A review of domestication effects on stocked fishes, strategies to improve post stocking survival of fishes and their potential application to threatened fish species recovery programs in the Murray–Darling Basin

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Cover Image: VIE marked silver perch being released in an experimental stocking. Photo Michael Hutchison, Queensland Department of Agriculture, Fisheries and Forestry.

Introduction

A number of Australian native fish species in the Murray–Darling Basin 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 icon species such as Murray cod (*Maccullochella peelii)*, trout cod (*Maccullochella macquariensis)*, Macquarie perch (*Macquaria australasica)*, silver perch (*Bidyanus bidyanus)* and eel-tailed catfish (*Tandanus tandanus),* as well as small bodied species like the southern purple spotted gudgeon (*Mogurnda adspersa)* and the olive perchlet (*Ambassis agassizi)* (Murray–Darling Basin Commission 2004).

The Murray–Darling Basin Commission (now Murray–Darling Basin Authority) has developed a Native Fish Strategy (the Strategy) with the long-term goal of restoring native fish populations to 60% of their pre-European colonisation levels. One of the objectives of the Strategy is to devise and implement recovery plans for threatened fish species. Driving actions of the Strategy include rehabilitating fish habitat, protecting fish habitat, managing riverine structures (barriers to migration), controlling alien fish species, protecting threatened fish species and managing fish translocation and stocking (Murray–Darling Basin Commission 2004). Although all these actions are likely to have positive effects on the recovery of threatened fishes, in some catchments of the Basin these fish have already become locally extinct, or declined so drastically that carefully managed conservation stocking of hatchery-reared fish may become a necessary part of any recovery program.

If the driving actions of the Native Fish Strategy are successful, then reintroduced hatchery-reared threatened fish that survive should go on to produce self-sustaining populations. However, conservation stockings are not always successful. Much of this has been attributed to domestication effects of captive rearing. The basis of this review is to investigate why stocking of hatchery-reared fish is not always successful and how to improve the post-stocking survival of hatchery-reared fish. The review also includes an investigation of current hatchery practices in eastern Australia to determine likely domestication effects on threatened Murray–Darling Basin species.

Effects of hatchery domestication on fish

Fish stocking is widely used as a fisheries enhancement tool. In eastern Australia there have been experimental stockings of estuarine recreational species (Butcher *et al*. 2000; Taylor *et al.* 2007) and stocking has been used to create recreational fisheries for native species in impoundments (Hutchison *et al*. 2006; Simpson *et al*. 2002). Stocking is also used as a conservation tool to restore threatened fish stocks. Within Australia, hatchery-reared Mary River cod (*M. p. mariensis)* and trout cod have been stocked as part of the recovery programs for these species (Simpson & Jackson 1996; Lintermans & Ebner 2006).

However, it has been recognised for some time that stocking of hatchery-reared fish does not always deliver dramatic improvements in fish stocks (Blaxter 2000; Hutchison *et al.* 2006; Larscheid 1995). Recognition of poor post-release survival rates of hatchery-reared fish has been noted by fisheries scientists for over a century

(Brown & Day 2002). 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. They also noted the difference in mortality rates between hatchery-reared and wild fish is especially large when size and age are taken into account. Similarly, fish raised in hatcheries can exhibit behavioural deficits that influence their survival after release (Olla *et al*. 1994; Stickney 1994). A range of behavioural deficits have been recorded in hatchery-reared fish - these are explored in further detail in the current review.

Response to predators

Predation risk is an important factor that influences the survival of stocked fish. One key deficit in many hatchery-reared fish is their failure to recognise or respond appropriately to predators such as seeking refuge. Experiments by Alvarez and Nicieza (2003) showed second generation hatchery brown trout (*Salmo trutta)* and hatchery-reared offspring of wild brown trout were not sensitive to predation risk, whereas brown trout from natural populations reacted to the presence of a piscivorous fish by increasing their use of refuges. Hatchery bred trout were active in the daylight, regardless of predation risk, whereas wild fish shifted towards nocturnal activity in the presence of predators. Malavasi *et al.* (2004) compared the response of wild and hatchery-reared juvenile sea bass (*Dicentrarchus labrax)* to the presence of a predator, the European eel (*Anguilla anguilla)*. Schools of wild sea bass aggregated more quickly and reached greater shoal cohesiveness than hatchery-reared sea bass in the first 20 seconds after exposure to an eel. Similar results were obtained by Stunz and Minello (2001) who found that hatchery-reared red drum (*Sciaenops ocellatus)* were more susceptible to predation by pinfish (*Lagodon rhomboids)* than were wild red drum. Survival of wild red drum was higher in structurally complex habitats such as seagrass and oyster reefs than in open non-vegetated bottoms, but the habitat effect was not significant for hatchery-reared red drum. This suggests that hatchery-reared fish failed to use cover effectively.

Studies on Australian species demonstrate similar results when comparing wild and hatchery-reared stocks. Radio-tracking of hatchery-reared (310-429 mm total length (TL)) and wild (370-635 mm TL) trout cod (*Maccullochella macquariensis*) by Ebner and Thiem (2006) in a lowland reach of the Murrumbidgee River, revealed that even large hatchery-reared fish have much poorer survival rates than wild fish. After 13 months, 9% of the hatchery fish were alive, compared to 95% of the wild fish. A related study by Ebner *et al*. (2006) used radio-telemetry to follow hatchery-reared trout cod (330-424 mm TL), released into the Murrumbidgee and Cotter rivers in the upper Murrumbidgee catchment. After 7 months there was 100% mortality of individuals in the Cotter River and 86% mortality of individuals in the upper Murrumbidgee River. Ebner *et al*.(2006) presented evidence suggesting predation by cormorants (*Phalocrocorax carbo)* may have been one of the primary causes of mortality.

According to Johnsson *et al.* (1996), fish bred in hatcheries over several generations experience no selection by predators against risky foraging behaviour. This can lead to boldness in off-spring, or other inappropriate behaviours in the presence of predators. Huntingford (2004) also found that farmed fish are selected for traits such as rapid growth and that there is scope for unplanned natural selection for different behavioural phenotypes in a hatchery environment. The following examples support these ideas and some experiments demonstrate behaviours that may increase the risk of hatchery-reared fish to predation in external environments.

- Under chemically simulated predation risk, domesticated masu salmon (*Oncorhynchus masou)* were found to be more willing to leave cover and feed than were wild fish (Yamamoto & Reinhardt 2003).
- Wild Japanese flounder (*Paralicthys olivaceus)* were observed by Furuta (1998) to make rapid feeding movements, returning to the bottom near their starting point. In contrast hatchery-reared Japanese flounder spent more time in the water column and re-settled on the bottom farther from their starting point than wild flounder. Increased time in the water column may make hatchery-reared flounder more susceptible to predation
- Berejikian (1995) found steelhead fry (*O. mykiss)* reared from wild collected ova, in the same conditions as a hatchery derived fry, showed better survival against predation by prickly sculpin (*Cottus asper),* even though both groups were not exposed to predators during the rearing process.

Feeding

In some hatcheries fish are *fed a die*t of artificial pellets. However prolonged exposure to an artificial diet could potentially condition fish so that they fail to recognise natura*l or wild food*s or may alter foraging behaviour. In addition, if fish are conditioned to come to the surface to take pellets this could potentially make them more susceptible to bird predation once stocked. Several studies (outlined below) have examined some of the effects of hatchery domestication on feeding and foraging behaviour in fish.

The authors have observed that long-term pellet fed, tank reared Murray cod refuse live prey in preference for pellets. Research by Brown *et al.* (2003) compared the foraging behaviour of hatchery-reared Atlantic salmon, fed on pellets or live bloodworms in both standard hatchery tanks and habitat enriched tanks. When exposed to novel live prey, those fish reared in the enriched tanks and previously exposed to bloodworms showed the most enhanced foraging behaviour. However, Olla *et al*. (1994) state that many pellet reared fish readily switch to live prey food under laboratory conditions, a position supported by work of other authors. For example, Massee *et al*. (2007) found that juvenile sockeye salmon (*O. nerka)* reared either on pellets, *Artemia* (a common live prey fed to hatchery fish) or a combination of both, showed no significant difference in their ability to capture pellet, *Artemia* or mosquito larva prey. Massee *et al*. (2007) concluded there was no need to alter existing hatchery practices by providing live food to salmon prior to release. However, Olla *et al.* (1994) indicate that though fish in the laboratory often seem to be able to adapt to new diets, studies on released hatchery-reared fish showed they often experienced poor growth, low survival and consumed less food and fewer food types than wild fish. Results from Ersbak and Haase (1983) are illustrative of poor foraging success in hatchery fish when compared with wild counterparts. They found that resident brown trout were twice as successful as stocked brook trout (*Salvelinus fontinalis)* in obtaining food and that stocked brook trout condition declined post-stocking, while resident trout condition remained stable. The resident trout showed greater flexibility in switching to new prey items as they became available.

Poor foraging success in hatchery-reared fish may be explained by evidence of an onset of physiological changes in response to pellet diets. Norris (2002) found that whiting (*Sillago maculata)* reared on a diet of pellets developed physiological changes that caused an increase in the number of taste receptors (except in the gular region). In contrast, whiting fed a mixture of hatchery pellets and live food exhibited an initial increase followed by a slight decrease in taste bud density after 30-60 days. Taste bud densities were significantly higher in fully pellet fed whiting than in whiting that received live food.

Norris (2002) also explored other reasons for differences in feeding success including the influence of the duration a diet is fed (potential conditioning of fish to a diet) and the influence of chemical and visual stimuli on feeding. This work found that the time taken to locate prey was closely correlated to the length of time spent on a diet. Initially, there was no significant difference in the time taken to locate either pellets or live prey between fish from each diet group. After 30 days on specific diets, fish fed live prey were significantly faster at locating live prey, but there was no significant difference in the time taken to locate pellets. After 60 days on their respective diets, fish of both diets were significantly faster at locating the prey corresponding to their diet. After 120 days the time difference between locations of each prey type was again highly significant. Responses to chemical and visual stimuli were also tested using a series of transparent, opaque and perforated tubes. Results of this experiment suggested fish raised on a diet of live food relied more on visual stimuli than those raised on pellet foods, whereas fish reared on pellet foods relied more on olfactory cues than the live food group. This could have implications for survival of stocked fish.

Pellet diets not only influence feeding behaviour of stocked fish, they can also impact on other behaviours if the pellets are deficient in some nutrients. Koshio (1998) compared the behaviours of ayu (*Plecoglossus altivelis*), yellowtail (*Seriola quinqueradiata*) and red sea bream (*Pagrus major*) fed on diets containing different levels of ascorbic acid. Fish raised on diets containing 480 mg kg^{-1} (or more) ascorbic acid showed behaviours most like those of wild fish. For example ayu displayed more territorial behaviour, yellowtail had higher schooling rates and red sea bream displayed greater frequencies of predator avoidance tilting-behaviour, than fish fed on low, or no ascorbic acid diets.

The practice of feeding artificial food is common for rearing of trout fingerlings in Australia, but in Australian native fish hatcheries this would appear to be more common only in hatcheries or grow-out facilities that rear fish to large sizes (see section on hatchery practices below. Under these conditions, fish may exhibit domestication effects on feeding and foraging behaviour that need to be overcome to increase their chances of survival upon stocking.

Movements and other ecological deficiencies

Other than predator avoidance and feeding behaviour, there are other differences between hatchery-reared and wild fishes that could influence survival in the wild. Some of these additional traits are discussed below.

According to Petersson and Jaervi (1999), sea ranched salmonids of hatchery origin differ from wild fish in a number of ways. They grow faster in a hatchery, but are less afraid of predators, have lower survival, are less aggressive, have poorer mating success and different migration patterns when released in the wild compared to wild fish. Dispersal patterns also appear to differ between wild and hatchery-reared fish. A radio-telemetry study showed hatchery-reared sub-adult trout cod in the Murrumbidgee River were found to have different dispersal patterns to wild trout cod in the same area (Ebner & Thiem 2006). A similar result was obtained in a radiotelemetry study of rainbow trout (Bettinger & Bettoli 2002). Rainbow trout stocked in a tailrace in the Clinch River, Tennessee dispersed rapidly with 93% of the stocked fish either dying or emigrating from the tail race. In comparison resident rainbow trout persisted longer and were less active. The rapid long range movements of the stocked fish were energetically inefficient and probably exposed them more to predation.

Some fish species display territorial behaviour. The ability of a fish to maintain a territory can be important for their survival. This could be particularly relevant to outcomes for trout cod and Murray cod. Metcalfe *et al*. (2003) studied outcomes of territorial interactions between wild and hatchery-reared Atlantic salmon. They found that although Atlantic salmon originating from hatcheries were more aggressive than wild fish, the hatchery environment reduced their ability to compete for territories with wild resident fish. Wild resident fish also out-competed wildorigin fish that were hatchery-reared. Further examples of hatchery-origin fish suffering in competition with wild fish are provided by Olla *et al*. (1994).

Minimising domestication effects and other strategies to improve post stocking survival

The evidence presented above suggests that in most cases hatchery-reared fish have a number of deficits that may impair their survival post-release into the wild. However, research has begun to examine whether some of the hatchery domestication effects can be reduced prior to stocking. Conservation biologists have long recognised the importance of conditioning captive bred mammals prior to release and using soft release strategies to improve post-release survival (Brown & Day 2002). One approach used with Australian native mammal (and bird) reintroductions has been controlling introduced predators prior to reintroduction. For example, foxes (*Vulpes vulpes)* were controlled prior to and post-reintroduction of yellow-footed rock wallabies (*Petrogale xanthopus celeries)* into south-western Queensland (Lapidge 2003). Foxes were also controlled to enhance survival of released captive-reared malleefowl (*Leipoa ocellata)* (Priddel & Wheeler 1997).

Localised removal of exotic predators like redfin perch (*Perca fluviatilis*) by electrofishing immediately prior to stocking threatened fishes could be a possible strategy to employ in the Murray–Darling Basin. However, most predators of native fish in the system are going to be native fish and birds. Removal of native predators would be considered unethical and may have unintended side-effects. Conditioning hatchery-reared fish for survival in the wild is therefore a more appealing option.

There are numerous examples of pre-release conditioning and training of captive mammals and birds. In a review of the reintroduction of captive born animals to the wild, Beck *et al.* (1994) found that 36% of projects involving mammals and 48% of projects involving birds had undergone some type of pre-release training. Beck *et al.* (1994) also found that 82% of the mammal projects and 83% of the bird projects had used some type of acclimatisation to the site, before full release.

Primates have been trained to learn to orientate in vegetation and to forage for natural foods (Box 1991). In Australia, brush-tailed phascogales (*Phascogale tapoatafa)* were trained to forage for food prior to release. Meal worms were hidden under bark and in holes drilled into logs and branches. Phascogales were also provided with moths, crickets and dead mice (Soderquist & Serena 1994). Black-footed ferrets (*Mustella nigripes)* have been trained to hunt in outdoor enclosures (Miller & Vargas 1994) and reared in large outdoor cages to allow pre-release conditioning in prairie dog burrows (Biggins & Thorne 1994). Masked bobwhite quails (*Colinus virginianus ridgwayi)* have been deliberately harassed by humans, dogs and hawks and permitted to escape to condition them with a fear of predators (Carpenter *et al.* 1991).

Kleiman (1989) states there are six main areas to consider when developing pre-release training programs for mammals. They are:

- predator avoidance
- acquisition and processing of food
- proper interaction with conspecifics
- finding shelter or constructing nests
- locomotion through complex terrain
- orientation and navigation in a complex environment.

Kleiman (1989) also states training for fear of humans is important. Most of these areas have potential to be applied in some way to fishes. Brown and Day (2002) recommend applying conservation biology techniques like these to fish stock enhancement programs. Kleiman (1989) recommends pairing captive reared animals with wild caught individuals to assist with life-skills training after using this technique with golden lion tamarins (*Leontopithecus rosalia)*. Carpenter *et al.* (1991) paired masked bobwhite quails with a related wild subspecies to enhance their food finding and predator avoidance training.

Can fish learn?

For training or acclimation programs to work for fish, fish must have a capacity to learn. Recent research supports the concept that fish can learn. Hughes *et al.* (1992) and Warburton (2003) found evidence that fishes can optimise foraging behaviour through learning. Mosquitofish (*Gambusia affinis)* can learn to orientate to avoid predation (Goodyear 1972). Brown (2003) suggests that many prey species do not show innate recognition of potential predators and that such knowledge is acquired through the pairing of alarm cues with the visual and/or chemical cues of the predator. For example, Brown *et al*. (1997) demonstrated that a population of 80,000 flathead minnows in a 4 ha pond, learned to recognise the chemical cues of northern Pike within 2 to 4 days.

Social learning (learning from conspecifics) may be important in some fish species. Brown and Laland (2003) reviewed research into social learning in fish and they presented unequivocal evidence for social learning For example, predator avoidance behaviour, migration, orientation and foraging can all involve social learning. In particular, social learning of predator avoidance is apparently widespread among fish. 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. However, it is of interest to note that species with a similar morphology and ecology can express different predator avoidance behaviour, resulting in different survival rates (Nannini & Belk 2006). Brown and Laland (2001) in a review of social learning and life skills training in hatchery fishes provided ample evidence of predator naïve fish being able to rapidly acquire predator avoidance skills with training. They are strong advocates for using life-skills training techniques.

Avoiding early predation is critical

Most mortality occurs immediately after stocking, *i.e* .in the first few days, rather than first few weeks (Sparrevohn & Stoetrupp 2007, Brown & 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 hatchery-reared barramundi (*Lates calcarifer)* fingerlings into an impoundment. Gut contents of predators were examined for batch tagged fingerlings. Hutchison *et al.* (2006) found that variation in 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. This suggests that if fish are able to survive the early stages of stocking, they have a much better chance of surviving to adult size. Predator naïve fish that survive the first day or two probably acquire predator avoidance behaviours. If fish already have predator avoidance behaviours at the time of stocking, then perhaps survival can be enhanced.

Predator avoidance and recognition training.

One of the earliest attempts to train hatchery-reared fish to avoid predators was by Fraser (1974). Fraser used an electrified plastic model loon (a type of predatory bird) moving through a hatchery raceway to train brook trout fingerlings to avoid loons. The experiment was repeated over two different years. In 1970 fish were trained for 9 days and in 1972 fish were trained for 8 days. An untrained control group were maintained in an adjacent raceway. Following training, both groups of fish were released into a small lake. Intensive post-stocking sampling showed no significant difference in survival between the two groups. Mean survival of trained fish and untrained fish was estimated to be 16% and 18% respectively. Fraser attributed the failure of the training technique to the fact that fish only learned to move aside from the model some 50 cm to avoid being shocked. This response would not protect the fish from a real predator, as a real predator would turn and chase its prey.

Training of fingerlings to avoid fish predators has been more successful than Fraser's attempt at bird recognition training. Model predatory fish were used to train Nile tilapia (*Oreochromis niloticus)* by associating the model predator with a negative stimulus (simulated capture with an aquarium net) (Mesquite & Young 2007). After 12 training sessions the conditioned tilapia expressed a new anti-predator response, but untrained control fish responded to the model predator as a novel object. Whether or not the responses to the model by trained fish led to appropriate responses in the presence of a real predator was not tested. Järvi and Uglem (1993) in a lab-based experiment trained Atlantic salmon (S. Salar) smolts using two techniques non-contact and contact training. Non-contact training exposed smolts to a predator (cod [*Gadus morhua*]) through transparent netting whereas contact training exposed smolts to a free roaming predator. Järvi and Uglem (1993) were also interested in physiological stress interactions between adaptation to seawater during the smolt migration, response to, and prior experience of predators. Considering only the predator response behaviours, they found that the predator naïve smolts behaved less appropriately towards predators than the two trained smolt groups and the non-contact trained smolts behaved less appropriately than the contact trained smolts. In contrast, Hawkins *et al*. (2007) found no difference between survival of predator–exposed and predator–naïve *S. salar* smolts released into the Bran River, Scotland. They attributed their findings to the overriding migratory behaviour of smolts.

Brown (2003) stated prey fishes are known to possess chemical alarm cues. Chemical alarm cues, when detected by conspecifics or heterospecifics elicit a variety of overt and covert responses. These cues alone or as part of a predator's prey item's odour can provide reliable information on predation risk. Vilhunen (2006) conditioned hatchery-reared Arctic charr (*Salvelinus alpinus)* to odours of Arctic charr-fed pikeperch (*Sander lucioperca).* Arctic charr exposed just once showed improved predator avoidance behaviour relative to unexposed control fish, and Arctic charr exposed to odours four times did not become conditioned to the odour, but rather improved their anti-predator response.

Ferrari and Chivers. (2006) conditioned predator naïve fathead minnows (*P. promelas)* six times to brook char (*S. fontinalis)* odour paired with either high or low concentration alarm cues. Alarm cues were derived from fathead minnow pulverised skin extract. The intensity of the minnows' anti-predator response to char odour was related to the concentration of their last alarm cue exposure.

Alarm cue odours appear to be an effective conditioning agent. Pairing of a novel odour (lemon essence) with a damage released alarm cue showed a significant increase in alarm responses to lemon odour in Atlantic salmon (Leduc *et al*. 2007). The odour sensitivity of fish to alarm cues and predators is quite remarkable. For example, the predator avoidance response varies according to the intensity of alarm cues (Brown *et al.* 2006) and intensity of predator odours (Ferrari *et al* 2006a). Fish can even distinguish odours of individual predators of the same species to determine density of predators (Ferrari *et al*. 2006b). Recognition of predator odours and alarm cues could be very important in turbid environments such as those in the Murray–Darling Basin. Odour cued training may therefore be a useful methodology.

Prey species are not only cued by odours, they may also be cued by visual and vibration stimuli. Mikheev *et al*. (2006), found that in daylight hours perch (*Perca fluviatilis)* relied more on visual cues to respond to predatory pike. Olfactory cues enhanced the effects of the visual cues. A number of researchers have used visual cues for training of fish. Berejikian (1995) visually exposed steelhead fry (both wild origin hatchery-reared and hatchery origin hatchery-reared) to predation of sacrificial steelhead fry by sculpin (*Cottus asper),* for 50 minutes. Berejikian then compared the response of trained wild origin hatchery-reared and hatchery origin hatchery-reared fry and their respective control groups to direct exposure to sculpin. Wild origin trained fry survived the best, followed by wild origin naïve fry, hatchery origin trained fry and hatchery origin naïve fry. This result suggests that there may be a hereditary component to the outcome, but survival can still be improved with training.

Direct but controlled exposure to predators is another training technique. Olla and Davis (1989) exposed groups of 12-14 coho salmon juveniles (*O. kisutch)* to predation by ling cod (*Ophiodon elongatus)* in a 5 m diameter plastic lined pool. After 60 minutes, surviving coho salmon were removed and held in a 700 litre tank. After five days these surviving fish were mixed with predator naïve fish then exposed to predation by ling cod as before. The pre-trained fish had better predator avoidance rates than the naïve fish. Olla and Davis (1989) also found that just two 15 minute exposures to predators could improve survival relative to naïve fish.

Arai *et al.* (2007) compared two training techniques for Japanese flounder (*Paralichthys olivaceous*). They were direct exposure to predators and allowing juvenile flounder to observe predation of conspecifics. In subsequent tests both predator-exposed and predator-observed fish were better at avoiding predators than predator naive control fish. Reaction distance was greatest in predator exposed fish, followed by predator observed fish, then predator naïve fish. It would appear that observational learning of predation is an effective training technique, but direct exposure is more effective.

Fish may also cue their predator responses from the behaviour of conspecifics. Mathis *et al.* (1996) showed that pike (*Esox lucias)* experienced fathead minnows (*Pimephales promelas)* were able to transfer predator fright responses from pike chemical stimuli to predator-naïve minnows. They were also able to transmit a fright response interspecifically to predator naïve sticklebacks (*Culaea inconstans)*. In the absence of pike-experienced minnows, naïve fish did not show a fright response to pike. This suggests that for some species, wild predator-experienced conspecifics or predator-experienced individuals of different species could be used to assist in predator awareness training of hatchery-reared fishes.

Training for foraging

Norris (2002) found that the longer whiting (*Sillago maculata)* were maintained on a particular diet, the faster they were able to locate that food item. 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) showed that combining habitat enrichment in a tank with exposure to live food prior to release enhanced the ability of Atlantic salmon parr to generalise from one wild prey type to another. Fish exposed to live food in standard hatchery tank conditions, pellet feeds in standard tanks and pellet feeds in habitat enriched tanks were less able to generalise from one prey to another. However the pellet reared fish from the enriched environment were the next most successful. It is not certain how hatchery enrichment helps, but it appears to induce more natural behaviour in fish and may enhance learning by providing greater sensory feedback to the brain (Brown *et al*. 2003).

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. Perhaps wild conspecifics could be used to assist with social training in foraging behaviour.

Semi-natural rearing

Habitat enrichment has already been noted above as important for fish to enhance their ability to generalise from one prey item to another. Semi-natural rearing techniques have been shown to assist in producing fish better equipped to survive in the wild.

Olson *et al.* (2000) compared the survival of intensively reared large (120-140 mm) walleye (*Stizostedium vitreum)* fingerlings and extensively reared small (30-50mm) fingerlings stocked into four small lakes. Intensively reared fish were raised in hatchery tanks, beginning on a diet of brine shrimp, then switching to pellets. Extensively reared fish were raised in 0.3 to 0.5 ha earthern ponds and fed on zooplankton species. Relative survival varied between the lakes. In two lakes survival of pond reared fingerlings was better than the larger intensively reared fish. Pond reared fingerlings were actually larger than the intensively reared fingerlings by the end of the growing season. This experiment was possibly confounded by different release times of the large and small fingerlings, but these timings reflected when the fish were available.

McKeown *et al.* (1999) examined the interaction between stocking size and rearing method on post-stocking survival of Muskellunge (*Esox masquinongy)* in New York State. Greater length at stocking led to better survival, but after accounting for length at stocking, pond reared fish had better survival than trough reared-pond finished fish, which in turn had better survival than totally trough reared fish.

Conservation stockings for North American salmonids are moving towards semi-natural rearing and away from intensive rearing methods. Fuss and Byrne (2002) compared survival from smolt stage to adult, for coho salmon extensively reared in a semi-natural rearing pond, containing large woody debris and rock. Density of fish in the pond was only 5% that of fish reared in conventional hatchery ponds. Survival of smolts to adult stage was higher in fish reared in semi-natural ponds, than by conventional methods. However, according to Fuss and Byrne (2002) the increased survival did not offset the increased adult yield that would have been realised from standard hatchery production techniques. Nevertheless Fuss and Byrne (2002) did agree that semi-natural rearing methods would have value for fish to be released in recovery programs.

In a review of rearing techniques for Pacific salmons, Maynard et al. (2004) found that semi-natural rearing techniques such as providing natural substrates, structure and overhead cover usually improves survival. They also found that supplementing diets with live food often enhances the ability of fish to hunt live prey. The authors concluded that in most cases conditioning to predators improves post release survival.

Acclimatisation and habituation to release sites

In tank based experiments, 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. Experimental tanks were divided into two open water quarters and two habitat enriched quarters containing either potted willows or limestone rocks. Survival of fish released into open water did not differ significantly from survival of fish released into cover, but fingerlings spent most time in the cover environments, whether released into open water or not. Fish that entered open water were often harassed by predators. Holding fish in predator free cages for more than 15 minutes did not improve survival any further, but all habituation periods produced better survival relative to non-habituated fish.

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. Use of predator free enclosures may improve survival by giving stocked fish time to overcome transport and handling stress. It may also give fish time to become aware of their surroundings and the chemical cues within.

Schlechte and Buckmeier (2006) tested the hypothesis that stocked largemouth bass placed in a setting that protects them from predators may become conditioned to natural stimuli and experience an improved ability to avoid predation. They also tested whether ability to avoid predation may be further enhanced by availability of suitable habitat. Experiments were conducted 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. Schlechte and Buckmeier (2006) concluded the complex natural cover provided a refuge from predators in the same way as exclusion cages and enabled them to habituate in those environments. However Hutchison *et al.*(2006) found that releasing barramundi , Australian bass (*Macquaria novemaculeata),* golden perch (*Macquaria ambigua)* or silver perch (*B. bidyanus)* fingerlings into "floating" artificial cover in impoundments provided no significant improvement to survival. Hutchison *et al.* (2006) did not evaluate the effect of natural cover releases.

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) cites further examples of the benefits of habituation or acclimatisation at release.

Other strategies

Size at release

Size at release has been shown by many researchers to have a significant influence on survival. Hutchison *et al.* (2006) showed that fingerlings of barramundi, Australian bass, golden perch or silver perch 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 waterbody. For example, in the absence of spangled perch, 35-45 mm silver perch fingerlings survived almost as well as 50-65 mm fingerlings. Based on prevailing hatchery prices at the time of their experiments Hutchison *et al.* (2006) concluded that the 50-65 mm fingerlings were the most cost effective to stock in impoundments with high densities of predators.

Similar conclusions have been reached for stocking experiments conducted with other species. For example larger size at stocking has been linked to increased survival in red drum (*Sciaenops ocellatus)* (Willis *et. al.* 1995), whitefish *(Coregonus lavaretus)* (Jokikokko *et al*. 2002), largemouth bass (*M. salmoides)* (Miranda & Hubbard 1994), mullet (*Mugil cephalus)* (Leber & Arce 1996), lake trout (*Salvelinus namaycush)*, (Hoff & 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 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[1]; Pearce*,* pers comm[2].). However, the experience of Ebner and Thiem (2006) and Ebner *et al.*(2006) with poor survival of large hatchery-reared trout cod suggests that hatchery domestication can have the potential to remove the advantages of large size-at-release. Similarly Koike *et al*. (2000) had better returns for masu salmon stocked in spring as 0+ fry, compared to larger 0+ parr stocked in autumn and 1+ smolts stocked in spring. Stocking of fertilised eggs had the poorest success rate.

Spreading the risk

Hutchison *et al.* (2006) found that chance distribution of schools of predators at the time of stocking can impact on the outcomes of stocking. They suggested stocking large batches of fish in several locations (scatter stocking) to spread the risk, rather than releasing all fish in a single location. Single location stocking (point stocking) could result in success or could result in heavy mortalities. Cowx (1999) has also advised against point stocking and has advocated trickle stocking through frequent planting of small numbers of fish throughout the water body. Cowx (1994) also suggests scatter stocking has better outcomes than point stocking.

Minimising transport stress

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: Carneiro & Urbinati 2001). Fish should be starved 24 hours before transportation, to reduce oxygen demand and ammonia build up during transport (Cowx 1994). It is important not to withhold feed longer than this as it 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, transport water should be gradually mixed with water from the receiving environment to equilibrate temperatures and water chemistry to avoid shocking the fish (Simpson *et al.* 2002).

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Timing of release and release location

Various researchers have experimented with timing of release and release location to improve survival. Hutchison *et al*. (2006) recommend stocking fingerlings as early as possible in order to take advantage of the spring and summer growing season. Fingerlings stocked late, at the onset of the winter low growth period, are likely to remain small for several months, and are therefore more susceptible to a wider range of predators for a longer period than fish stocked in warm months. Other researchers have also suggested stocking early in the season improves chances of survival (Sutton *et al*. 2000; Leber *et al.* 1997; Leber *et al*. 1996).

Hutchison *et al.* (2006) found that four species of Australian native fish stocked during low water levels had poorer survival than when stocked at high water levels. This was attributed to less available cover, less available prey and predators being more highly concentrated in the reduced volume of water. Studies of recruitment of reservoir sport fish species in the USA have positively correlated recruitment to the fishery with water level when the fish were age 0 (Sammons & Bettoli 2000; Sammons *et al.* 1999). This is consistent with poorer survival during low water levels.

Stocking the right habitat may also be important. Schlechte and Buckmeier (2006) have suggested stocking largemouth bass into complex cover can improve their survival. Survival of mullet (*M. cephalus)* was enhanced when mullet fingerlings were stocked into freshwater streams rather than bay environments (Leber *et al.* 1996). Freshwater is generally used as a nursery habitat by mullet. Russell and Rimmer (1997) found survival of stocked barramundi to be higher if released into floodplain wetland habitats rather than directly into the main river. Elrod (1997) found that releasing lake trout fingerlings offshore in Lake Ontario, rather than near shore, improved survival. He suggested offshore stocked fish were less susceptible to predation by double crested cormorants (*Phalacrocorax auratus).* In contrast Australian bass fingerlings were found to have lower survival if stocked in deep water, rather than in shallow water areas or artificial cover (Hutchison *et al*.2006). This was attributed to large schools of predatory adult bass being present in the open waters of the impoundment experiment sites.

Taylor *et al.* (2007) conducted telemetry studies of stocked and wild mulloway (*Argyrosomus japonica)*. They recommended stocking mulloway into their preferred habitat of deep estuarine holes and basins to minimise movements. They also recommend stocking densities should be based on estimated area of key habitat in the estuary to improve survival. To a large extent stocking location and timing should be based on knowledge of the ecology of the species being stocked and the predator and prey composition of the receiving waters.

Practices of hatcheries and grow-out facilities producing Murray–Darling Basin threatened fish species

A questionnaire was sent to 84 private and government hatcheries and grow-out facilities in eastern Australia that produce fish native to the Murray–Darling Basin (see appendix A). Hatcheries hold brood-stock fish for production of fingerlings for stocking, the aquarium trade or to supply grow-out facilities. Grow-out facilities on-grow fish to larger sizes, either for the aquarium trade or for human consumption and occasionally for stocking. Some grow-out facilities may have their own hatchery, but most rely on other hatcheries to supply them.

The questionnaire was designed to determine the degree of domestication of threatened Murray–Darling Basin fish species produced in hatcheries and grow-out facilities in south-eastern Australia. This was to enable planning for appropriate experiments designed to reduce potential hatchery and grow-out domestication effects in Murray–Darling Basin species.

Responses were received from 26 (31%) of the facilities contacted. The responses were from 10 grow-out facilities and 16 hatcheries. Predation by fish (excluding cannibalism in cod) was reported by 6.25% of hatcheries and by 0% of grow-out facilities. The rare instance of predation by fish was related to invasion of ponds by eels. Exposure to fish predators (excluding cannibalism) was therefore rare or absent.

Predation by birds (cormorants, herons, pelicans) was reported as common by 37.5% of hatcheries and 40% of grow-out facilities. Predation by birds was reported as rare by 37.5% of hatcheries and 10% of grow-out facilities. No predation by birds was reported by 12.5% hatcheries and 50% of grow-out facilities. Bird control was practised by 50% of hatcheries and 20% of grow-out facilities. Bird control was not necessary in grow-out facilities where fish were maintained indoors in tanks.

None of the respondents produced Macquarie perch and only two hatcheries produced trout cod. The main threatened species produced were silver perch, Murray cod and eel-tailed catfish. Tables 1 to 9 summarise the feeding and rearing regimes for these species and their degree of exposure to predators. Trout cod data are not presented, but they are essentially reared in the same way as Murray cod. Among the respondents, three hatcheries and two grow-out facilities indicated that they produce catfish, the grow-out facilities producing catfish did not complete all questions on this species. Therefore only the hatchery data is presented in this paper for that species.

Table 1: Rearing facilities used for Murray cod by hatcheries and grow-out facilities. (Hatcheries n=8 Grow-out facilities n=5)

	Pond	**Tank**	**Both**
Grow-out	20%	100%	20%
Hatchery	87.5%	37.5%	25%

Table 2: Feeding regime for Murray cod used by hatcheries and grow-out facilities. (Hatcheries n=8 Grow-out facilities n=5)

	Pellet	**Live**	**Both feeds**
Grow-out	100%	0%	0%
Hatchery	25%	87.5%	12.5%

Table 3: Exposure of Murray cod to predation in hatcheries and grow-out facilities. (Hatcheries n=8 Grow-out facilities n=5)

	Cannibalism	**Fish**	**Bird**
Grow-out	40%	0%	20%
Hatchery	62.5%	0%	87.5%

Table 4: Rearing facilities used for silver perch by hatcheries and grow-out facilities. (Hatcheries n=8 Grow-out facilities n=7)

	Pond	**Tank**	**Both**
Grow-out	85.7%	14.3%	0.%
Hatchery	100%	12.5%	12.5%

Table 5: Feeding regime for silver perch used by hatcheries and grow-out-facilities. (Hatcheries n=8 Grow-out facilities n=7)

	Pellet	**Live**	**Both feeds**
Grow-out	85.7%	14.3%	14.3%
Hatchery	75%	100%	75%

Table 6: Exposure of silver perch to predation in hatcheries and grow-out facilities. (Hatcheries n=8 Grow-out facilities n=7)

	Fish	**Bird**
Grow-out	0%	57%
Hatchery	12.5%	100%

Table 7: Rearing facilities used for eel-tailed catfish by hatcheries (n=3)

	Pond	**Tank**	**Both pond & tank**
Hatchery	100%	0%	0%

Table 8: Feeding regime for eel-tailed catfish used by hatcheries (n=3)

	Live	**Pellet**	**Both feeds**
Hatchery	100%	0%	0%

Table 9: Exposure of eel-tailed catfish to predation in hatcheries.

	Fish	**Bird**
Hatchery	0%	100%

A common trend across all species is that hatchery-reared fish tend to be produced in ponds and exposed to live foods. There is also exposure to predation by birds. Excluding cannibalism in cod, there is virtually no exposure to predation by fish. All grow-out facility reared silver perch and cod are pellet fed. The bulk of grow-out facility reared cod are tank reared and not exposed to predation by birds. In contrast silver perch are pond reared and just over half are exposed to birds.

It is probably not necessary to train hatchery-reared fish to take live foods, as all are exposed to these foods. Most fish are also exposed to some level of bird predation, but approximately half the hatcheries that produce these fish only encounter birds rarely or not at all. Therefore there may be some merit in bird training for some of these fish. As most fish are not exposed to fish predation (excluding cannibalism) then predatory fish training can be expected to be of benefit.

Grow-out facility reared fish would probably benefit from live food training. As grow-out facility produced fish are large, they would most likely not be susceptible to predation by fish post–stocking, unless very large Murray cod were present. However the work of Ebner *et al*. (2006) suggests that fish stocked at large sizes can still be vulnerable to predation by birds.

Four businesses indicated they would be willing to undertake training of fish before supplying for stocking, two businesses indicated they were unwilling to provide training and the remaining twenty respondents were undecided. The majority of respondents could not decide whether it was more appropriate for hatcheries or government agencies to train fish prior to stocking.

Conclusions

Hatchery rearing of fishes can lead to domestication effects that may contribute to poor survival relative to wild fish. Pond or extensively reared hatchery fish are likely to have better survival post-release than intensively tank-reared fish, although in many cases the increased size of the tank reared fish may confer on them a survival advantage.

Taking into account the effect of stocking size and the type of hatchery rearing, further improvements in post-stocking survival can probably be made through pre-release predator awareness training, live food foraging training and pre-release tank or pond habitat enrichment. Minimising transport stress and acclimation or habituation at time of release can also improve the ability of newly stocked fish to avoid predation. Release into complex natural cover (if present) rather than into open water may also enhance post-release survival in some cases.

It is recommended the above techniques be used in threatened fish conservation stocking programs in the Murray–Darling Basin, with priority being given to piscivorous fish awareness training prior to release of fingerlings. There may also be some benefit in training fingerlings to recognise piscivorous birds. However, at least half of all hatchery-reared fish have frequent bird exposure. Therefore bird training may not always be necessary. We believe controlled exposure of hatchery-reared fish to predatory fish combined with alarm cues derived from pulverised skin extract of conspecifics is the method most likely to achieve improved predator response. Such methods could relatively easily be implemented by hatcheries.

In cases where large grow-out facility reared fish are to be released in the wild, they would probably benefit from pre-release live food foraging training. Most grow-out facility fish are probably too large to be taken by the majority of predatory fish. However, they could still be taken by predatory birds, especially cormorants and pelicans. We recommend pre-release predatory bird training for grow-out facility reared fish through a combination of controlled predator exposure and use of alarm cues. Reducing transport stress and including habituation at release would probably be of benefit to both fingerlings and larger fish.

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Appendix 1: Hatchery Questionnaire

Hatchery Questionnaire

Hatchery or aquaculture facility name ______________________________

Address____

____

Government or Private hatchery? _________________________________

Please complete the following questionnaire by writing short answers in the boxes provided. Leave blank boxes that don't apply to your hatchery operation.

1. Do you rear or produce any of the following species?

Species	**Yes/No**
Murray cod (*Maccullochella peelii peelii*)	
Trout cod (*Maccullochella macquariensis*)	
Macquarie perch (*Macquaria australasica*)	
Silver perch (*Bidyanus bidyanus*)	
Eel tailed catfish (*Tandanus tandanus*)	

2. Do you normally produce these fish for stocking programs, on-sale to other producers for growout, aquarium trade or human consumption (tick appropriate boxes)?

Species	**Stocking**	**On-sale**	**Aquarium trade**	**Human consumption**
Murray cod (*Maccullochella peelii peelii*)				
Trout cod (*Maccullochella macquariensis*)				
Macquarie perch (*Macquaria australasica*)				
Silver perch (*Bidyanus bidyanus*)				
Eel tailed catfish (*Tandanus*				

tandanus)				

3. If you produce any of the above species, what is the maximum size in mm to which you normally rear these fish before sale

Species	**Maximum size mm (stocking)**	**Maximum size mm (other)**
Murray cod (*Maccullochella peelii peelii*)		
Trout cod (*Maccullochella macquariensis*)		
Macquarie perch (*Macquaria australasica*)		
Silver perch (*Bidyanus bidyanus*)		
Eel tailed catfish (*Tandanus tandanus*)		

4. Do you normally pond rear or tank rear your post larval fish? Please tick the appropriate box.

Species	**Pond**	**Tank**	**Both**
Murray cod (*Maccullochella peelii peelii*)			
Trout cod (*Maccullochella macquariensis*)			
Macquarie perch (*Macquaria australasica*)			
Silver perch (*Bidyanus bidyanus*)			
Eel tailed catfish (*Tandanus tandanus*)			

5. Are your post-larval fish normally reared on commercial pellet feeds or on live food? Tick appropriate box.

Species	**Pellet**	**Live food**	**Both**
Murray cod (*Maccullochella peelii peelii*)			
Trout cod (*Maccullochella macquariensis*)			
Macquarie perch (*Macquaria australasica*)			
Silver perch (*Bidyanus bidyanus*)			
Eel tailed catfish (*Tandanus tandanus*)			

6. If your fish are reared on live food please provide details of type of live food here.

____

____

7. Are your fish subject to any predation by other fish species? Leave blank for species you don't produce.

Species	**Predation by other fish**	
	Yes	**No**
Murray cod (*Maccullochella peelii peelii*)		
Trout cod (*Maccullochella macquariensis*)		
Macquarie perch (*Macquaria australasica*)		
Silver perch (*Bidyanus bidyanus*)		
Eel tailed catfish (*Tandanus tandanus*)		

Comments:

____

____

8. If your fish are preyed on by other species of fish, what are the main predatory species involved? Tick the appropriate boxes.

Species produced	**Fish predators**				
	Spangled perch	**Redfin perch**	**Golden perch**	**Murray cod**	**Other fish**
Murray cod (*Maccullochella peelii peelii*)					
Trout cod (*Maccullochella macquariensis*)					
Macquarie perch (*Macquaria australasica*)					

Silver perch (*Bidyanus bidyanus*)					
Eel tailed catfish (*Tandanus tandanus*)					

Comments

____

____

9. Are your fish subject to predation by birds?

Species	**Predation by birds**		
	Yes common	**Yes rare**	**No**
Murray cod (*Maccullochella peelii peelii*)			
Trout cod (*Maccullochella macquariensis*)			
Macquarie perch (*Macquaria australasica*)			
Silver perch (*Bidyanus bidyanus*)			
Eel tailed catfish (*Tandanus tandanus*)			

10. If your fish are subject to predation by birds what species are involved?

Species	**Bird predators**			
	Cormorants or shags	**Herons or egrets**	**Pelicans**	**Other birds**
Murray cod (*Maccullochella peelii peelii*)				
Trout cod (*Maccullochella macquariensis*)				
Macquarie perch (*Macquaria australasica*)				
Silver perch (*Bidyanus bidyanus*)				

Eel tailed catfish (*Tandanus tandanus*)				

10. Do you have protective measures in place to prevent bird predation?

Yes/No (circle one)

Comments

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____

11. Would your hatchery be willing to introduce measures for training fish to recognise predators prior to stocking. This would only apply to conservation stockings. Measures developed are intended to be simple, involve minimum losses and have a short time duration.

Yes/No/Undecided (circle one)

12. Would you prefer conservation or fisheries agencies to undertake the measures to improve predator avoidance or would you prefer it be done at your hatchery?

Agencies/Hatchery/Undecided (circle 1)

Thank you for your time.

Please return this questionnaire in the enclosed stamped envelope or by e-mail if you received the questionnaire electronically.