $p = 0.015$ ) simulated bird attack (Figure 37). Trained sub-adult Silver perch tended to use cover more than the untrained sub-adult Silver perch before simulated predatory bird attack. Simulated predatory bird attack also increased cover use by trained Silver perch marginally. Use of cover did not change in untrained fish but became slightly more variable.

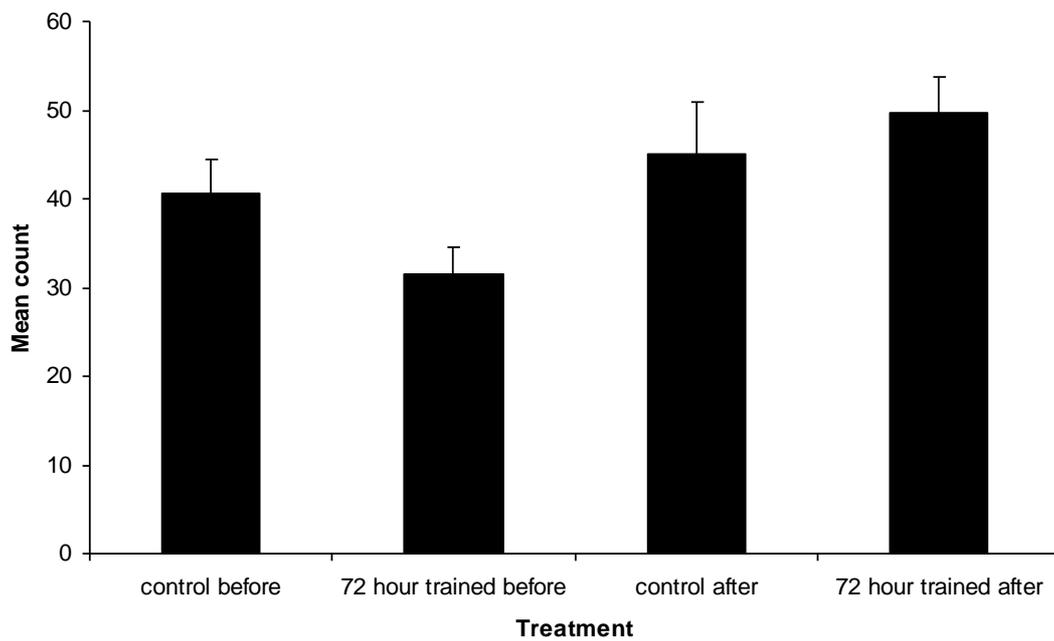


**Figure 37:** Use of cover by trained and untrained sub-adult Silver perch before and after exposure to simulated predatory bird. Error bars show one standard error of the mean.

Both control and trained sub-adult Murray cod showed some tendency to increase use of cover and to move away from the predator end of the tank, but the response was variable, with no significant difference between trained and untrained fish in the use of any of the cells. Figures 38 and 39 summarize use of tank cells by Murray cod.



**Figure 38:** Mean use of cells by untrained control and 72 hour trained sub-adult Murray cod before and after simulated predatory bird attack. Cells are the simulated predator cell (P), near the predator cell (N) and the far cell (F). Error bars show one standard error of the mean.



**Figure 39:** Mean use of cover cells by untrained control Murray cod and by 72 hour trained Murray cod, before and after simulated bird predator attack. Error bars show one standard error of the mean.

### ***Feeding trials***

Small adult Silver perch pairs in 1000 L tanks did not behave normally. They refused to feed during live feeding trials. In contrast during training in 7000 L tanks Silver perch readily took live shrimp, even on the first day. Given the behaviour of Silver perch in the 1000 L tanks this experiment was abandoned. It was clear during the training that Silver perch readily accepted live shrimp. Within an hour of being returned to a 7000 L tank Silver perch were readily feeding again. Unfortunately 7000 L tanks could not be used for the validation experiments as the larger tank size made accurate observations of predation more difficult and there were also insufficient large tanks available for simultaneous replication due to the requirement to use the large tanks for housing other species at the research centre.



**Figure 40:** Shrimp are noticeable on the bottom of a 5000 L tank (left) free to roam, without being preyed on by sub-adult cod, even when directly in front of, and in close proximity to sub-adult cod (right).

Sub-adult pellet reared cod could not be coaxed to take live shrimp during the training process, even after one month of training. Shrimp were free to roam around the 7000L tank and even when dropped directly in front of cod were not taken (Figure 40). Therefore it was apparent that these pellet reared cod could not be trained to take live shrimp and the experiment was abandoned. The cod immediately returned to feeding on pellets and dead foods after the cessation of live food training.

### **Tag retention trials**

#### ***VIE tags***

Five months after tagging VIE tags could only be found in 55% of tagged Freshwater catfish. This most likely reflected tag shedding, rather than loss of readability as the tags had been injected into translucent tissue.

#### ***PIT tags***

Six weeks after gut cavity tagging with PIT tags, PIT tags were detected in 100% of tagged Silver perch. There were no post-tagging mortalities in this period and all tagged fish showed no signs of post tagging infection or injuries.

### **Field validation trials**

A total of 211 VIE marked cod and 528 VIE marked Silver perch were recaptured in this study. With the exception of Storm King Dam, where a local stocking group had released unmarked fingerlings in addition to our research stocking, very few unmarked fish in the appropriate size range were recaptured. At Caliguel Lagoon where there was no supplementary stocking 97.8% of cod and 99.3% of Silver perch captured had visible VIE tags.

Shortly after stocking Reilly's Weir with Silver perch a flood occurred. Waters began rising rapidly during the 24 hours post-stocking survey. By the following day waters had risen to flood level. We believe that juvenile Silver perch were able to disperse widely from the stocking site during this event. Only 9 Silver perch were recaptured at this site despite extensive searches. Murray cod stocked at this site were compromised by a transport incident. A vehicle breakdown, led to bagged fish in the vehicle being exposed to high temperatures en-route. As a consequence cod fingerlings became stressed leading to high mortalities in some bags. Even though the cod that survived the journey were still stocked at this site it was felt that the additional transport stress would have compromised results. Further flooding events also occurred at this site during the course of the study. Therefore data from Reilly's Weir was not analysed for this study.

Recapture rates at Cotswold Dam of both cod and Silver perch were low (16 and 19 fish respectively). A sustained period of wet conditions led to Cotswold Dam backing up into fringing riparian forest and dead standing timber for the duration of the project. This provided extensive habitat for stocked cod and Silver perch, but this inundated area was difficult to sample efficiently, with access to the shoreline for the electrofishing boat blocked by vegetation, and the area far too extensive to sample with a back pack electrofisher. In a drier year dam levels would have dropped as water was used for irrigation, creating an accessible shoreline. However this did not occur, with extensive inundated timber habitat remaining available to stocked fingerlings for the duration of the project. The dam also spilled over twice during the course of the project which could have resulted in the loss of some fingerlings downstream.

In contrast access to shorelines by electrofishing boat was much easier in Storm King Dam and Cotswold Lagoon for most of the project. Recaptures of stocked fingerlings were much higher at these sites. Silver perch recapture rates for the different treatments at these two sites ranged from 3.6% to 6% and cod recapture rates ranged from 0.16% to 4.26%. Data analyses were run for all three sites (Storm King Dam, Caliguel Lagoon and Cotswold Dam) and then repeated using data from just Caliguel Lagoon and Storm King Dam only. The reason for the second analysis was to determine if the low catch rates at Cotswold Dam (probably related to prevailing environmental conditions) were masking any trends at the sites with higher recapture rates. Recapture rates are indicative of relative survival and are not intended to be used as estimates of absolute survival.

### ***Silver perch***

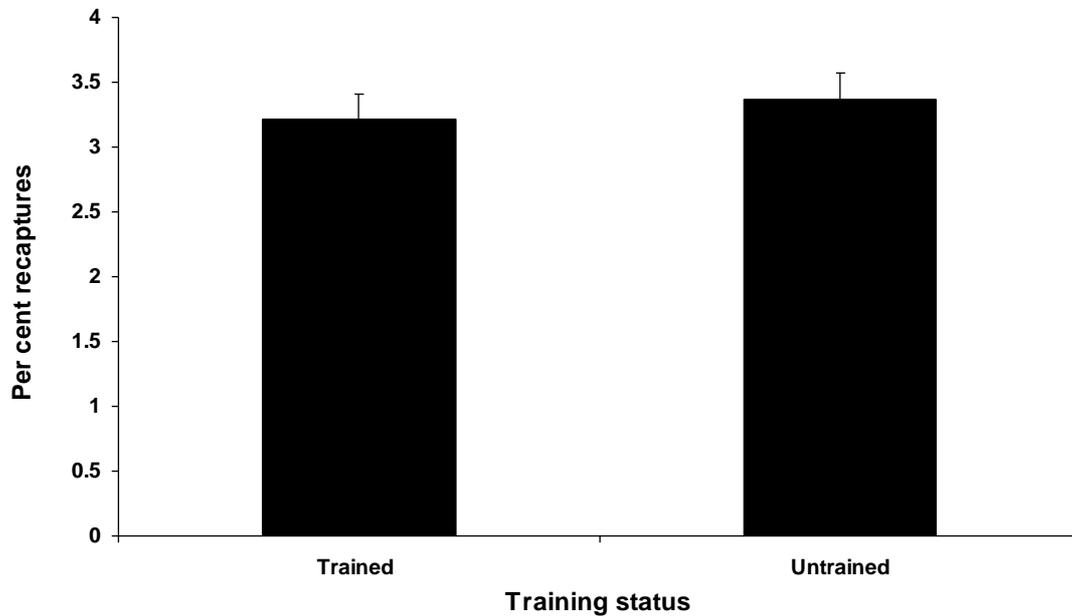
The GLM of binomial proportions for recaptures of Silver perch across all three sites found only one significant effect "site", which accounted for most of the deviance. Training status and release strategy were retained in the model but neither were significant parameters. The model is summarised in Tables 7 and 8. Adjusted mean recapture rates for Silver perch across the three sites by training status and release strategy are shown in Figures 41 and 42.

**Table 7:** Summary of analysis for Silver perch recaptures data from Cotswold Dam, Caliguel Lagoon and Storm King Dam. Dispersion parameter is fixed at 1.

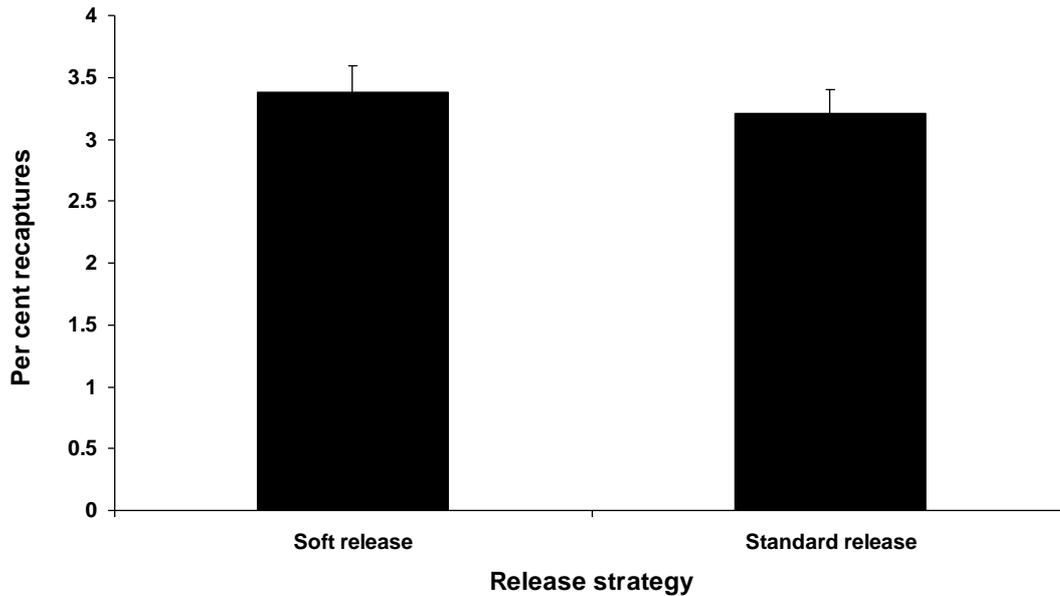
	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx chi probability
Regression	4	283.656	70.914	70.91	<0.001
Residual	7	4.310	0.6157		
Total	11	287.966	26.1788		

**Table 8:** Significance levels of parameters in the GLM of binomial proportions for recaptures of Silver perch at Cotswold Dam, Caliguel Lagoon and Storm King Dam. Parameters for factors are compared with reference levels "Site Caliguel lagoon", "Status trained" and "Release soft."

Parameter	t probability
Constant	<0.001
Site Cotswold Dam	<0.001
Site Storm King Dam	<0.002
Status untrained	0.570
Release standard	0.547



**Figure 41:** Adjusted mean recapture rates by training status for Silver perch from Storm King Dam, Cotswold Dam and Caliguel Lagoon. Means have been adjusted by averaging over the levels of some factors. Error bars are one standard error of the mean.



**Figure 42:** Adjusted mean recapture rates by release strategy for Silver perch from Storm King Dam, Cotswold Dam and Caliguel Lagoon. Means have been adjusted by averaging over the levels of some factors. Error bars are one standard error of the mean.

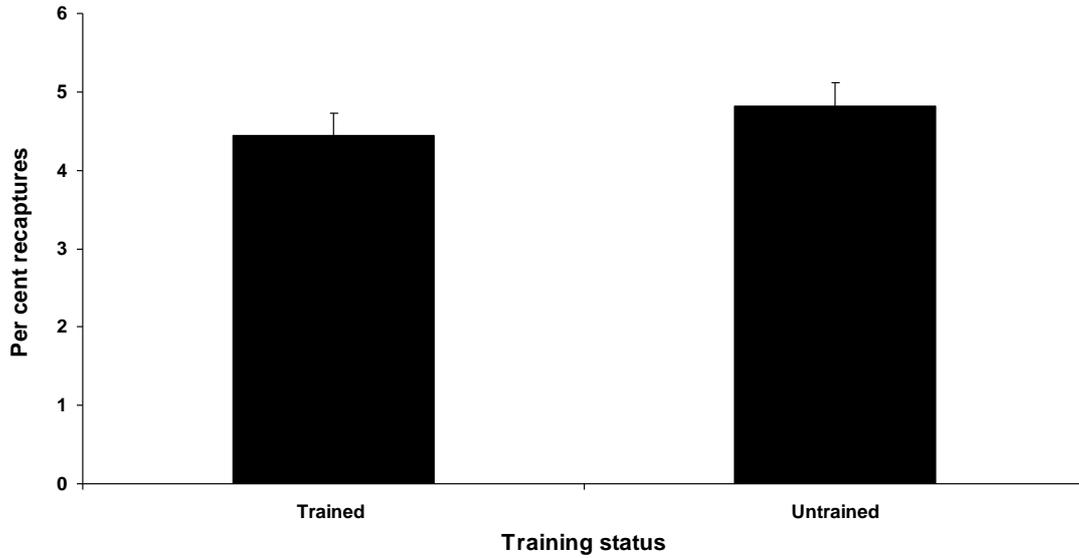
In the GLM of binomial proportions for Silver perch recapture data from Storm King Dam and Caliguel Lagoon, site was no longer a significant parameter. In this model predator index emerged as the only significant parameter explaining recapture rates. Training status and release strategy remained non-significant effects. The model is summarised in Tables 9 and 10. Adjusted mean recapture rates for Silver perch across the two sites by training status and release strategy are shown in Figures 43 and 44. The relationship between predator index (predation pressure) and recapture rates of Silver perch is shown in Figure 45.

**Table 9:** Summary of analysis for Silver perch recaptures data from Caliguel Lagoon and Storm King Dam. Dispersion parameter is fixed at 1.

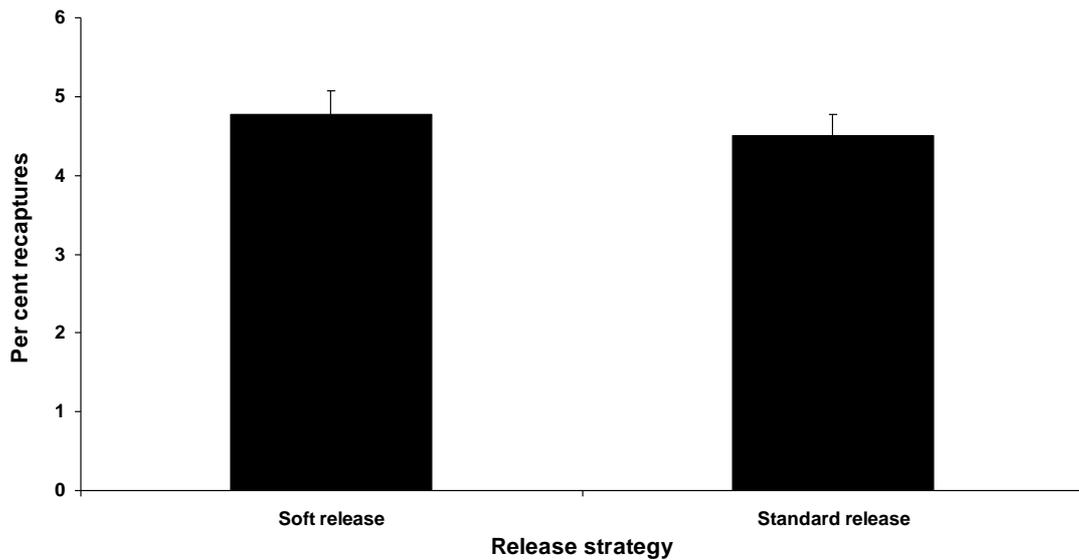
	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx chi probability
Regression	3	9.104	3.0348	3.03	0.028
Residual	4	3.679	0.9197		
Total	7	12.783	1.8262		

**Table 10:** Significance levels of parameters in the GLM of binomial proportions for recaptures of Silver perch at Caliguel Lagoon and Storm King Dam. Parameters for factors are compared with reference levels “Status trained” and “Release soft.”

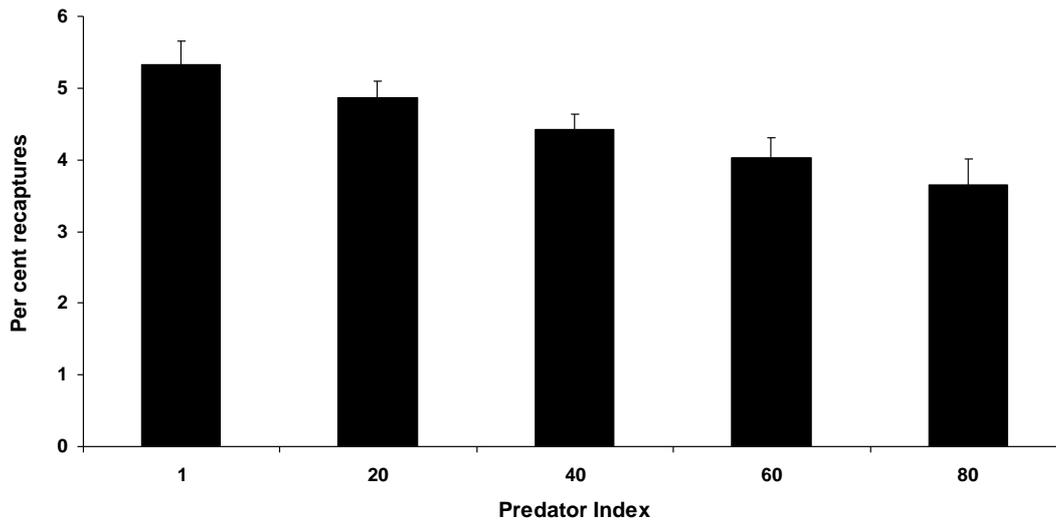
Parameter	t probability
Constant	<0.001
Predator index	0.003
Status untrained	0.369
Release standard	0.510



**Figure 43:** Adjusted mean recapture rates by training status for Silver perch from Storm King Dam and Caliguél Lagoon. Means have been adjusted by averaging over the levels of some factors. Error bars are one standard error of the mean.



**Figure 44:** Adjusted mean recapture rates by release strategy for Silver perch from Storm King Dam and Caliguél Lagoon. Means have been adjusted by averaging over the levels of some factors. Error bars are one standard error of the mean.



**Figure 45:** Adjusted mean recapture rates of Silver perch compared to the predator index at release locations (derived from recapture data at Storm King Dam and Caliguel Lagoon). The parameters release strategy and training status have been held constant over levels of other factors. Error bars are one standard error of the mean.

### *Murray cod*

The GLM of binomial proportions for recaptures of Murray cod across all three sites found several significant effects on recapture rates. These were sampling effort, training status, release strategy and predator index. Adjusted mean values for recapture rates of trained fish are significantly higher than for untrained fish and fish released by standard release methods had better survival than soft released fish. Site could not be included in the model due to aliasing with sampling effort. Sampling effort or site both account for the lower recapture rates at Cotswold Dam. The model is summarised in Tables 11 and 12. Adjusted mean recapture rates for Murray cod across the three sites by training status and release strategy are shown in Figures 46 and 47.

**Table 11:** Summary of analysis for Murray cod recaptures data from Cotswold Dam, Caliguel Lagoon and Storm King Dam. Dispersion parameter is fixed at 1.

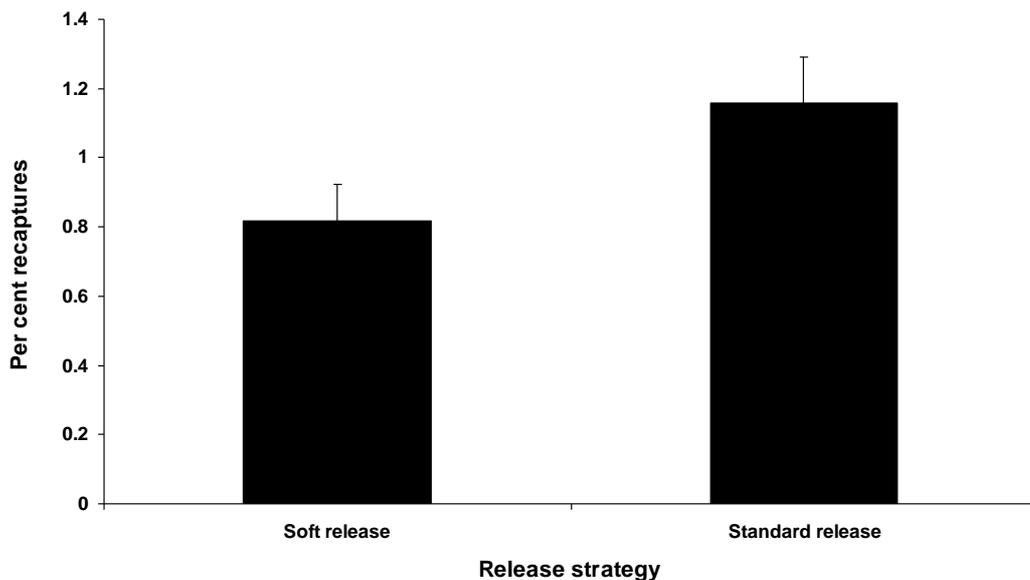
	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx chi probability
Regression	4	174.53	43.632	43.63	<0.001
Residual	7	20.97	2.995		
Total	11	195.49	17.772		

**Table 12:** Significance levels of parameters in the GLM of binomial proportions for recaptures of Murray cod at Cotswold Dam, Caliguel Lagoon and Storm King Dam. Parameters for factors are compared with reference levels "Status trained" and "Release soft."

Parameter	t probability
Constant	<0.001
Sampling effort	<0.001
Status untrained	0.012
Release standard	0.015
Predator index	0.047



**Figure 46:** Adjusted mean recapture rates by training status for Murray cod from Storm King Dam, Cotswold Dam and Caliguel Lagoon. Means have been adjusted for fixed values of predator index (25.41) and sampling effort (4.916). Marginal weights have been held constant over other factors. Error bars are one standard error of the mean.



**Figure 47:** Adjusted mean recapture rates by release strategy for Murray cod from Storm King Dam, Cotswold Dam and Caliguel Lagoon. Means have been adjusted for fixed values of predator index (25.41) and sampling effort (4.916). Error bars are one standard error of the mean.

In the GLM of binomial proportions for Murray cod recaptures at the Storm King Dam and Caliguel Lagoon, sampling effort was no longer a significant parameter. In this model release strategy remained as a significant effect as did predator index. There was also a significant interaction between training status and predator index. This interaction suggests that at low levels of predator abundance trained and untrained fish survive at similar rates, but as predator density increases survival of untrained fish declines at a faster rate than for trained fish. Adjusted mean values for

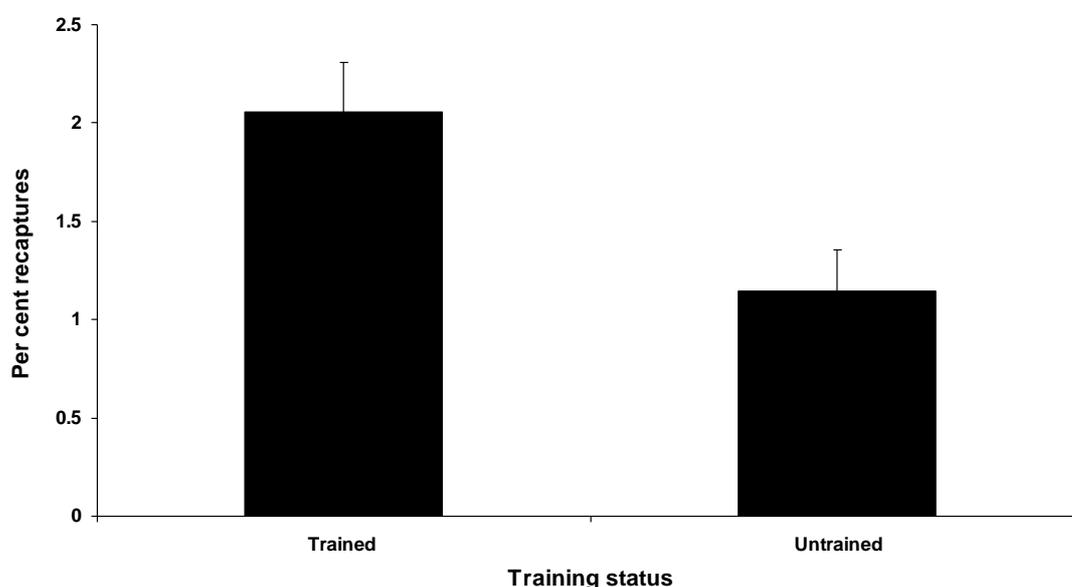
recapture rates of trained fish are significantly higher than for untrained fish and fish released by standard release methods had better survival than soft released fish. The model is summarised in Tables 13 and 14. Adjusted mean recapture rates for Murray cod across the two sites by training status and release strategy are shown in Figures 48 and 49 respectively. The interaction between predator index and training status is shown in Figure 50.

**Table 13:** Summary of analysis for Murray cod recapture data from Caliguel Lagoon and Storm King Dam. Dispersion parameter is fixed at 1.

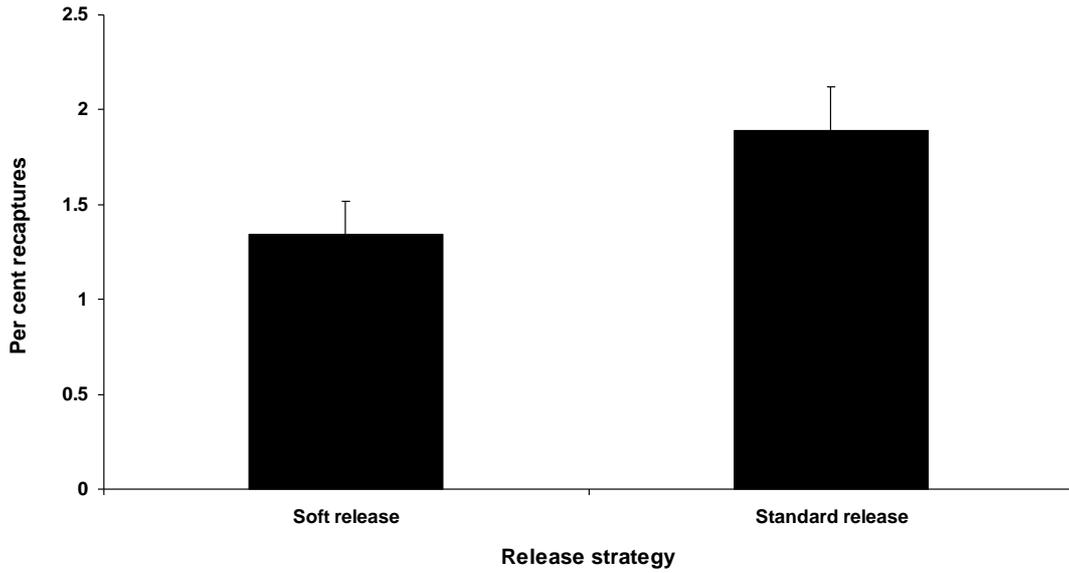
	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx chi probability
Regression	3	104.103	34.701	34.70	<0.001
Residual	4	4.061	1.015		
Total	7	108.164	15.452		

**Table 14:** Significance levels of parameters in the GLM of binomial proportions for recaptures of Murray cod at Caliguel Lagoon and Storm King Dam. Parameters for factors are compared with the reference level "Release soft."

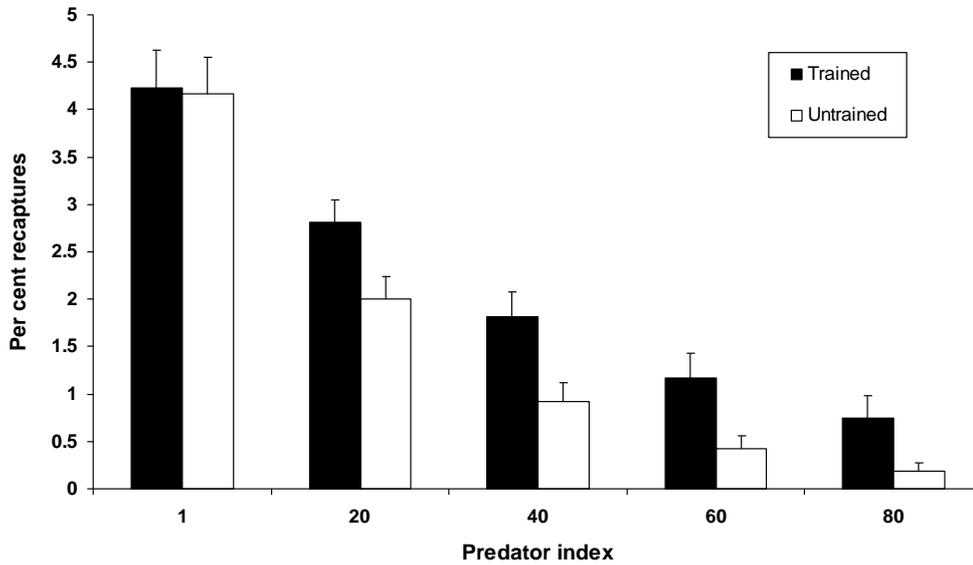
Parameter	t probability
Constant	<0.001
Predator index	<0.001
Predator index.Status untrained	0.008
Release standard	0.021



**Figure 48:** Adjusted mean recapture rates by training status for Murray cod from Storm King Dam and Caliguel Lagoon. Means have been adjusted for a fixed value of predator index (34.34) and marginal weights have been held constant over levels of other factors. Error bars are one standard error of the mean.



**Figure 49:** Adjusted mean recapture rates by release strategy for Murray cod from Storm King Dam and Caliguél Lagoon. Means have been adjusted for a fixed value of predator index (34.34) and marginal weights have been held constant over levels of other factors. Error bars are one standard error of the mean.



**Figure 50:** Interaction between predator index and training status of Murray cod stocked into Storm King Dam and Caliguél Lagoon. Recapture rates are adjusted mean values. Marginal weights have been held constant over levels of other factors. Error bars are standard errors of the mean.

## Discussion

### Tag retention trials

Although VIE tagged Freshwater catfish and gut cavity PIT tagged Silver perch were not used in field trials in this project, the information gained from the two tag retention studies in this project will be useful for other research projects involving catfish or Silver perch. The VIE tag retention rate in Freshwater catfish of only 55% after 5 months is relatively low compared to some other species. For example the retention rates were 97.8% for Murray cod and 99.3% for Silver perch recaptured in the field component of this study.

One option for researchers wishing to follow small batch marked catfish for a short period of time could be double tagging, using a VIE tag in each jaw. Alternatively batch marking with sub-cutaneous wire tags, using different body locations for different batches could be a marking method for future investigation.

The high tag retention rate of gut cavity PIT tagged Silver perch (100%) and low post tagging mortalities (0%) recorded in this study over a six week period are promising. This suggests that gut cavity PIT tagging is a suitable marking method for use in short term mark recapture studies of Silver perch. Especially studies where the identity of individual fish is required to be known. Gut cavity tagging eliminates the risk of accidental ingestion by anglers, which always remains a possibility for musculature tagged fish. PIT tags have the advantage over external dart tags and T bar tags in that they do not lead to the external lesions that are often associated with external tags.

### Tank based validation trials

#### *Response to predatory fish by fingerlings*

All three species of fingerling fish used in tank-based validation trials showed some significant changes in response to predatory fish following training. The type of response differed between species. The changes in behaviour suggest that all three species could benefit from pre-release training prior to stocking for conservation or other purposes.

Silver perch fingerlings trained for 72 hours displayed the most consistent behavioural changes. These were significantly more consistent use of far cell by 72 hour trained fish and a downward trend in use of other cells after introduction of a predator; and significantly reduced use of the middle and upper water column after introduction of a predator. F-tests run with the two sample t-tests showed that variances between 72 hour trained fish and control fish were unequal in all cases ( $p < 0.001$ ). This significant difference emphasises the inconsistent response of control fish versus the consistent response of the 72 hour trained fish.

In the case of Murray cod, although there was no significant difference between treatment groups in mean frequency of use of different cells and cover post-introduction of a predator, there was a behavioural change that influenced efficacy of cover use. Murray cod fingerlings have a tendency to show territorial aggression toward each other. This territorial behaviour leads to sparring between individuals and therefore movement within and between cells. In 72 hour trained fish this movement and sparring was significantly reduced compared to control fish following introduction of a predatory fish. This reduced movement within cover should assist

cod in being less obvious to a predator than cod which continue to spar and move about in cover.

Reduction of territorial interactions when predators are present could be important for the survival of stocked fingerlings, as fingerlings are normally released in large batches and the chances for territorial interactions shortly after release are high. Trained cod appeared to conceal themselves better in the cover cells than control fish, although this was not quantified. Our observations suggest while untrained fish favoured cover cells, they were frequently exposed in these cells.

In the case of Freshwater catfish there was a significant increase in use of the far cells by 48 hour trained catfish after introduction of a predator and there was a tendency for other trained groups to increase their use of far cells relative to the untrained fish. There was also a significant reduction in use of near cells by 48 hr trained catfish and although not significant at the five per cent level, time series and other figures suggest a tendency for other trained fish to do the same. There was also a trend towards increased use of cover by 72 hour trained catfish but this was not significant at the five per cent level. It is possible that 48 hours training is sufficient for catfish. Why the 72 hour trained fish do not have a statistically significant improvement in predator avoidance behaviour compared to the control fish when the 48 hour trained fish do is unknown.

Pair-wise testing showed 72 hour trained catfish to be not significantly different from both the 48 hour trained fish and the control fish. It is possible that further replication would have reduced variance and led to significant differences between control catfish and 72 hour trained catfish also. The trends in the data as displayed in the bar graphs and time series are certainly in the direction to be expected with improved predator recognition and avoidance behaviour.

### ***Response to simulated bird attack by fingerlings***

The fact that there was no significant difference between trained and untrained fingerlings of all three species in response to simulated bird attack is not surprising. It is quite likely that all fingerlings had some limited prior exposure to birds in hatchery ponds prior to purchase for this study. The survey by Hutchison *et al.* (in press) suggests that most hatchery reared fingerlings in south-eastern Australia have some bird exposure. All groups (trained and untrained) displayed some form of predator avoidance behaviour when exposed to simulated bird attack. This result suggests that for the majority of native fish stocked as fingerlings, pre-release training for birds is probably unnecessary as it is already likely to have occurred due to limited bird exposure in hatchery ponds. If fingerlings have not had prior bird exposure, then some form of bird training may be beneficial. Although we used simulated birds, with real cormorant odour in this study, it would be preferable to use a live bird for the training. A real bird would have the advantage of providing additional stimuli such as swimming vibrations. Some limited exposure to real predation and direct predator contact is also likely to enhance training (Jarvi and Uglem 1993; Berejikian, 1995). Predation would need to be limited/controlled in the training tank or training pond to prevent high mortalities of fingerlings.

### ***Response to simulated bird attack by sub-adult fish***

Sub-adult Silver perch showed some positive responses to simulated bird attack training. If it was ever proposed to stock sub-adult Silver perch as part of a recovery program, then bird avoidance training will probably be beneficial. Evaluating outcomes in the field may be difficult. Generally fewer numbers of large fish are stocked when compared to fingerlings. A favoured way of monitoring survival of small numbers of large fish is by radio-telemetry. However, O’Conner *et al.* (2009) report poor radio-tag retention rates (18%) for Silver perch. Therefore it is probably a wasted investment to try radiotelemetry on stocked sub-adults of this species. Alternative systems such as PIT tags and PIT tag reader arrays may be required. When dealing with smaller numbers of fish recapture rates and detection rates can be problematic for interpretation of results.

In contrast to Silver perch, sub-adult Murray cod showed poor responses to simulated predation after training. It appears that sub-adult Murray cod were unable to learn the appropriate responses in the 72 hour training period. Poor survival and deficient behaviours of stocked sub-adult Trout cod. *M. macquariensis* have been reported by Ebner and Thiem (2006) and Ebner *et al.* (2006). It was hoped that pre-release training of sub-adult Trout cod might improve future outcomes. Based on the results of the current project, it is probable that domestication effects on *Maccullochella* spp held until adult or sub-adult stage in captive situations are difficult to overcome.

### ***Live food training***

It was reported in the results section that there were problems with evaluation of live food training for both sub-adult Murray cod and adult Silver perch. The problems between the two groups were contrasting. Silver perch had no difficulty adapting to live feed in the large training tank, with live food being consumed very rapidly. Silver perch were either not comfortable in the 1000 L evaluation tanks, or required to be in groups larger than two fish to stimulate feeding behaviour. Unfortunately larger groups of sub-adult fish could not be trialled due to logistical problems in holding larger numbers of sub-adult/adult fish on site. Based on our observations in the training tank we believe that Silver perch would have little difficulty in adapting to live feeds after stocking into riverine or lacustrine environments.

In contrast, sub-adult Murray cod refused to take live feed in the training tank even after one month. They had no difficulty in taking pellet foods or dead feed immediately after the training period. As for the bird predator avoidance training, it would appear that sub-adult Murray cod are extremely difficult to train to overcome long-term domestication effects. Stocked sub-adult cod sourced from a grow-out facility are likely to suffer from foraging difficulties in the wild. This could be expressed in erratic roaming behaviours as noted for Trout cod by Ebner and Thiem (2006). Given the poor training result for both bird avoidance and live food foraging, we recommend against using sub-adult cod sourced from grow-out facilities for conservation stocking programs. It would be far better to either translocate wild adults to new sites or to use hatchery pond reared fingerlings. Fingerlings have generally been raised on live foods (Hutchison *et al.*, in press), appear to retain bird avoidance behaviours and are trainable to avoid predatory fish.

### Field trials

Recapture rates of micro-tagged Silver perch and Murray cod at the non-flood affected sites of Storm King Dam and Caliguel Lagoon are comparable to those reported from other studies of stocked fingerlings. For example recapture rates have been reported at between 0.4% and 6% for Barramundi (Hutchison *et al.*, 2006), 3.28% for Striped mullet (Leber *et al.*, 1996) and 0.28% for Red drum (Winner *et al.*, 2001).

In contrast to the laboratory based experiments where Silver perch showed a significant change in behaviour following training, no significant difference in recapture rates of trained and untrained Silver perch was detected in the field. Although trained and untrained Silver perch were stocked at least 1 km apart, and up to 2 km apart, by the time of the 24 hour post stocking survey, very few Silver perch were captured near their release points. Untrained Silver perch were captured near trained fish release points and visa versa. It wasn't unusual to capture trained Silver perch alongside untrained Silver perch. This suggests rapid dispersal from stocking points and formation of mixed schools. Silver perch are a grunter species and are known to vocalise (Stuart *et al.*, 2009) It is possible that their vocalisation behaviours assisted them in locating each other and forming mixed groups of trained and untrained fish.

Social learning from conspecifics and cueing responses from fish around them is an important way for fish to learn to avoid predators (Brown and Laland, 2001; Brown and Laland 2003). Brown and Laland (2003) concluded that it is conceivable that hatchery reared fish could be trained *en masse* to recognise predators and prey using social learning protocols. Berejikian *et al.* (2000) suggested one of the problems with past attempts to assess the effects of training fingerlings on the success of field releases is that both trained and untrained fish have been released together. This enables the control fish to rapidly acquire anti-predator behaviour from the trained fish through social learning processes, but the improved survivorship of the control fish offsets the apparent effect of the training procedure by reducing differences in mortality between test and control fish. We tried to avoid this by stocking trained and untrained Silver perch at least 1 km apart, but it seems we underestimated their rate of dispersal and ability to find each other.

If untrained Silver perch are able to rapidly learn off trained conspecifics when stocked into the wild, this could be an advantage and streamline *en masse* training in the hatchery. It would only be necessary to train a sub-group of the fish to be stocked and these could be stocked in a mixed batch of trained and untrained fish. For example, if stocking 20,000 Silver perch fingerlings, it may only be necessary to train 5000 of them.

In contrast to Silver perch, Murray cod are not a schooling or very social species. They are territorial and cannibalistic. Juvenile cod also appear to be much more sedentary than Silver perch. During the 24 hour post stocking survey, Murray cod fingerlings were only captured within 500 m of their initial release points. This contrasted with Silver perch which were caught up to 2 km from where they were released within 24 hours of stocking. This more sedentary and territorial behaviour probably means that trained and untrained cod did not intermingle in the first 24 hours after stocking. This may explain why a significant difference in recapture rates was

detected between trained and untrained Murray cod. On average it would appear that survival of trained Murray cod was twice that of untrained cod. If released near high densities of predators, survival of trained Murray cod could be up to 4 times that of untrained cod. Training of Murray cod fingerlings is therefore highly recommended for conservation stocking programs for this species. It is likely that this result could be transferable to other *Maccullochella* spp. also.

Contrary to expectation, predator free release cages did not confer any survival advantage to Silver perch or Murray cod. In the case of Murray cod these release cages actually seem to have been detrimental to survival. Hutchison *et al.* (2006) also found a similar negative effect for Golden perch *Macquaria ambigua* stocked into floating brush cover devices. Hutchison *et al.* (2006) speculated that predators may have gathered around the cover device and waited for the fingerlings to emerge.

Murray cod often drop to the bottom substrate when stocked. It is possible that the cod in our release cages did this too. Turbid conditions prevented any observations of cod behaviour in most release cages. The release cages were only 1.8m diameter and 650-700 cod were released per cage. It is probable that territorial interactions may have occurred between cod during the 90 minutes they were in the cage. This sparring could have weakened some fish or drawn the attention of predators, which could have been attracted to the area by the time the cage was lifted.

Schlechte and Buckmeier (2006) successfully used predator exclusion cages of a similar design to ours to improve post release survival of large mouth bass fingerlings in ponds pre-stocked with predators. Their cage design was 0.61 m in diameter, 1.22 m high and constructed from 3mm-mesh nylon netting with a flotation ring on the top. A lead line was sewn to the bottom of the netting to contour the net to the bottom so that fish could not leave the device prematurely. Our cages were larger in diameter (1.8 m), deeper (1.6 m) and made from slightly larger mesh (6 mm), but the design concept was the same, including the flotation ring on the top and leadline on the bottom. Schlechte and Buckmeier (2006) stocked 250 fingerlings into each of their exclusion devices, whilst we stocked 650 to 700 fish into each of our larger devices. Densities were therefore similar in both devices. It would appear that behavioural differences between species being stocked may alter the outcomes from predator exclusion cages.

GLMs of binomial proportions for both Silver perch data and Murray cod data selected predator index as a significant parameter explaining recapture rates of both species. Survival was higher in both trained and untrained groups if fish were released near a point with lower predator densities. It is not always possible to predict what predator abundances are going to be at any given release point. However it is clear that abundances vary throughout a site. We therefore recommend that for conservation stockings, more than one release point should be used to spread the risk. We suggest three or four release points be used to release large batches of fish. A large batch may have the advantage of helping to swamp predators such that reasonable numbers of fingerlings escape predation while acclimating to the receiving waters. Pre-release training should further enhance survival.

## Conclusions

Silver perch, Murray cod and Freshwater catfish fingerlings all showed some improvements in predatory fish avoidance behaviour following mass training. It would appear that the best results are achieved with at least 72 hours training in Silver perch and Murray cod. However 48 hours training was sufficient to produce some significant changes in the behaviour of Freshwater catfish. Whilst sub-adult/small adult Silver perch appear trainable with respect to predator avoidance and live food foraging, hatchery reared-sub-adult Murray cod do not respond well to training and should be avoided for conservation stocking programs.

Field trials confirmed training to be beneficial for the survival of stocked Murray cod fingerlings. Trained Murray cod fingerlings can be expected to survive better than untrained fingerling in locations with moderate to high predator densities. Predator free release cages appear to disadvantage cod fingerlings. Until alternative predator exclusion designs with proven results are developed, stocking of Murray cod fingerlings should be done directly into the receiving waters.

In contrast to tank based validation results, there were no significant differences detected between trained and untrained Silver perch stocked into the wild. One possible explanation is that Silver perch are a schooling fish. Rapid dispersal from the stocking sites and amalgamation into mixed schools may have led to rapid social learning of the untrained fish from the trained fish. Based on the laboratory results and the likelihood that social interactions confounded the field results, we recommend that pre-release training still be used when stocking Silver perch fingerlings for conservation purposes.

Predator free cages neither advantaged nor disadvantaged stocked Silver perch. Therefore it would appear to be acceptable to release Silver perch directly into the receiving water.

Predator abundance was a significant parameter influencing survival outcomes for both Murray cod and Silver perch. The patchiness of predator distributions within a site means it is appropriate to use several release points at a site, when stocking for conservation (or recreational) purposes to spread the risk. Large batches should be stocked at each release point to ensure some swamping of predators.

## **Key recommendations**

- Pre-release training for predatory fish avoidance should be used when stocking Murray cod fingerlings for conservation purposes.
- Pre-release training should be used when stocking Silver perch fingerlings for conservation purposes.
- To reduce predation risk several release points should be used at any given site. Fingerlings should be stocked in large batches at each release point.
- Until alternative predator exclusion designs with proven results are developed, stocking of Murray cod fingerlings should be done directly into the receiving waters.
- It is acceptable to release Silver perch directly into the receiving waters.
- Further research should be conducted into the influence of social learning on Silver perch predator avoidance, to determine what proportion of stock need training to benefit untrained fish released at the same time and location.

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