Enhancement of Mulloway (*Argyrosomus japonicus*) in Intermittently Opening Lagoons

D. Stewart Fielder, William J. Bardsley and Geoff L. Allan

NSW Fisheries
Port Stephens Research Centre
Taylors Beach Road
Taylors Beach, NSW, 2316, Australia

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Non-technical summary

95/148 Enhancement of Mulloway (*Argyrosomus japonicus*) in Intermittently Opening Lagoons

Principal Investigator: Mr Stewart Fielder
NSW Fisheries
Port Stephens Research Centre
Taylors Beach Road
Taylors Beach, NSW, 2316

Tel: (02) 4982 1232 Fax: (02) 4982 1107
Email: fielders@fisheries.nsw.gov.au

Objectives:

1 to evaluate the production of mulloway juveniles using intensive and extensive techniques.

2 to stock two intermittent lagoons with some 50,000 juvenile mulloway.

Mulloway, *Argyrosomus japonicus* (formerly *A. hololepidotus*) is an economically important species targeted by commercial and recreational fishers in all Australian states except Tasmania. In New South Wales legal size limits have been introduced to increase protection of the fish stocks. Commercial catches in New South Wales have declined from 154 t in 1992/93 to 88 t (value $640,000) in 1997/98 (ABARE, 1995; 1998). Consequently, interest in the development of techniques for the production of mulloway to enhance wild stocks and for aquaculture of table-fish has increased.

Reseeding or enhancement of wild marine stocks is not widely practised in Australia but is common in Japan and is receiving increased consideration as a means of augmenting declining fisheries world-wide. Reseeding is usually considered when the natural population has been reduced by some event, e.g. overfishing of stock, localised depletion, or when the size of the natural stock is limited by the lack of recruitment, such as may occur when intermittently opening lagoons are closed when larvae or juveniles would otherwise enter. At least the latter is relevant for mulloway.

Increasing the numbers of any species through stock enhancement may impact on other

aspects of the ecosystem and should therefore be considered before large-scale enhancement programs commence. Prior to such an evaluation, the first question is whether it is possible to produce sufficiently large numbers of juveniles for stock enhancement and if the survival once stocked is high enough to be considered as an option for fisheries management. This study addressed these questions.

NSW Fisheries has been assessing the potential for the hatchery production of mulloway since 1990. Wild-caught, mature broodstock have been domesticated and held in tanks for many years. Maturation of mulloway has been controlled with photoperiod and temperature and fish have been hormone-induced to spawn. Mulloway larvae were reared for the first time in 1993 using intensive hatchery techniques. The hatchery-reared mulloway were easily weaned from live feeds to pellet diets, and ongrown to market-size in sea cages.

This research used the intensive larval rearing method which utilised dedicated, controlled facilities, high input of labour by skilled technicians, and relied on artificial propagation of live rotifers and brine shrimp for food. Mulloway were successfully reared using this technique, but it is expensive and difficult to produce sufficient numbers for large-scale enhancement. An alternative method is extensive larval rearing. This method utilises large-scale ponds, relatively low input of labour from skilled pond managers, and relies on propagation of natural zooplankton following addition of fertilisers to the ponds. Prior to this study, mulloway had not been reared using this technique, although many other marine fish species, including the closely related American red drum, have been reared in large numbers using fertilised ponds. If this method works for mulloway, large-scale enhancement may be possible.

The potential for reseeding and aquaculture of mulloway is also supported by the success of reseeding other sciaenids such as red drum, *Sciaenops ocellatus* in estuaries in the USA (Rutledge, 1989). Red drum are very similar to mulloway in their life history and breeding requirements and strong similarities exist in the larval rearing of the two species.

This report describes the results of trials designed to evaluate intensive and extensive methods for production of juvenile mulloway. Eight separate larval rearing trials and three large-scale experiments in commercially operated, earthen, brackishwater ponds were completed. The report also describes results of the first attempts to reseed juvenile mulloway into three intermittently-opening coastal lagoons in New South Wales. Recommendations for the next phase of research are presented.

Objective 1: To evaluate the production of mulloway juveniles using intensive and extensive techniques.

In excess of 100,000 juvenile mulloway were successfully produced using intensive clearwater, intensive greenwater, and extensive fertilised pond larval rearing methods. Intensive hatchery production of mulloway required expensive, dedicated live food and larval fish rearing facilities and a high input of labour from skilled technicians. By contrast, extensive pond rearing was possible in multi-purpose earthen ponds using relatively low input of experienced labour. The live feeds, rotifers and brine shrimp were cultured specifically to feed to mulloway larvae reared in intensive clearwater and greenwater tanks, however a natural bloom of rotifers and copepods was available to mulloway larvae reared in extensive ponds.

Growth and survival of mulloway larvae to juvenile fish was generally lower in intensive tanks (0.3-0.5 mm/d length increment; ~2% survival to 45 dah) than in extensive ponds (1.2-1.7 mm/d length increment; >20% survival to 45 dah). Cannibalism was a problem in intensive tanks, despite regular size grading, and may have accounted for the high mortality rates experienced. Infestation of a parasite, *Amyloodinium* sp. also caused major mortality, particularly in the intensive greenwater trials.

The optimum salinity for growth and survival of larvae and juvenile mulloway was determined. Although both larvae and juveniles grew well over a wide range of salinities from 5-35 g/L, larvae grew fastest and survival was generally best at low salinities of 5-12.5 g/L. Trends in data for juvenile mulloway growth also suggested that low salinity of 5 g/L was better.

Approximately 100,000 juvenile mulloway were produced in extensive ponds. Survival of larvae generally increased when older larvae were stocked into ponds. The optimum number of larvae to stock into ponds was similar to other fish species and ranged from 200,000-650,000 larvae/ha. Larvae grew up to five times more quickly in extensive ponds (1.2-1.7 mm/d) than in intensive tanks (0.3-0.5 mm/d). Some variability in performance of mulloway in ponds occurred and was probably due to uncontrolled environmental effects. Predation by cormorants also caused major mortality of juvenile mulloway grown in an un-netted 1.0 ha pond.

The best strategy to maximise survival and sustainable production of juvenile mulloway, may be a combination of initial larval rearing in intensive tanks, followed by ongrowing in fertilised ponds.

Objective 2: To stock two intermittent lagoons with some 50,000 juvenile mulloway.

Three intermittently opening lagoons were each stocked with about 25,000 juvenile mulloway. All mulloway had been marked with a non-toxic chemical for identification before release. Systematic, pre-stocking and post-stocking surveys were conducted in two lagoons (Khappinghat Creek and Swan Lake) to assess their suitability for stocking (e.g. no juvenile mulloway present) and the success of the stocking exercise. A third lagoon (Smiths Lake) was also stocked even though no pre-stocking survey was conducted.

Post-stocking surveys failed to capture juvenile mulloway in two lagoons, although abundance of benthic macrophytes made the sampling unreliable in Swan Lake and both lagoons were open to the sea for several weeks after stocking. Large numbers of mulloway were recaptured from Smiths Lake. The captured mulloway were identified as being hatchery-reared fish. Growth was rapid over many months after stocking and did not slow down during winter. Mulloway reached market-size (~1.2 kg) in the lagoon at about 16 months old. Commercial fishers reported catches of mulloway (2.5-2.7 kg) in Smith Lake at the time of writing (25 months after stocking).

Our results demonstrate that large numbers of juvenile mulloway can be propagated, and stock enhancement of intermittently opening lagoons is feasible. The next phase is to determine the environmental, social, and economic impacts of stocking large numbers of predatory fish. This would involve co-ordinated effort between researchers and managers from a number of disciplines such as aquaculture, fisheries and conservation.

1. Introduction

1.1 Background

NSW Fisheries has been assessing the potential for hatchery production of mulloway *Argyrosomus japonicus* (previously described as *A. Hololepidotus*) (Griffiths & Heemstra, 1995) for aquaculture to determine whether wild catches can be enhanced by reseeding, and an industry developed for cultured mulloway. Mulloway is a widely distributed temperate species (Sciaenidae), commanding high prices. It is also highly fecund, euryhaline and grows quickly (Battaglene & Bell, 1991; Gray & McDonall, 1993; Battaglene & Talbot, 1994).

Preliminary research conducted by NSW Fisheries has demonstrated that mulloway should be suitable for reseeding and aquaculture. Broodstock can be collected from the wild, and domesticated and grown quickly to maturity at approximately 8 and 10 kg for males and females respectively. Further, broodstock can be hormone-induced to spawn; larvae can be reared under intensive hatchery conditions by feeding rotifers and brine shrimp (Battaglene & Talbot, 1994) and juvenile mulloway can be weaned easily onto artificial pellet diets and grown quickly with minimal mortality.

The potential for reseeding and aquaculture of mulloway is supported by the success of reseeding other sciaenids such as red drum, *Sciaenops ocellatus* in estuaries in the USA (Rutledge, 1989). Red drum are very similar to mulloway in their life history and breeding requirements and strong similarities exist in the larval rearing of the two species.

Mulloway is an economically important species targeted by commercial and recreational fishers in all states except Tasmania. Like many other estuarine and in-shore fisheries, catches of mulloway in NSW have declined. In 1980/81 some 250 tonnes were caught compared with 88 tonnes in 1997/98 (NSW Fisheries, 1999). In NSW this trend has led to increased restrictions on the minimum size limit and the adoption of bag limits. Conflict between commercial and recreational fishers has resulted from the belief that the large by-catch of juvenile mulloway, taken by prawn trawlers operating in estuaries, is partially responsible for the decline in mulloway catches.

One suggested solution to this conflict is to release hatchery produced fish at the end of the prawn trawling season to compensate for removal of large numbers of juvenile mulloway. Reseeding or enhancement of wild marine fish stocks is not currently practised in Australia. However, it is common in Japan and USA and receives increased consideration when the natural population has been reduced by some event, e.g. declines in adult stocks from

exploitation, or when the size of the natural stock is limited by a critical life-history stage, an example being the lack of recruitment of fish to intermittently opening lagoons.

The development of reseeding programs will depend on finding ways to produce and grow juvenile fish cheaply and ensure a sufficient proportion survive after stocking. The need for low culture densities to avoid cannibalism, and the euryhaline nature and fast growing attributes of the mulloway juveniles suggests that production of commercial quantities may be best achieved by extensive larval culture methods in ponds (Battaglene & Talbot, 1994). This extensive method of fingerling production is commercially used in Australia for catadromous species such as barramundi *Lates calcarifer* and Australian bass *Macquaria novemaculeata* (Rutledge & Rimmer, 1991; Battaglene *et al.*, 1992) and for large-scale production of red drum fingerlings in the USA (McCarty *et al.*, 1986), but has not been evaluated for mulloway.

The aims of this study were to evaluate the production of mulloway juveniles using intensive and extensive larval rearing techniques; produce large numbers of juveniles cheaply; and to stock the juvenile mulloway into two intermittently opening lagoons.

1.2 Need

NSW Fisheries started research on the hatchery production of mulloway as part of a larger program to develop marine fish farming in NSW. Research has commenced on the acclimation of broodstock, intensive and extensive larval rearing, and development of feeds; however, there is need to evaluate the most cost-effective method of mass producing juvenile mulloway.

Development of hatchery production of mulloway is necessary for two main reasons. First, successful production of juvenile mulloway, at low cost, will make assessment of reseeding proposals possible. Large-scale reseeding experiments are required to determine the feasibility of enhancing wild stocks in estuaries and intermittently opening coastal lagoons (Rutledge, 1989). Reseeding should reduce conflict between commercial and recreational fishers and ultimately lead to increased catches of mulloway. Second, a successful aquaculture industry for mulloway would reduce pressure on wild stocks, provide sustainable employment, and an alternative source of fish would help lower fish imports. The creation of new opportunities for commercial fishers would also arise as some fishers have the equipment and aptitude to develop the necessary husbandry skills to farm and market fish. In the face of possible new management regulations to reduce effort in most fisheries in NSW, the methods we develop will allow fishers to keep operating in areas which may otherwise be closed to commercial fishing.

1.3 Objectives

The objectives of this project were:

1 to evaluate the production of mulloway juveniles using intensive and extensive techniques.

2 to stock two intermittent lagoons with some 50,000 juvenile mulloway.

2. Fingerling production

2.1 Introduction

The first objective of the project was to evaluate the production of mulloway juveniles using intensive and extensive techniques. Intensive production techniques utilised high-technology, indoor, recirculating facilities, skilled hatchery technicians, and rearing methods developed at the Port Stephens Research Centre (PSRC) over many years for mulloway and other marine fish larvae such as snapper, *Pagrus auratus*, Australian bass, and sand whiting, *Sillago ciliata*. Extensive production techniques utilised small and large-scale, fertilised earthen ponds, and were managed by Mr Glen Searle, Searle Aquaculture, Palmers Island, NSW. Mr Searle has developed pond management techniques for production of several species of brackishwater fish larvae such as Australian bass and sand whiting, as well as estuarine prawns and freshwater fish.

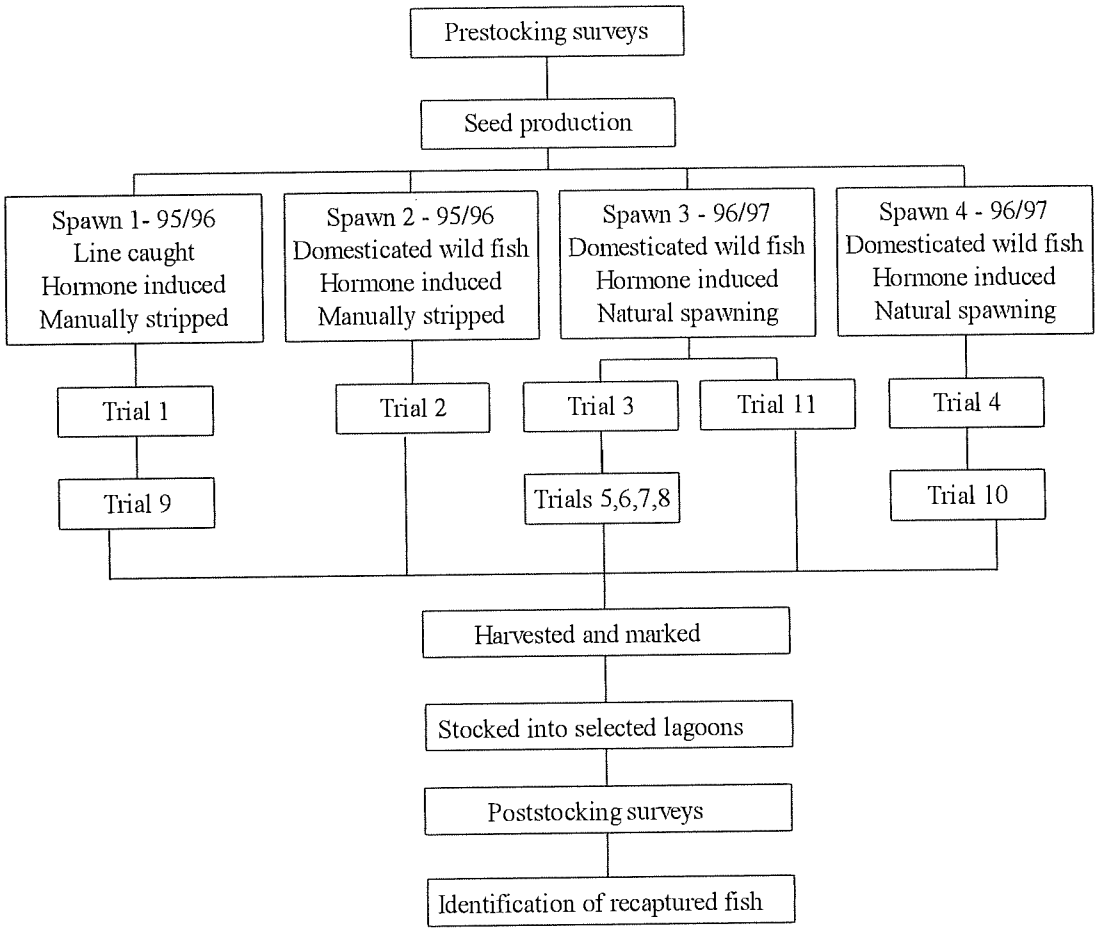
2.2 Materials and Methods

2.2.1 Intensive clearwater larval rearing

General

Intensive rearing trials were conducted to assess growth and survival of mulloway larvae in recirculating clearwater facilities. Trials were conducted over two summer breeding seasons. Spawn induction attempts during the December 1995 to March 1996 breeding season produced two viable spawns, which provided enough larvae for two hatchery trials. Two successful spawn induction attempts and subsequent intensive hatchery trials were also completed in the 1996/97 season. Larvae were sampled regularly (1-5 d) from each tank to estimate growth rate. At least 10 larvae/tank were sampled unless otherwise stated.

Table 1: Flow diagram of mulloway broodstock origin, spawn induction, larval rearing trials, and stocking into lagoons.



Larval Rearing Trials:

Trials 1, 2, 3, 4 - Clearwater tank rearing

Trials 5, 6 - Salinity tolerance experiments

Trials 7, 8 - Greenwater tank rearing

Trials 9, 10, 11 - Extensive pond rearing

Trial 1

(i) Induction

Fertilised eggs were obtained from wild captured broodfish. A 15 kg female was line captured from the mouth of Wallis Lake, NSW, injected with 1,000 IU/kg of human chorionic gonadotropin (hCG) and transported to PSRC in a 600 L fibreglass tank supplied with oxygen (1 L/min). The female mulloway was then placed into a 5,000 tank which was supplied with filtered (10 μm) seawater at 3 L/min. Milt was expressed from a male fish which had not survived capture and stored in 5 mL syringes in an ice slurry. Following ovulation, eggs were manually stripped into several 2 L buckets, mixed with sperm, and seawater added.

(ii) Rearing

Batches of eggs were split between a series of 45 L glass aquaria for incubation. Dead eggs were allowed to settle and were removed by siphon. Vigorous aeration was provided until eggs hatched. After hatching, larvae were transferred to floating 40 L tanks in each of three 2,000 L larval rearers for several hours to allow acclimation. Larvae were then released into the rearing tanks.

Larval rearing tanks were 2,000 L, recirculation tanks with black sides and white conical bottoms. Prior to stocking with larvae, tanks were filled with oceanic water and chlorinated (20 ppm active chlorine) *in situ* for 24 hours before neutralisation with sodium thiosulphate. Water was drawn through a perforated central standpipe covered with 300 μm mesh screen, and circulated through a 200 L biofilter filled with bioballs and shellgrit by an Eheim pump. After neutralisation of the seawater, the Eheim pump was filled with activated carbon.

Surface skimmers were added 3 days after hatch (dah) to prevent accumulation of surface films, which can prevent inflation of larval swimbladders. Cultured rotifers were offered to larvae from 3-15 dah. Rotifers were nutritionally boosted prior to feeding using either the algal species *Pavlova lutheri* or Tahitian *Isochrysis* aff. *galbana*, or DHA Super Selco (Artemia Systems, Belgium). Rotifer densities were maintained at 10/mL throughout the day. Water flow rates were increased from daily levels of 2 L/min to 5 L/min overnight to strip uneaten rotifers from the water column, and allow freshly boosted rotifers to be added at the beginning of each day. Brine shrimp were offered from 9 dah onwards. Until 17 dah, AF grade artemia nauplii were fed until the larvae were large enough to consume nutritionally boosted ongrown EG grade artemia. Artemia were initially offered at 0.1/mL/day. This was gradually increased to the equivalent of 2/mL/day, spread over several feeds, in keeping with the increased

demand of larger larvae. A weaning pellet diet (ML 400, Fukui & Co, Japan) supplemented live feeds from 32 dah to harvest.

Good water quality was maintained by regular siphoning of the tank bottom. Sterilised oceanic water was used to replace any wastewater. Water quality was monitored daily, with dissolved oxygen, pH, salinity, temperature and ammonia being measured routinely (Table 2).

Trial 2

(i) Induction

A 14 kg female mulloway which had been held in a 17,000 L broodstock tank at PSRC, and exposed to a truncated 120 day phototherm regime, was successfully induced to spawn following an injection of hCG (1,000 IU/kg). Eggs were manually hand-stripped from the female fish and were fertilised with sperm collected from a wild captured male mulloway. (see Appendix 6 for further information on management of broodstock).

(ii) Rearing

Incubation and larval rearing protocols were the same as Trial 1.

Trial 3

(i) Induction

Eggs for this hatchery trial were obtained from an hCG (1,000 IU/kg) induced, 13 kg female mulloway which had been held in a 30,000 L broodstock tank at the Fisheries Research Institute (FRI), Cronulla. Fourteen fish (1:1; M:F) were held in this facility and matured under an artificially controlled temperature and photoperiod regime designed to emulate ambient environmental conditions. A spermiating male fish of 10 kg was also injected with 500 IU/kg of hCG. Injections were carried out *in situ*, and the induced fish remained in the broodtank. Natural spawning occurred and eggs were collected by hauling a 500 μm plankton mesh net through the water column. Harvested eggs were stocked into a 600 L tank filled with 10μm filtered seawater (35 g/L; 23±1 °C) and supplied with oxygen at 1L/min and road transported to PSRC (4 h).

(ii) Rearing

Eggs were bucketed directly into six, 2,000 L larval rearing tanks and incubated. Dead eggs were removed from the tanks by siphon. Larval rearing protocols were the same as Trial 1.

Trial 4

(i) Induction

Fertilised eggs for this trial were also obtained from broodfish held at FRI. Two 13 kg female fish, and a 15 kg male were injected with 1,000 and 500 IU/kg of hCG, respectively and allowed to spawn naturally within the broodstock tank as in Trial 3.

(ii) Rearing

Handling and treatment of eggs and larvae was the same as Trial 3.

Trial 5

An experiment was conducted to determine the effect of salinity on growth and survival of mulloway larvae from 6-20 dah. The experiment was conducted using replicate 100 L recirculation tanks. Treatment salinities were 5, 12.5, 20, 27.5, and 35 g/L. Water temperature was maintained between 23.6-24 °C, and daylength was 14:10 h Light: Dark. (see Appendix 3 for detail of methods).

Trial 6

An experiment was conducted to determine the effect of salinity on growth and survival of juvenile mulloway for 28 days. Treatment salinities were 0.6, 5, 10, 20, and 35 g/L (see Appendix 3 for detail of methods).

2.2.2 Greenwater larval rearing

General

Greenwater tank trials were conducted to assess growth and survival of mulloway larvae in large-scale, non-recirculated, greenwater systems. This assessment was not part of the original project objectives, but was possible due to a surplus of mulloway larvae.

Trial 7

Production of mulloway larvae, live feed and algae was conducted in 10,000 L flat bottom tanks. Tanks had no provision for recirculation, and remained static unless water was exchanged. Tanks were filled with unsterilised seawater which was pumped through sand filters and a 10μm (nominal) cloth filter bag. Air was supplied to each tank from three submerged airstones. All tanks were maintained in an uncovered outdoor facility.

A single larval rearing tank was initially filled with seawater and inoculated with approximately 500 L each of cultured microalgae, *Tetraselmis chuii* and *Chaetoceros muelleri*, produced at the PSRC algal unit. Aquasol®, a soluble comprehensive plant fertiliser, was also added to provide 1 mg/L of available nitrogen. Rotifers, *Brachionus plicatilis*, were then inoculated at 1-2/mL into the tank. After six days, mulloway larvae were stocked into the tank.

To ensure that an algal bloom could be maintained within the larval rearing tank, separate algae cultures were produced in 10,000 L tanks. Tanks were drain harvested every 6-7 days and new algae cultures were established by inoculating with 1,000 L of algae from a tank with the greatest algal density. At times the larval tank developed self sustaining algae blooms. The density of these blooms required controlling by water exchange to avoid excessive diurnal fluctuation of dissolved oxygen and pH. Productive algal blooms are beneficial in maintenance of water quality by reducing ammonia concentration, enhancing nutritional status of live feeds, and reduction of light intensity.

Approximately 160,000 larvae (4 dah) were drain harvested from an intensive clearwater tank and, after acclimation in a floating 40 L perspex tank, released into the greenwater tank. Density of rotifers at stocking was 7/mL. Initially the rotifer population was self-sustaining around this concentration, with the algal bloom being consumed and requiring daily replacement from algae culture tanks. At 10 dah, rotifer densities were reduced by actively feeding mulloway larvae, and by 13 dah supplementary feeding with nutritionally boosted rotifers and AF grade artemia nauplii was necessary. Rotifers were offered daily to 16 dah, then in a supplementary fashion until 22 dah. Ongrown artemia were fed from 15 dah onwards. Water quality (dissolved oxygen [DO], pH, salinity, temperature and ammonia) was monitored daily (Table 2).

The onset of cannibalism made size grading necessary at 27 dah. Size-grading was conducted in standard floating graders, which had a perforated bottom of evenly spaced stainless steel bars, and solid walls. Fish smaller than the bar spaces swam through the grader and larger fish were retained.

Trial 8

A single greenwater tank was stocked with 39,000 larvae. Larvae were siblings of those used in Trial 5, and were drain harvested from an intensive, 2,000 L rearing tank at 11 dah. Larval rearing and water quality sampling protocols were the same as Trial 7.

2.2.3 Extensive Pond Trials

General

Attempts to rear mulloway larvae in a range of different size earthen ponds were conducted at Searle Aquaculture, Palmers Island, Yamba, northern NSW.

Experiments were conducted to determine the effect of age of stocking, and stocking density on growth and survival of mulloway larvae in extensive fertilised ponds. In addition to specific experimental stockings, unreplicated production runs provided further information on pond management techniques, growth rate and survival of larvae.

Experiments were undertaken in eight rectangular, centrally drained, 250 m^2, earthen ponds. Production trials were conducted in 1.0 and 0.25 ha ponds. Ponds were dried, smoothed and limed five to seven days prior to filling with brackish water. Rearing ponds were partially filled from large production ponds containing viable blooms of phytoplankton and zooplankton, and then filled to capacity with estuarine water. All influent water passed through a 1,000 μm filter screen. Ponds were then fertilised with aglime and urea. A single paddlewheel was provided to each pond.

Supplementary feeding of artemia was used on an as needs basis when blooms of natural feed were reduced.

Water quality parameters (DO, pH, temperature, turbidity, and salinity) were monitored routinely (Table 2).

Attempts were made to sample larvae from the ponds approximately every 3 days using a 500 μm ichthyoplankton net, which was towed through the water column along the longest axis of the ponds. Juvenile mulloway were harvested by draining the pond through a central drain into an external harvesting basket (~1 m^3). The approximate number of harvested fish was determined by weight. A random sample of fish provided the mean fish weight (n=100) and mean total length (n=20).

Trial 9

The aim of this trial was to determine the effect of age of stocking (3, 7, 15, and 20 dah) on the growth and survival of mulloway larvae in earthen ponds.

Mulloway larvae were obtained from the wild captured broodfish used in Trial 1. Larvae were reared in a 2,000 L intensive tank (Trial 1) at PSRC and stocked into two replicate ponds at 3, 7, 15, and 20 dah, respectively. Attempts were made to stock equivalent numbers of larvae into ponds at each treatment age; however, due to variable survival between intensive rearing tanks, difficulty in estimating the number of larvae harvested initially from rearing tanks, and postharvest mortality, the number of larvae varied for each stocking age. The number of stocked larvae was 7,614, 8,750, 10,085, and 5,250, for 3, 7, 15, and 20 dah, respectively (Table 3).
Larvae were drain harvested from rearing tanks and placed into 40 L plastic bags filled with approximately 10 L of sterilised seawater. Rotifers were added (10/ml) and the bags inflated with oxygen and sealed with rubber bands. Bags were placed into styrene boxes and transported by road from PSRC to Yamba (~ 8 h). Light penetrated the boxes and larvae were able to feed in transit.

Larvae were removed from bags at Yamba and placed into a single 500 L, conical-bottom tank. Larvae were acclimated to the pond conditions by slowly adding pond water to the tank over a three-hour period. The larvae were then mixed well in the tank by stirring, and an estimate of the total number of larvae was made by randomly selecting 500 mL aliquots (n=5). Larvae were distributed evenly from the acclimation tank to the treatment ponds (n=2) by bucket.

It was only possible to harvest four of the eight ponds at a time. Therefore, larvae that were stocked into ponds at 3 and 7, and 15 and 20 dah, were harvested on 29 and 38 dah, respectively (Table 3).

Trial 10

The aim of this trial was to determine the effect of stocking density on growth and survival of mulloway larvae. Experimental ponds were stocked with 11 dah larvae, harvested from intensive rearing tanks (Trial 4). Larvae were transported by road from PSRC to Yamba in two 600 L fibreglass tanks. Larvae were provided live rotifers in transit, and an internal light source (cyalume) was provided to enable larvae to feed. Oxytetracycline hydrochloride (OTC;

100 mg/L) was added to the tank water as a prophylaxis against post harvest bacterial infection. A fish counter (Jensorter, Model FC-22, Oregon, USA) was used to estimate the number of larvae stocked into treatment ponds. Larvae were acclimatised to pond conditions using the same technique as in Trial 9. After acclimation, eight ponds (two replicates of four treatments) were stocked with larvae. Treatment ponds were stocked at 5,000, 10,500, 16,000 and 26,000 larvae/pond. Ponds were managed in similar fashion to Trial 9.

After 30 days (41 dah), all ponds were harvested and fish counted and measured to estimate survival and growth.

Trial 11

Large-scale production of mulloway larvae was attempted in two different size ponds. Larvae were obtained from spawn induction of broodfish described in Trial 3. Larvae were harvested and transported from PSRC to Yamba using methods described in Trial 9. Approximately 438,000 of a mix of 2, 3 and 4 dah larvae were stocked into a 1.0 ha pond. A second 0.25 ha pond was simultaneously stocked with approximately 265,000, 4 dah larvae. Feeding larvae (4 dah) were provided with rotifers (10/ml) and light in-transit. Ponds were managed as in Trial 7.

Juvenile mulloway were progressively harvested from the ponds when they were required for stocking into intermittently opening lagoons. Harvesting involved herding of fish into a plastic-mesh trap (~2 m^3) using a shade cloth barrier net. Once concentrated in the trap, juveniles were caught with dip nets, weighed and placed into transportation tanks.

As juvenile fish size increased, fish remaining in the pond were weaned onto a pellet diet (Kinta, Yarrawonga, Victoria), by broadcasting pellet from the pond bank at least twice a day.

2.2.4 Statistical analyses

Trials 5, 6, 9 and 10 were designed for analysis using single factor analysis of variance (ANOVA). Homogeneity of variance was assessed using Cochran's Test. Multiple comparisons among means were assessed using Student-Newman-Kuels procedure. The significance level was set at $P<0.05$.

For trial 9, it was only possible to harvest four of the eight ponds on the same day. Therefore, two harvest events were conducted. Consequently, larvae stocked at different ages were grown in ponds for different periods, and were 18, 22, 23 and 26 days for the 20, 7, 15 and 3 dah stocking treatments, respectively. However, we analysed the data as if ponds were

harvested at the same time, and assumed that the different lengths of time that larvae were in ponds had no effect on survival or growth of larvae.

Table 2. Water quality environment parameters for intensive clearwater, intensive greenwater and extensive fertilised ponds for rearing mulloway larvae.

Trial	pH	Dissolved Oxygen (mg/L)	Temperature (^{0}C)	Salinity (g/L)
Trial 1- Intensive	6.83 - 8.32	4.94 - 8.00	19.6 - 28.1	20.9 - 36.8
Trial 2 –Intensive	7.19 - 8.06	4.22 - 7.30	22.6 - 25.8	32.2 - 35.5
Trial 3 –Intensive	8.05 - 8.48	4.88 - 6.48	21.1 - 26.4	29.9 - 34.4
Trial 4 –Intensive	7.84 - 8.26	4.29 - 6.25	23.1 - 23.8	27.9 - 33.1
Trial 5 –Intensive	8.3 - 8.9	>6.0	23.6 - 24.0	5.0 - 33.8
Trial 6 –Intensive	8.3 - 8.9	>6.00	17.5 - 22.6	0.6 - 34.5
Trial 7 & 8 – Greenwater	8.09 - 8.67	4.59 -10.46	21.6 - 27.6	24.8 - 35.1
Trial 9 –Extensive	7.1 - 8.9	4.3 - 13.6	21.0 -31.0	17.0 - 27.0

2.3 Results

2.3.1 Intensive Rearing Trials

Trial 1

(i) Induction

The female mulloway began ovulating after a latency period of 36 h. Eggs were hand-stripped from the female at 36, 36.5 and 37 h after hormone injection. Approximately 1,500,000 eggs were spawned. Fertilisation rate was 20.3±6.7% (n=3).

(ii) Rearing

A total of 271,000 larvae were stocked into three larval rearing tanks. One tank was harvested at 20 dah, and produced 10,500 fish with an average size of 7.72±1.1 mm (mean total length

[TL] ± sd, mm) and survival of 13%. Daily mean increment in TL to 20 dah was 0.28 mm/d. The remaining two tanks were combined and graded into two size classes at 31 dah (Fig. 1). The larger fish were harvested at 39 dah, and produced 1,250 fish with mean TL of 23.7±2.6 mm. The final tank was harvested at day 45, and produced 1,150 fish with mean TL of 29.1±4.3 mm. Survival of mulloway to 39 and 45 dah was approximately 1.3%.

Trial 2

(i) Induction

Ovulation occurred after 32.3 hours, when 800,000 eggs were hand-stripped. Approximately 14% of eggs were fertilised. A further 200,000 eggs were hand-stripped 30 min later with no fertilisation.

(ii) Rearing

Approximately 100,000 larvae were divided evenly between two rearing tanks. By 29 dah, fish averaged 17.8±1.8 mm and 16.4±1.4 mm in the two tanks, respectively (Fig 2). Daily mean increment in TL to 29 dah was 0.55±0.02 mm/d (n=2 tanks). Tanks were combined and graded into two size classes at 32 dah. Total unexplained mortality occurred the following night. Counting and measuring of dead fish showed that a total of 157 fish with mean TL of 24.7±4.1 mm, and 3,043 with mean TL of 18.1±3.1 mm had been produced. Approximately 3.2 % of the fish survived to 32 dah.

Trial 3

(i) Induction

Fish spawned naturally in the broodfish tank on three consecutive nights. An estimated 5,000, 000 eggs were released on the first night, with a fertilisation rate of 30%. On the second night of spawning a total of 224,000 eggs with fertilisation rate of 66% were collected. Collection of eggs from a spawn on the third night included some previously uncollected eggs and larvae. Fertilisation of the final spawning episode was approximately 95%, and 490,000 viable eggs and larvae were collected.

(ii) Rearing

All six available larval rearers were stocked with larvae from the first spawning event. Survival rates could not be determined, as tanks were all partially harvested at early growth stages to

Fig. 1. Change in length of mulloway larvae in 2000 L recirculation tanks. Data are means ± sd (n=3 tanks to 20 dah; n=2 tanks 20-30 dah). Trial 1

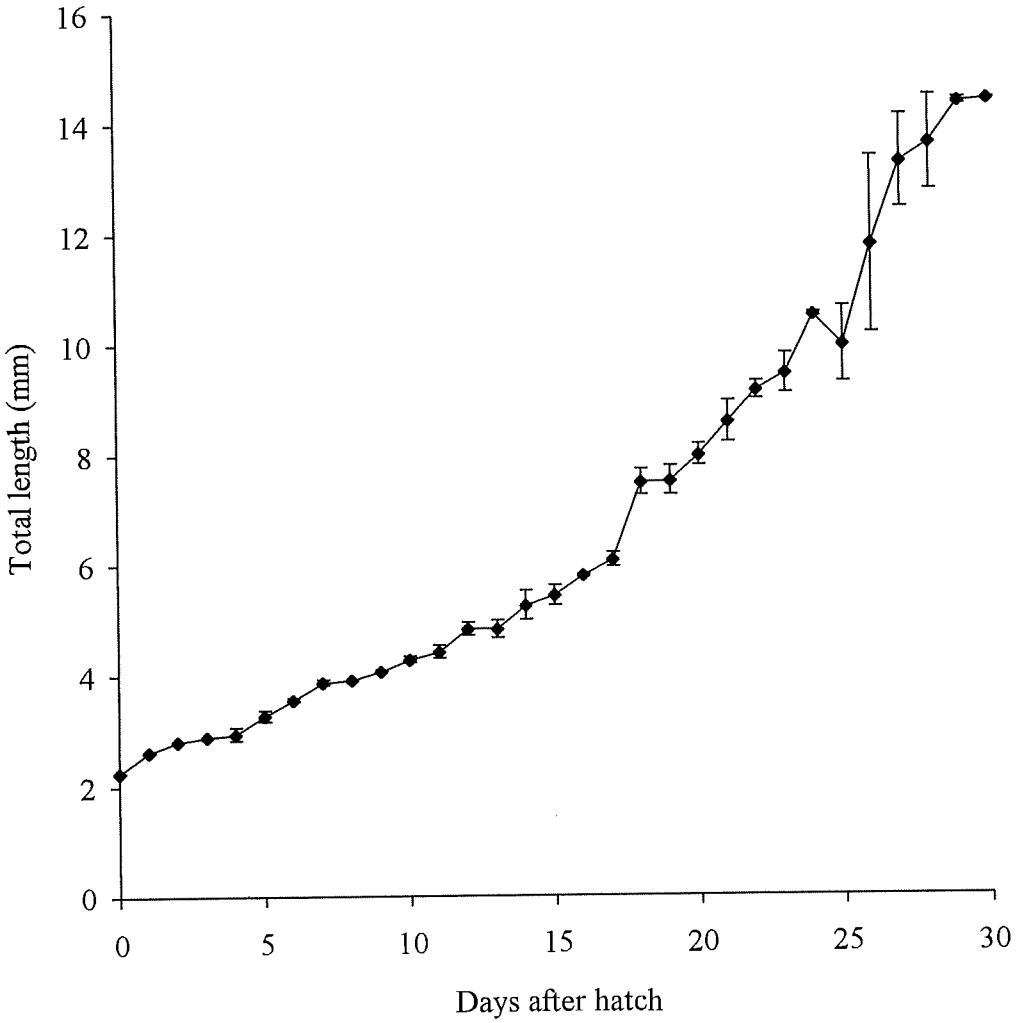
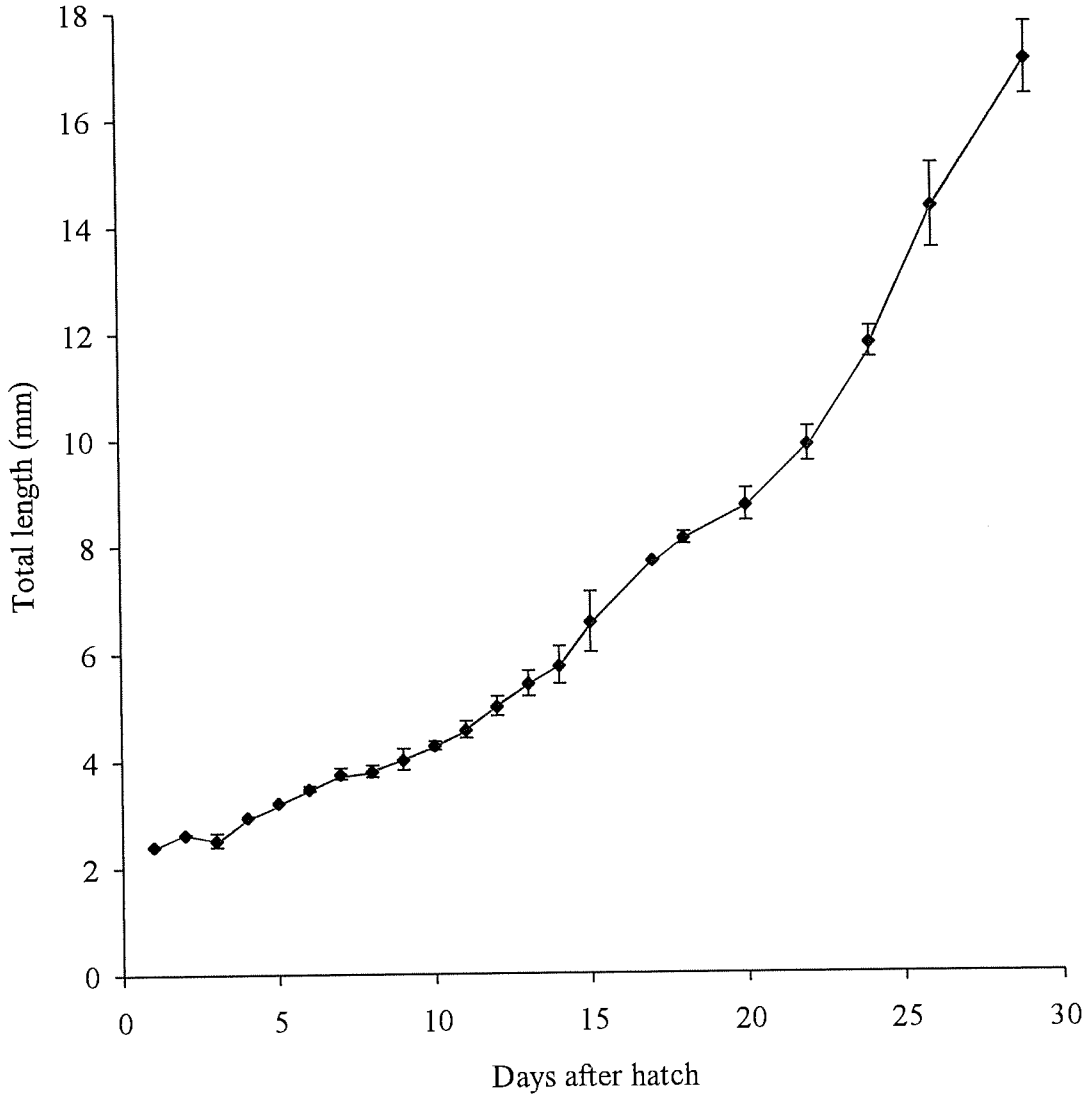


Fig. 2. Change in length of mulloway larvae in 2000 L recirculation tanks. Data are means ± sd (n=2 tanks). Trial 2.



provide larvae for other extensive larval rearing trials.

Trial 4

(i) Induction

Broodfish spawned naturally over three consecutive nights. Spawning on the first night produced 4,785,000 eggs, with a fertilisation rate of 34%. The second spawn produced 597,750 eggs with a fertilisation rate of 73%. Approximately 150,000 eggs were spawned on the final night but all eggs were unfertilised.

(ii) Rearing

Approximately 117,000 larvae were placed into each of six rearing tanks. One tank was drained and all larvae were sacrificed following infestation of an ectoparasite, *Amyloodinium* sp. One tank was harvested when larvae were 6 dah, for stocking of a salinity tolerance experiment. Approximate survival to 6 dah was 26%. The remaining four tanks were harvested at 11 dah to stock an extensive larval rearing trial. Just prior to harvest (8 dah), mulloway larvae were 3.83±0.17 mm in total length. Approximate survival to 11 dah was 24.5% (n=4 tanks).

Trial 5 and 6

The results for experiments determining the effects of salinity on growth and survival of mulloway larvae and juveniles are presented in Appendix 3.

Salinity had a significant ($P<0.05$) effect on growth and survival of mulloway larvae. Larvae grew at all salinities from 5-35 g/L but were longest at 5 g/L. Survival of larvae was in general highest at lower salinities of 5-12.5 g/L. Juvenile mulloway grew and survived well at all salinities from 5-35 g/L, but trends in data suggest that 5 g/L was best.

2.3.2 Greenwater Tank Trials

Trial 7

Survival of the 160,000 larvae initially stocked appeared to be good by visual observation, until the onset of an infestation of an ectoparasite, *Amyloodinium* sp. at 48 dah. Infested mulloway stopped feeding and were lethargic. Attempts were made to treat the infestation with chronic exposure of fish to 25 mg/L formalin. After 24 h some improvement in fish

behaviour was observed and fish began feeding, but fish were still heavily infested with parasites. It was not possible to attempt treatment with high concentrations of formalin (100 - 200 mg/L) due to the inability to exchange high rates of influent seawater. Total eradication of *Amyloodinium* sp. was never achieved. The severity of the initial infestation combined with chronic parasitism resulted in continued daily mortality of fish, ending in death of all fish by 61 dah.

Growth rates were similar to those displayed by fish reared in intensive tank trials (Fig.3). Prior to size grading at 25 dah fish had a mean TL of 11.8±2.1 mm (mean TL increment, 0.4mm/d).

Trial 8

Mulloway larvae with a mean TL of 4.5±0.4 mm were harvested from a 2,000 L tank (Trial 5) and stocked at 11 dah into a greenwater tank. By 25 dah fish had grown to 9.2±1.6 mm (mean TL increment, 0.33 mm/d). The fish were size-graded at 25 dah and combined with fish from Trial 7. Consequently the fish were exposed to the *Amyloodinium* sp. Infestation of the parasite and death of all fish occurred.

2.3.3 Extensive Pond Trials

Trial 9

The age of larvae at stocking had a significant ($P<0.05$) effect on survival (Table 3). In general, survival increased as age of larvae at stocking increased, although there was no difference in survival between larvae stocked at 3 and 15 dah. Difficulty in maintaining algal and zooplankton blooms in the 15 dah treatment ponds was experienced 7 days after stocking. Also, 15 days after this treatment was stocked, an infestation of ectoparasitic ciliates (unidentified) was noticed on some larvae.

There was no significant ($P>0.05$) effect of age of larvae at stocking on the final harvest weight, final total length and total length increment of mulloway fingerlings (Table 3). The increment in total length of larvae grown in the intensive hatchery tanks from 3 to 20 dah was approximately 0.3 mm/d. However, when larvae for all age treatments were stocked into ponds, the total length increment increased by approximately 5-6 times to 1.4-1.7 mm/day (Table 3; Fig. 4).

Fig. 3. Change in length of mulloway larvae in 10,000 L greenwater tanks. Data are means ± sd (n=2 tanks). Trial 7.

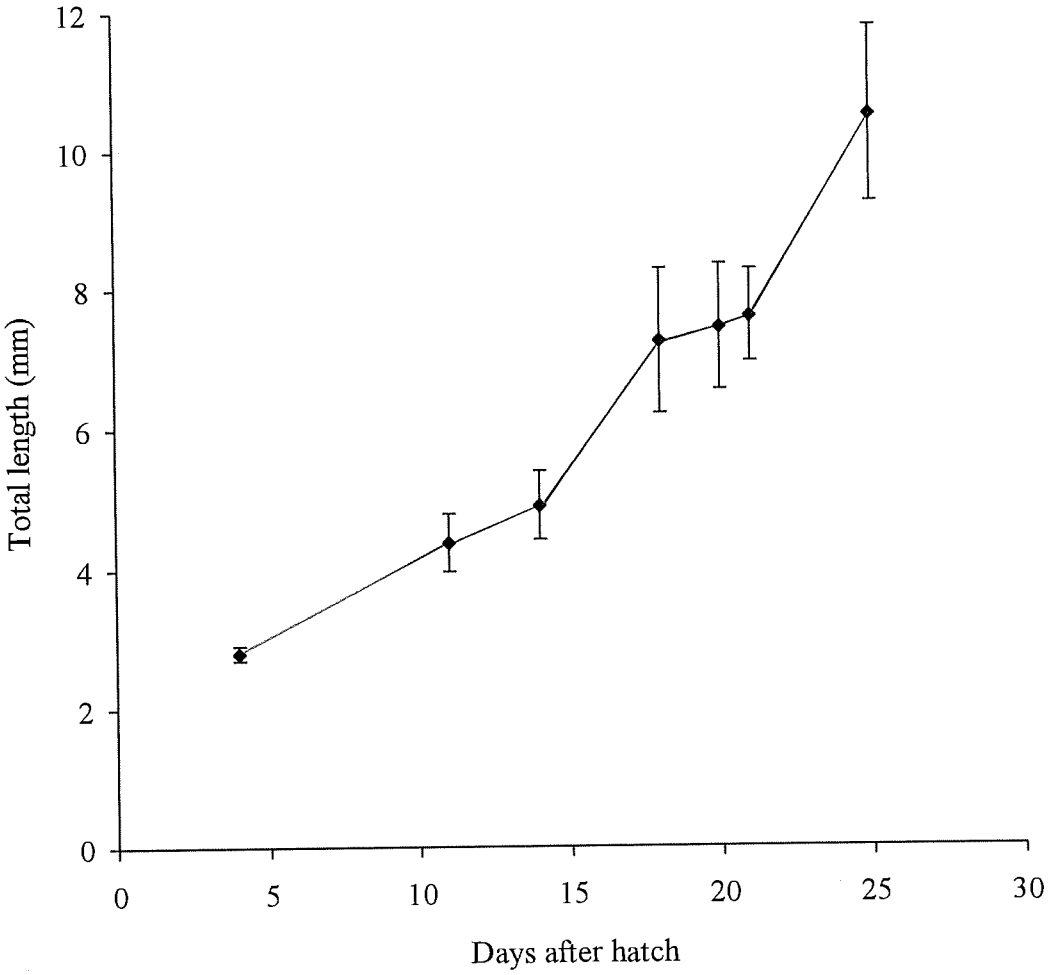


Fig. 4. Change in length of mulloway larvae stocked from a 2,000 L tank to 250 m^2 ponds at 3, 7, 15, and 20 dah. Data are means ± sd (n=2 ponds). Trial 9.

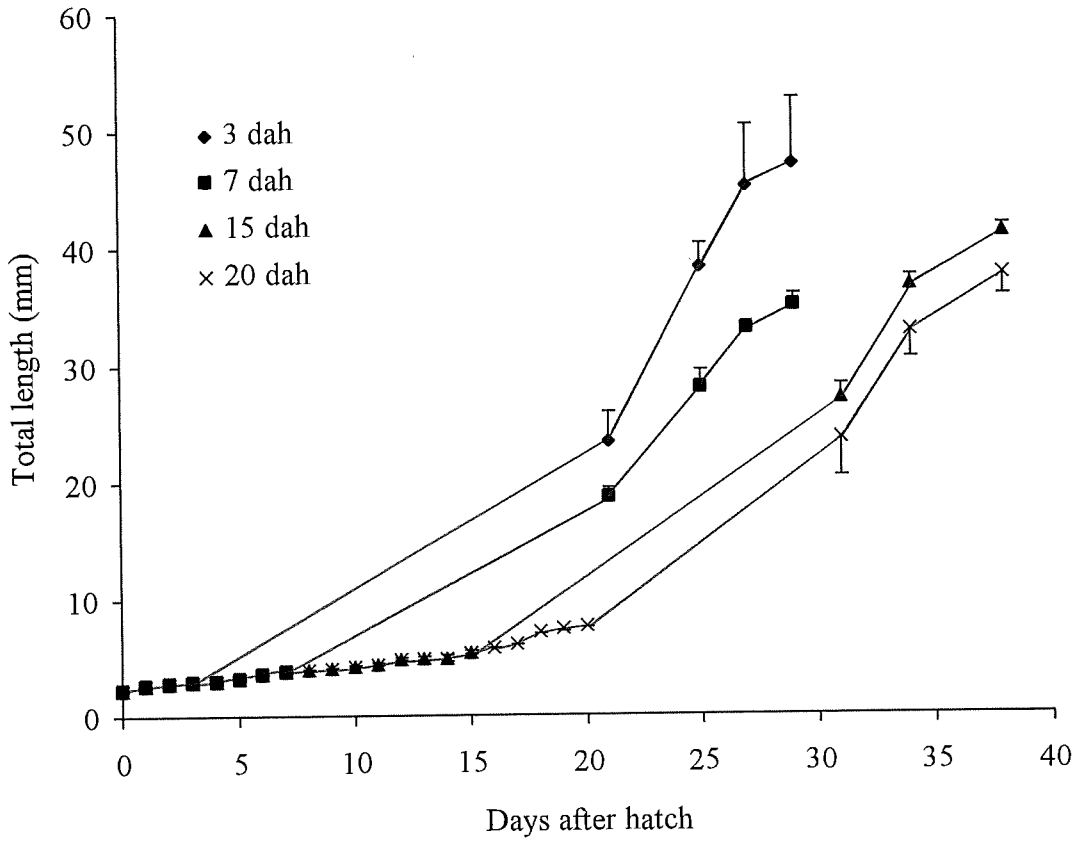


Table 3. Growth performance and survival of mulloway *Argyrosomus japonicus* larvae grown in earthen ponds and stocked at 3, 7, 15 and 20 dah.[1]

Date of stocking	Age at stocking (days)	Age at harvest (days)	Days in pond[2]	Number larvae stocked	Initial total length (mm)	Harvest weight (g)	Harvest total length (mm)	Growth rate (mm/d)	Total number harvested	Survival (%)
18-Dec-95	3	29	26	7614	2.9±0.1	1.08±0.3[a]	47.1±5.6[a]	1.7±0.2[a]	1658±66[a]	21.8±0.9[a]
22-Dec-95	7	29	22	8750	3.9±0.2	0.44±0.1[a]	35.0±1.0[a]	1.4±0.1[a]	3167±305[b]	36.2±3.5[b]
30-Dec-95	15	38	23	10085	5.4±0.2	0.86±0.1[a]	41.2±0.6[a]	1.6±0.02[a]	1594±199[a]	15.8±2.0[a]
4-Jan-96	20	38	18	5250	8.0±0.2	0.59±0.1[a]	37.7±1.9[a]	1.7±0.1[a]	4002±41[c]	76.2±0.8[c]

[1] Data are means ± standard errors for n=2 ponds. Means in each column with the same superscript are not significantly different (P>0.05).
[2]Larvae were held in ponds for different times as it was not possible to harvest all ponds at the same time. However, data were analysed as if ponds were harvested at the same time, and assumed that the different lengths of time that larvae were in ponds had no effect on survival or growth of larvae.

Table 4. Growth performance and survival of 11 day old mulloway *Argyrosomus japonicus* larvae grown in earthen ponds for 30 days and initially stocked at different densities.[1]

Number larvae stocked	Initial total length (mm)	Harvest total length (mm)	Growth rate (mm/d)	Total number harvested	Survival (%)
5000	3.8±0.2	48.3±5.3	1.5±0.2	254±115	5.1±2.3
10500	3.8±0.2	46.5±6.0	1.4±0.2	947±341	9.1±3.3
16000	3.8±0.2	39.2±0.5	1.2±0.02	986±156	6.2±1.0
26000	3.8±0.2	53.8±0.9	1.7±0.03	192±24	0.7±0.1

[1] Data are means ± standard error for n=2 ponds. Means within columns are not significantly different ($P>0.05$).

Fig 5. Change in length of 11 dah mulloway larvae in 250 m^2 ponds and initially stocked at 5,000, 10,500, 16,000, 26,000 larvae/pond. Larvae were grown in a 2,000 L tank to 11 dah. Data are means (n=2 ponds). Means were not different at 41 dah. Trial 10.

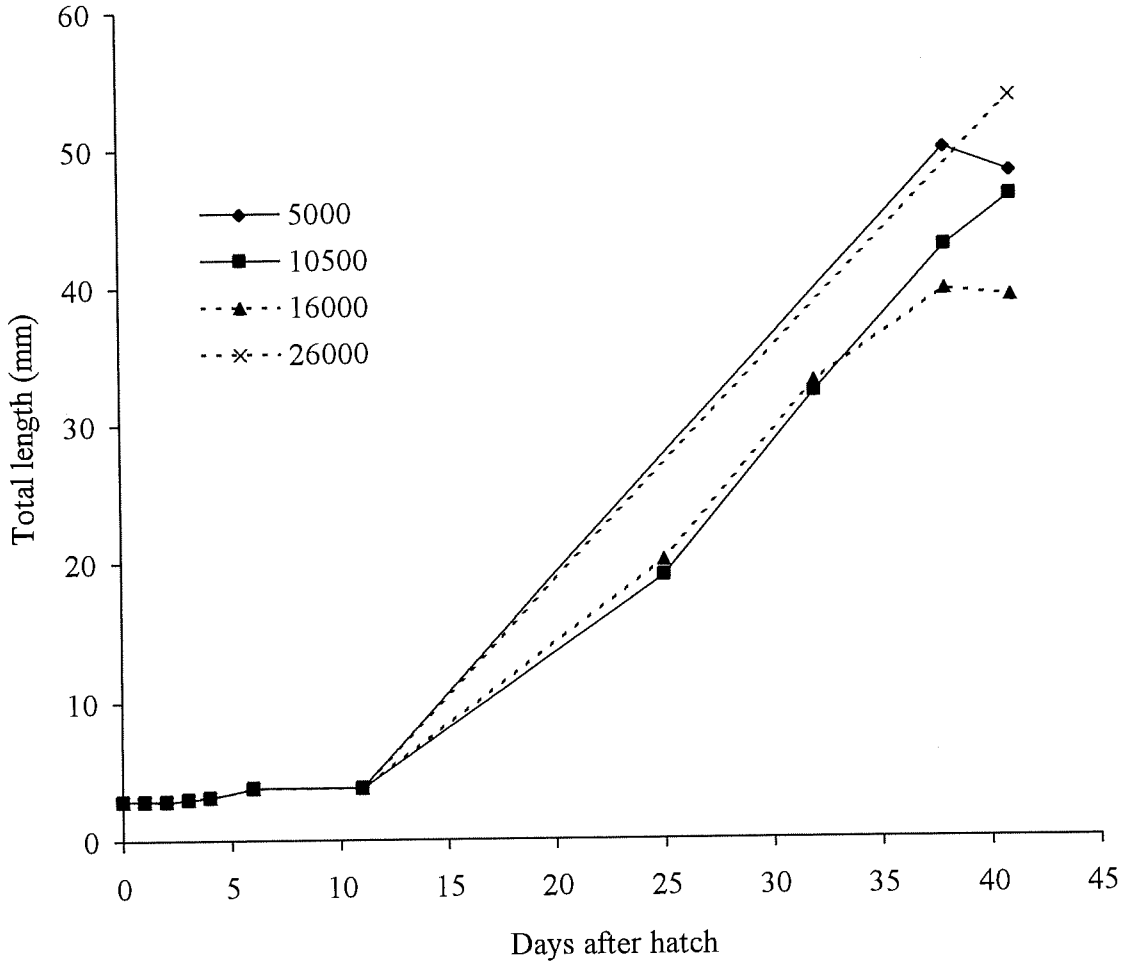
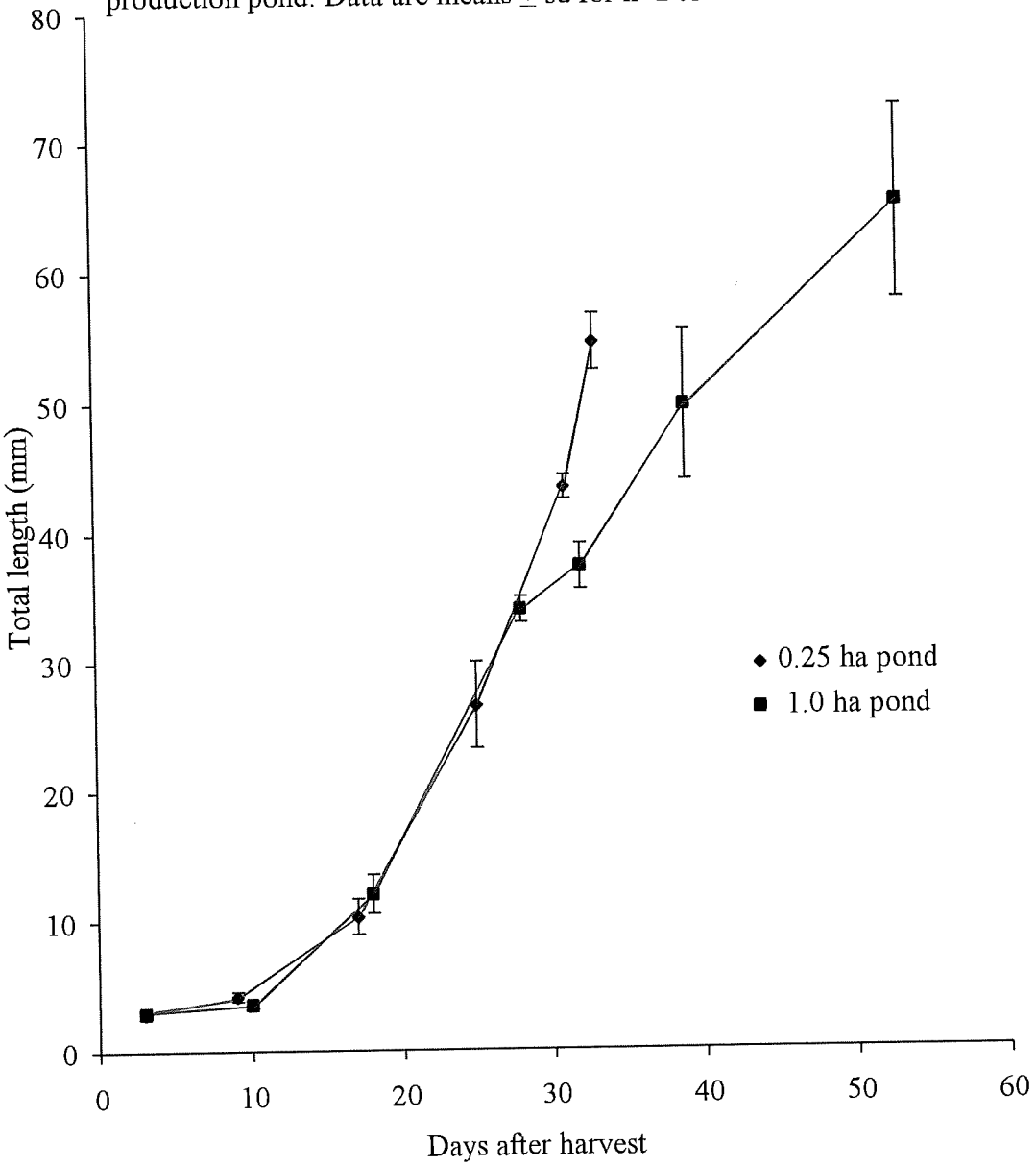


Fig. 6. Change in length of mulloway larvae reared in a 0.25 ha and 1.0 ha production pond. Data are means ± sd for n=2 to 10 larvae. Trial 11.



Trial 10

Mulloway larvae grew in all ponds and initial stocking density did not affect significantly ($P>0.05$) the survival, final total length and total length increment of larvae (Table 4; Fig. 5). However power analysis of mean number of fish harvested, and survival, showed that power of the experiment was low (0.3) due to few replicate ponds and high variability within ponds. Trends in data showed that larvae initially stocked into ponds at densities of 5,000, 10,500 and 16,000 larvae/pond had similar survival and was approximately 10 times greater than survival when larvae were stocked at 26,000 larvae/pond. Total daily length increment was high for all treatments and ranged from 1.2-1.7 mm/d.

Trial 11

Mulloway grew well in both production ponds (Fig. 6). A total of 52,064 juvenile mulloway were harvested from the 0.25 ha pond, representing a survival of 19.6% from stocking. Partial harvests were conducted on 48, 50, 56 and 100 dah and mulloway ranged in TL from 50-106 mm (1.7-11.8 g).

A total of 26,000 juvenile mulloway up to 11.8 g in size were harvested from the 1.0 ha pond prior to inundation of the pond by a flock of cormorants. The cormorants caused complete loss of remaining fish; however based on observation of fish in the pond prior to cormorant attack, it was estimated that approximately 15,000-20,000 mulloway were in the pond. Estimated survival up to cormorant inundation was therefore about 9-10.5% (G. Searle, pers. comm.).

2.4 Discussion

During this study, wild-caught and captive, tank-held broodstock mulloway were successfully induced to spawn viable eggs. However, capture and transport of mature mulloway from the wild to the hatchery for spawn induction was often difficult. It was often not possible to capture both mature male and female mulloway at the same time. Therefore, it was necessary to collect and store milt for short periods (up to 3 d) in syringes, to allow fertilisation of hand-stripped eggs. Death of broodfish in transporting tanks soon after capture also occurred regularly if fish were caught at depths greater than 3-4m, despite rapid deflation of the swimbladder with a hypodermic needle. We therefore attempted to catch fish by angling or gill netting from ocean beaches and shallow rocky headlands. Mulloway were generally caught in these areas in smaller numbers and less frequently than from deeper offshore reefs.

Eggs were collected from wild-caught mulloway by hand-stripping, while captive fish were either hand-stripped or allowed to spawn spontaneously in tanks following injection with hCG. Although female fish were similar in size (13-15kg), the number of eggs produced by spontaneous spawning was approximately 3 times greater (4.25×10^6 eggs; n=3 fish) than the number produced by hand-stripping (1.25×10^6 eggs; n=2 fish). It was only possible to hand-strip eggs from females once following hormone induction, however eggs were spawned spontaneously for three consecutive nights after induction. Fertilisation of eggs was also generally higher for spontaneous spawns (range 0-95%; 49.7±34.5%, mean±sd) than for hand-stripped spawns (range 0-20.3%; 11.3±10.3%, mean±sd).

It is well known that stress associated with handling of broodfish can have major deleterious effects on the quality of spawned eggs, which can result in poor production and fertilisation of eggs (Carragher & Pankhurst, 1991; Pankhurst & Sharples, 1992). The effects of stress also can be exacerbated with wild-caught fish (Battaglene & Talbot 1992; Pankhurst & Carragher, 1992). Hand-stripping of eggs also relies on prediction of timing of ovulation, and egg quality can be poor due to over or underrippening (Scott *et al.*, 1993). Variations in the latency period may be influenced by a number of factors such as water temperature, hormone dosage, initial oocyte development, and between fish variability. We manipulated hand-stripped broodstock as little as possible, but most fish were handled at least four times. Several hand-stripped female mulloway also died as a result of stress. Similar reports have been made for other sciaenids such as red drum (Henderson-Arzapalo, 1992). Alternatively, broodstock, which were allowed to spawn spontaneously, were only handled once to administer hCG, and produced good quality eggs and remained healthy.

Juvenile mulloway were successfully produced using intensive clearwater, intensive greenwater, and extensive fertilised pond larval rearing methods. Intensive clearwater and greenwater techniques required dedicated, controlled larval rearing and live food production facilities, and high input of labour from trained technicians. Growth of larvae varied slightly between our intensive trials but total length of larvae generally increased by 0.3-0.5 mm/d. This growth was similar to other studies on intensive rearing of mulloway and red drum (Holt, 1990; Battaglene & Talbot, 1994). Survival in clearwater and greenwater cultures was variable between trials but was generally low, and was estimated at 25.0 and 13.0% to 11 (Trial 4) and 20 (Trial 1) dah, respectively. Survival of larvae to fingerlings (25-45 dah) decreased with age and ranged from approximately 1.3 % (Trial 1) to 3.2% (Trial 2). These results are similar to studies conducted by Battaglene & Talbot (1994) who showed that survival of mulloway larvae grown in two intensive tanks and harvested at 23 and 26 dah was 7.3 and 0.002% respectively.

Factors which influenced survival in clearwater and greenwater cultures included cannibalism and infestation of an ectoparasite, *Amyloodinium* sp. Cannibalism was observed around metamorphosis (~25 dah) and continued while fish remained in tanks, despite size-grading every 3-5 d to reduce heterogeneity of fish size. Cannibalism has caused significant problems in intensive culture of mulloway and other sciaenids such as red drum and white sea bass, *Atractoscion nobilis* (Arnold *et al.*, 1976; Soletchnik *et al.*, 1988; Orhun, 1989; Battaglene & Talbot, 1994).

Infestation of larvae and juvenile mulloway by an ectoparasite, *Amyloodinium* spp. occurred in indoor 100 and 2,000 L intensive clearwater tanks, and all outdoor greenwater tanks. Treatment with continual exposure to 25 mg/L formalin was largely ineffective in controlling the parasite and total mortality of infested juvenile mulloway occurred. Infestation by *Amyloodinium* spp. has been reported to cause major mortality during culture of marine fish larvae, juveniles and adults (Lawler, 1977) including sea bream, *Sparus aurata* (Paperna, 1984) and red drum (Johnson, 1990; Sandifer *et al.*, 1993). Control of *Amyloodinium ocellatum* infestation on red drum has been achieved with the antiprotozoal drug, chloroquine (Johnson, 1990). We were unable to use chloroquine in our trials due to the time taken to purchase the drug after identification was made of Amyloodinium sp.. Low salinity (<12 g/L) reduced infestation of sea bream, however it is likely that different populations of *Amyloodinium* spp. have different salinity tolerance (Paperna, 1984).

All intensive production trials in our study were conducted using techniques similar to those developed by Battaglene & Talbot (1994). We therefore used oceanic water (30-35 g/L) in clearwater and greenwater rearing trials. However, aquaculture of mulloway was in its infancy, and little was known of optimum salinity for rearing of larvae and juveniles. Results of preliminary investigations (Trial 5 and 6) showed that larvae and juvenile mulloway grew well at all salinities from 5-35 g/L. However total length and survival of larvae was best at lower salinities of 5-12.5 m/L. Survival of larvae grown at 5-12.5 g/L to 20 dah was 40-60%, which was 4-6 times greater than at 35 g/L. Therefore we were possibly using a suboptimal salinity for the production trials and survival may have been improved if lower salinities had been employed.

During this study, approximately 100,000 juvenile mulloway were produced in experimental (0.025 ha) and production (0.25 and 1.0 ha), fertilised earthen ponds. A highly experienced manager, Mr G. Searle, and a part-time farm technician operated the ponds. Larvae grew to juveniles in all pond rearing attempts, however the age and density at which larvae were stocked affected survival. Care should be taken in interpretation of the data for the trial, which investigated the effect of age of larvae at stocking on survival and growth in ponds (Trial 9). It was not possible to harvest all trial ponds at the same time and larvae from different age

treatments were held in ponds for slightly different periods. We assumed that the different lengths of time that larvae were in ponds had no effect on survival or growth of larvae. In general, survival increased as age of larvae was increased. Stocking of 3 dah larvae in a replicated experiment and in a 0.25 ha pond resulted in approximately 20% survival of juvenile fish; however when larvae were stocked at 20 dah, survival of juvenile fish was 76%. Some variation in survival occurred with stocking of larvae at intermediate ages in these and subsequent density trials, highlighting the fact that production of larval feeds, phytoplankton and zooplankton, in ponds can be variable due to physical and chemical factors such as temperature and salinity, and pond preparation (Rutledge & Rimmer, 1991).

The optimum stocking density of 11 dah larvae into extensive ponds was not clearly defined because of low power (0.3) of the experiment due to high variability within treatments and low number of replicate ponds (Searcy-Bernal, 1994). However, trends in data suggest that there was no difference in survival after 30 days when ponds were stocked initially with 200,000 and 640,000 larvae/ha (5-9% survival). Larvae stocked at a density of 1,040,000 larvae/ha had poor survival (0.7%). These results are similar to production of red drum in fertilised ponds where 2-6 dah larvae are typically stocked at densities of 156,000-1,225,000 larvae/ha however 750,000 is considered optimum (Colura *et al.*, 1976; McCarty *et al.*, 1986; Ray, 1989).

Mulloway larvae grew very quickly in ponds, regardless of age or stocking density. Total length growth rate of larvae in ponds ranged from 1.2-1.7 mm/d, and was 3-5 times faster than larvae grown in intensive tanks. Consequently, much larger juvenile mulloway were harvested from ponds than from intensive tanks after the same growing period. Red drum larvae and juveniles have also been recorded to grow rapidly at 0.7-1.7 mm/d in fertilised ponds (Colura *et al.*, 1976; Arnold *et al.*, 1977; Crocker *et al.*, 1981). A major difference between intensive and extensive pond production, was the lack of requirement for size-grading of fish in ponds. Even without size-grading, survival of larvae to juvenile fish in ponds was much higher than survival in intensive tanks. A reason for this difference in survival may relate to stocking density of fish and cannibalism. Cannibalism can cause catastrophic losses of intensively and extensively reared predatory fish, however it has been reduced in red drum by reducing fish density (Soletchnik *et al.*, 1988). In our study, the final stocking density of intensively reared mulloway was approximately 600-800 fish/m^3, however the maximum density of mulloway reared in ponds (0.25 ha, Trial 11) was approximately 21 fish/m^3.

Cormorants can cause catastrophic losses of pond grown fish (Barlow, 1995) and it was estimated that half of the juvenile mulloway produced in a 1.0 ha pond were consumed by a flock of cormorants. One of the only known methods to avoid this problem is complete enclosure of ponds with bird-proof mesh.

Clearly, production of large numbers of juvenile mulloway was most successful in fertilised ponds. However variability in survival between ponds stocked at similar or different times demonstrates the potential for unreliable production of fingerlings, particularly if newly hatched larvae are stocked. Survival of first feeding fish larvae is strongly influenced by size and density of live food (Tucker, 1992). Zooplankton production in ponds is in turn influenced by uncontrollable climatic factors, thus making availability of optimum food and environmental conditions difficult to control. Alternatively, it is possible to have a high degree of control over supply of live feeds for first-feeding larvae, and environmental parameters, in intensive production systems. Having the option of short-term intensive culture may provide time to optimise pond conditions. Therefore the best strategy to maximise survival and sustainability of production of juvenile mulloway, may be a combination of larval rearing, from first-feeding to 7-14 dah, in intensive tanks, followed by ongrowing in fertilised earthen ponds.

3. Site selection for stock enhancement

3.1 Introduction

The second objective of the project was to stock two intermittent lagoons with some 50,000 juvenile mulloway. Khappinghat Creek (32°01'S, 152°34'E), near Taree, and Swan Lake (35°11'S, 150°33'E), near Nowra, NSW were selected as stocking sites. In addition, a nearby and similar lagoon to each of the stocked sites was selected as a control (unstocked) lagoon. The control lagoons were Deep Creek (30°36'S, 153°00'E) and Lake Wollumboola (34°57'S, 150°46'E). A fifth lagoon, Smiths Lake (32°23'S, 152°28'E), near Forster, NSW was also selected for stocking of juvenile mulloway when Khappinghat Creek was flowing with freshwater and open to the sea as a result of heavy, extended rainfall.

Prestocking fauna surveys of all lagoons (except Smiths Lake) were conducted to determine the suitability of the lagoons for stocking of mulloway; the presence of a wild mulloway population; and as a possible gauge of potential impact of mulloway stocking on local fish and decapod crustacean communities. Hatchery reared mulloway have not been stocked previously into the selected lagoons.

A preliminary toxicant status of Khappinghat Creek and Swan Lake was also investigated. A sampling regime was designed to indicate the general environmental toxicant background into which mulloway was released, and to highlight whether further investigation was necessary. The sampling regime was not scaled to assess the risk of consumption of stocked or native fish associated with any previous use of the environment surrounding the lagoons (Appendix 4).

3.2 Materials and Methods

3.2.1 Prestocking surveys

a) Prestocking sampling of fish and decapod populations of Khappinghat Creek and Deep Creek

A beach seine and gill nets were used to sample fish and decapod crustacean communities. The seine net had a total length of 20 m, a drop of 2 m, and mesh size of 6 mm. Approximately 150 m^2 of shallow water adjacent to the shoreline was sampled with each seine shot. Gill nets consisted of four joined 10 m long panels with a 1m drop. Panels were 25, 50, 75 and 100 mm mesh, and were butted together in random order. Each creek was subjected to four seine shots, conducted during daylight hours in water generally shallower than 1.8 m. Four bottom-set gillnets were set in each creek 30 minutes before nightfall. Nets were set in the deepest sections of the waterways, in water generally 2- 4 m deep. Clearing of the first net began 2 h after setting.

A secchi disc and a multifunction electronic meter were used to measure water quality parameters.

b) Prestocking sampling of fish and decapod populations of Swan Lake and Lake Wollumboola

Fauna samples were conducted with a beach seine and gill nets. The beach seine and hauling technique was the same as for Khappinghat and Deep Creeks. A total of 10 replicate hauls were completed. Gill nets consisted of ten 5 m panels of 13, 19, 25, 32, 38, 45, 50, 50, 55, 60 and 70 mm meshes butted together. Nets had a fall of 2 m, and were set in 2-4 m deep water. Setting of nets was difficult due to an abundance of benthic macroalgae, *Ruppia* spp. in both lakes. Therefore, the number of available sites for independent sampling in both lakes was restricted.

A secchi disc and a multifunction electronic meter were used to measure water quality parameters.

c) Precursory assessment of the nutrient and toxicant status Khappinghat Creek and Swan Lake.

Samples of water, sediment, fish and invertebrates were taken for analysis for heavy metals and organochlorines. Four sites in each lagoon were selected for water and sediment samples.

Tissue samples were taken from specimens collected at accessible sites. All samples were placed immediately on ice, and frozen as soon as possible after collection (4-8 h) (Appendix 4).

d) Preliminary samples of fauna and sediment for Smiths Lake.

Preliminary samples of fauna and sediment were not completed for Smiths Lake due to the limited time following a decision to stock juvenile mulloway. When juvenile mulloway were available for stocking in 1997, the selected lagoon, Khappinghat Creek, was flowing to the sea and completely freshwater and was therefore not suitable for stocking.

3.3 Results and Discussion

3.3.1 Prestocking surveys

a) Prestocking sampling of fish and decapod populations of Khappinghat Creek and Deep Creek

There was an abundance of potential forage species for juvenile mulloway in both lagoons, however native stocks of mulloway were not encountered. The degree of sample replication was sufficient to measure species diversity, but was not enough to provide an estimate of species abundance. The seine net generally captured small, non-commercial and juvenile commercial fish species less than 15 cm in length. Gill nets mainly captured commercial fish species greater than 7 cm in length. At the time of sampling, Deep Creek was strongly influenced by heavy rainfall, which resulted in low salinity (Table 5). However the salinity in Deep Creek was still suitable for survival of juvenile mulloway (Gray & McDonall, 1993; Fielder & Bardsley, in press).

b) Prestocking sampling of fish and decapod populations of Swan Lake and Lake Wollumboola

No wild mulloway were captured from either of the southern lagoons, however an abundance of benthic macroalgae made it impossible to effectively set gill nets in areas, such as deep holes, where mulloway may have been present. The sampling regime was sufficient to show population diversity, however estimation of the abundance of some of the more patchily distributed fish species was not possible. The seine net generally captured small non-commercial and juvenile commercial species less than 15 cm in length. Gill nets mainly captured commercial species greater than 7 cm in length. Species diversity in the southern lagoons was much lower than the northern lagoons with only 20 fish species and no decapods captured, compared to 42 fish and 9 decapod species, respectively. Consequently, the variety

of potential mulloway forage was less; with the prolific numbers of small mouth hardyhead (81% of catch) being the most likely staple diet. Water quality parameters for both lagoons were similar and considered suitable for juvenile mulloway (Table 6).

Table 5. Characteristics of the northern lagoons which were selected for stocking of juvenile mulloway.

	Khappinghat Creek	**Deep Creek**
Location	south of Taree	North of Nambucca Heads
Surface area (km^2)	1.0	1.0
Depth range (m)	0-5	0-5
Likely opening regime	usually closed	usually open
Surrounding land use	native bush	bush, farm, some urban
Commercial fishery	yes	yes
Seagrass	yes	yes
Mangroves	no	yes
History of mulloway	yes	yes
Water quality measurements		
Salinity (g/L)	20	3-5
Temperature (°C)	23.6 - 26.6	22.5
Dissolved oxygen (mg/L)	1.7 - 2.4	4.2 - 5.8
Secchi depth (m)	0.3 - 0.45	0.3 - 0.45
Sampled catch		
Dominant fish species (>5%)	flathead gudgeon (30%) pacific blue-eye (25%) transparent goby (18%) southern herring (10%) port jackson perchlet (5%)	port jackson perchlet (65%) transparent goby (14%) ramsey's perchlet (5%)
Dominant decapods	acetes (~100%)	grass shrimp spp. (68%) common grass shrimp (30%)

Table 6. Characteristics of the southern lagoons which were selected for stocking of juvenile mulloway.

	Swan Lake	**Lake Wollumboola**
Location	east of Nowra	south of St. Georges Basin
Surface area (km^2)	14.1	6.2
Depth range (m)	0-5	0-3
Likely opening regime	usually closed	usually closed
Surrounding land use	mostly native bush	mostly native bush
Commercial fishery	yes	yes
Seagrass	yes (*Ruppia*)	yes (*Ruppia*)
Mangroves	no	no
History of mulloway	yes	none known
Water quality measurements		
Salinity (g/L)	15	18 - 19
Temperature (°C)	23.1 - 24.5	23.5 - 25.0
Dissolved oxygen (mg/L)	7.3 - 7.8	9.6 - 9.8
Secchi depth	clear	clear
Sampled catch		
Dominant fish species (>5%)	small mouth hardyhead (81%) mosquitofish (6%)	small mouth hardyhead (81%) yellowfin bream (7%) snub-nosed goby (6%)

4. Stocking of fingerlings into intermittently opening lagoons

4.1 Introduction

As juvenile mulloway became available from clearwater intensive trials at PSRC and extensive pond trials at Palmers Island, they were stocked into three intermittently opening lagoons to provide preliminary information on the feasibility of stock enhancement of mulloway. Although some stocked juvenile mulloway had been reared in different conditions, juveniles were of similar size, most had been trained to consume artificial pellet diets, and all mulloway were held in tanks at PSRC for several days following harvest from ponds. Therefore, it is unlikely that there were behavioural differences between intensively and extensively reared juveniles, which may have affected their fitness for survival after release into the lagoons.

4.2 Materials and Methods

General

Following harvest from tanks or ponds, juvenile mulloway were chemically marked for identification and transported to the lagoons for release. Fish were transported (maximum 8 h) in two, 750 L insulated, fibreglass tanks which were filled with filtered (10 μm) seawater (30-35 g/L) and provided with oxygen at 1-2 L/min. Fish were not fed in transit.

Prior to release into the lagoons, fish were acclimatised by draining half of the tank volume and then slowly refilling with lagoon water. This procedure was repeated at least twice to ensure minimal transfer shock. Fish were then scooped into 10 L buckets and released into the lagoon. The time of release into the three lagoons was similar and was between 1400-1800 h.

4.2.1 Chemical tagging of fingerlings

Attempts were made to chemically mark all mulloway juveniles before release into selected intermittently opening coastal lagoons. Two forms of marking were employed. Fish were either bathed in a solution of oxytetracycline hydrochloride (OTC; 50-100 mg/L) alone, or, bathed in a solution of OTC and strontium chloride (1-5 mg/L for 24 h).

(i) OTC marking

The antibiotic, OTC, was used to chemically stain the fish's bony structures. OTC is also routinely used to preserve water quality, by reducing bacteria proliferation, during transportation of fingerlings, and as a treatment for bacterial infection, which commonly manifests after harvesting and handling of fish. For these reasons, individual batches of juvenile mulloway had varying exposure to OTC, dependant on their time in transportation (4-8 h) and the need for post-harvest prophylactic treatment. Independent of the purpose of exposure, OTC was always administered as a bath at concentrations of 50-100 mg/L.

Approximately 100 juvenile mulloway from each of the marked and unmarked cohorts were retained in tanks (10,000-100,000 L) at PSRC to determine the retention time of the OTC marks. Some fish were sacrificed and processed at the time of release, while others were ongrown for comparison with recaptured fish from the stocked lagoons.

Fish were captured and killed and their saggital otoliths removed. Otoliths were set in resin, then thin sectioned transversely through the centre. Sections were glued to a microscope slide beneath a cover slip. Microscopic inspection under incident ultraviolet illumination was carried

out to detect fluorescent marks. Two operators evaluated otolith sections, and positive results were accepted only when both operators confirmed observation of a mark.

(ii) Strontium marking

Strontium can substitute for calcium in the bony structures of fish. Fish were bathed in strontium chloride at concentrations of 1 and 5 mg/L for 24 hours prior to release. Saggital otoliths were removed from control and recaptured fish. Digestion and analysis of whole otoliths was carried out at The Water Studies Centre, Monash University, Victoria, Australia. Otoliths were weighed and dissolved in nitric acid, and potassium chloride added as an ionisation suppressant. An atomic absorption spectrophotometer was used to analyse solutions over a nitrous oxide/acetylene flame. Calibration standards were compared against certified reference materials. The accuracy of strontium concentrations was validated through addition of known strontium spikes to some digestates to ascertain percentage recovery.

(iii) Statistical analyses

Incidence of OTC and strontium marks of otoliths was analysed using single factor ANOVA. Homogeneity of variance was assessed using Cochran's Test. Data for strontium concentrations of otoliths from mulloway captured in Smiths Lake were transformed by Log $(x+1)$ to satisfy the requirements of homogeneity of variance. Multiple comparison of means was assessed using SNK procedure. The significance level was set at $P<0.05$.

4.2.2 Stocking of fingerlings

A total of approximately 25,000 juvenile mulloway was produced in 1996. This production was much lower than anticipated and resulted in only one lagoon, Khappinghat Creek, being stocked in 1996 (Table 7).

Production of juvenile mulloway in 1997 was improved, and large numbers of fish were grown in earthen ponds. This allowed a further two intermittent lagoons to be stocked with juvenile mulloway. Swan Lake was stocked on two occasions, with a total of 28,000 juvenile mulloway of approximately 2.0 g/fish (Table 7). An initial post-stocking survey of Khappinghat Creek in December 1996 failed to recapture any mulloway. A second stocking of mulloway into Khappinghat Creek was therefore planned for early 1997, however extended periods of rainfall resulted in this lagoon opening and flowing rapidly to the sea, and reducing markedly in salinity, making it unsuitable for stocking. Although a prestocking survey had not been completed, a decision was made to stock a large, intermittently opening lagoon, Smiths Lake, which was located near Khappinghat Creek. Smiths Lake has an active commercial

fishery and mulloway have been captured previously (C. Bramble, commercial fisher, pers. comm,). Smiths Lake was stocked on three occasions, with a total of 21,600 juvenile mulloway ranging in size from approximately 2.0-11.8 g/fish (Table 7).

Table 7. Date, number and size of juvenile mulloway stocked into three intermittently opening lagoons in NSW.

Lagoon	Date of Stocking	Age of fish (dah)	Number of fish	Mean total length (mm)	Mean weight (g)
Khappinghat Creek	11/01/96	45	25 000	40.0	0.7
Swan Lake	24/02/97	45	11 000	50.0	1.7
	03/03/97	52	17 000	52.0	1.9
Smiths Lake	27/02/97	48	7 600	58.0	1.9
	07/03/97	56	10 000	65.0	2.5
	23/03/97	103	4 000	106.0	11.8

4.2.3 Post stocking surveys

Post stocking surveys were conducted to determine potential survival, growth and diet of stocked mulloway. Several recaptured mulloway were also analysed for toxicants (Appendix 4).

a) Assessment of stocking of Khappinghat Creek and Swan Lake

Attempts were made in both lagoons to recapture mulloway juveniles approximately six months after stocking, using replicated gill netting procedures. Gill nets were the same as those described in the prestocking sampling of Swan Lake. Four sites with a high probability of attracting juvenile mulloway, e.g. deep holes, were selected in each lagoon. The gill nets were set 30 min prior to sundown. Checking of the first net began two hours later, and clearing of the final net was completed seven hours after setting.

b) Assessment of stocking of Smiths Lake

Professional fishers assisted with collection of mulloway while targeting commercial species such as black bream and flathead. Commercial seine and flathead gill nets were used. If

juvenile mulloway were caught, a random sample of mulloway was taken. The sampled fish were frozen and transported to PSRC.
Sampled mulloway were then thawed, weighed and measured to assess growth rates. Saggital otoliths were removed to determine if they were chemically marked. Gut content of the second and third recapture groups were identified to assess diet. Fillets and livers of the second group of recaptures were analysed for toxicants.

4.3 Results

4.3.1 Post stocking surveys

a) Assessment of stocking of Khappinghat Creek and Swan Lake

No juvenile mulloway were captured with the gill nets in either lagoon. Effective gill netting was difficult in Swan Lake due to dense benthic macroalgae blooms. Both Khappinghat Creek and Swan Lake had opened to the sea following heavy rainfall in May 1996 (4 months after stocking) and May 1997 (3 months after stocking), respectively. Anecdotal information suggested that juvenile mulloway were observed swimming through the outlet to sea from Swan Lake (M. Angle, NSW Fisheries Officer, Nowra; pers. comm.).

b) Assessment of stocking of Smiths Lake

Professional fishers began reporting incidental capture of juvenile mulloway approximately five months after the first release of juveniles into the lagoon. Three samples of recaptured fish were collected from the deepest section of the lake using seine nets. Fishers indicated they were initially catching and releasing up to 200 juvenile mulloway in a single seine shot (C. Bramble, pers. Comm., 1997). A final sample was taken from flathead nets set around the lake's perimeter. Smiths Lake was manually opened to the sea for several weeks in June 1997 (2-4 months after stocking) due to rising water level (J. Gibson, pers. comm., 1997)

Toxicant levels in the second group of recaptured fish are detailed in Appendix 4.

4.3.2 Chemical tagging of fingerlings

(i) Oxytetracycline marking

Interpretation of saggital otoliths removed from marked fish, which were ongrown at PSRC, showed that initial marking had been successful. However, fluorescent marks were not observed on all control otoliths. This was probably due to the difficulty in sectioning the otoliths accurately through the centre where the mark was most likely to be evident. Otoliths

were removed from three groups of mulloway capture from Smith's Lake on three separate occasions. Fluorescent rings were detected in otoliths of each group of fish. There was no significant difference ($P>0.05$) between the rate of marking of the ongrown and recaptured groups (Table 8). These data indicate that many captured mulloway had been stocked from PSRC from February to April 1997.

Table 8. Number of otoliths of control and recaptured mulloway from Smiths Lake with fluorescent Oxytetracycline (OTC) mark.

Origin of otolith	Number of otoliths sectioned	Number otoliths with detected OTC mark	Marked otoliths (%)
Marked control siblings ongrown at PSRC	19	16	84
Recapture date			
11/08/97	21	13	62
30/09/97	23	20	87
03/11/97	15	11	73

The percent number of otoliths with fluorescing marks did not differ significantly (ANOVA, SNK, $P>0.05$).

(ii) Strontium marking

In 1996, preliminary evaluation of two groups of strontium bathed (1 mg/L) mulloway held in seawater tanks at PSRC for 10 d after marking showed that strontium had been successfully absorbed into the otoliths (Fig. 7). Concentration of strontium in otoliths of the two marked groups was significantly ($P<0.05$) higher than in unmarked control otoliths. There was no significant difference between the marked groups (Fig. 7).

Analysis of otoliths of mulloway stocked into Smiths lake in 1997 also showed that strontium had been successfully absorbed by mulloway after a 24 h bath in strontium chloride (1 mg/L) (Table 9). However, the concentration in the otoliths was half (2.0 mg/g) that of otoliths in fish from 1996 (approximately 4.0 mg/g).

Concentration of strontium in juveniles that were marked prior to stocking was significantly ($P<0.05$) greater than unmarked fish stocked 47 d later, and fish recaptured 110, 160 and 194 d after stocking. There was no significant difference between concentration of strontium in otoliths of recaptured mulloway (Fig. 8; Table 9).

Figure 7. Saggital otolith concentration of strontium achieved in three batches of mulloway, when immersed in 0 mg/L (control) or 1 mg/L (batch 1 and 2) strontium chloride solution for 24 h. Data are means ± se (n=20). Bars with common superscripts are not significantly different (P>0.05).

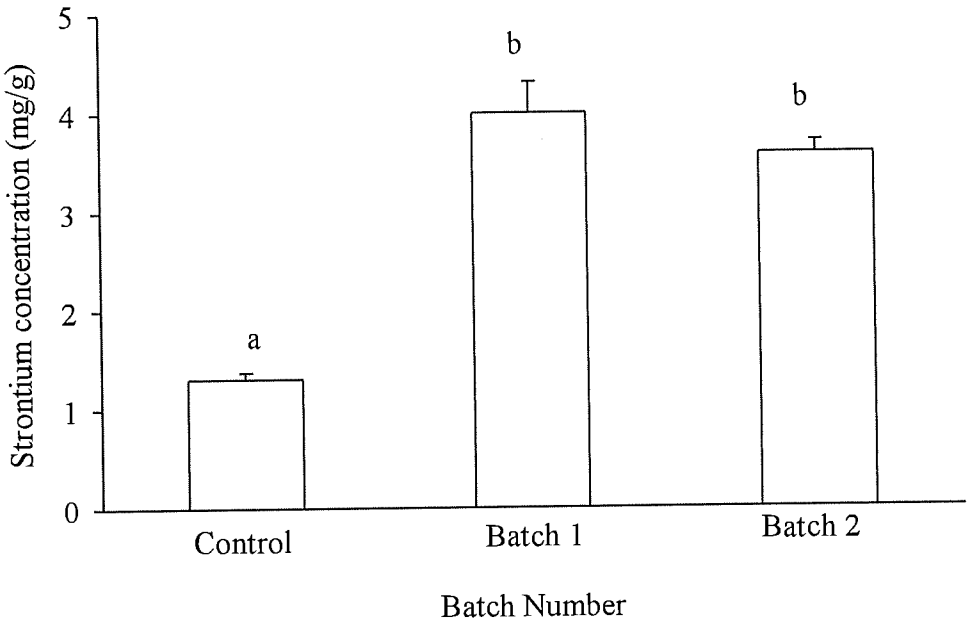


Table 9. Concentration of strontium in saggital otoliths of mulloway stocked into and recaptured from Smiths Lake.

Origin of otolith	Number of otoliths	Strontium concentration (mg/g)
Marked fish stocked 07/03/97	38	2.02 ± 0.09^{a}
Unmarked fish stocked 23/04/97	21	1.71 ± 0.12^{b}
Recaptured fish		
11/08/97	22	1.38 ± 0.05^{c}
30/09/97	25	1.32 ± 0.05^{c}
03/11/97	17	1.25 ± 0.08^{c}

Data are means ± se. Means for strontium concentration with the same superscript are not significantly different (ANOVA, SNK, P>0.05)

4.3.3 Growth of recaptured mulloway

Mulloway recaptured from Smiths Lake grew quickly after stocking. The daily increment in total length remained constant between sampling events (autumn-winter) and was approximately 1.0 mm/d (Fig. 9). It was possible to extrapolate the collected data to provide an estimate of the time taken for mulloway to reach legal length (450 mm). An estimate of approximately 500 days from hatch to legal size was validated by professional fishers who began catching and marketing legal length fish at this time (C. Bramble, commercial fisher, pers. comm, 1998) (Fig. 9).

4.3.4 Gut contents of recaptured mulloway

The gut content of the mulloway recaptured from Smiths Lake on 30/09/97 and 03/11/97 (249.5±12.5 [n=24] and 278.1±17.5 [n=15] mm; mean±sd TL, respectively) showed a variation in the primary food source (Appendix 5). Gut contents of mulloway recaptured on 30/09/97, consisted of approximately 58% prawns and 36% fish. Gut contents of mulloway which were captured 34 days later was different to the previous sample and showed a shift in dominant species to fish. Fish and prawns comprised approximately 86 and 14 % of prey items, respectively.

Fig. 8. Concentration of strontium in saggital otoliths of mulloway stocked into and recaptured from Smiths Lake. Points show relative mass of individual otoliths.

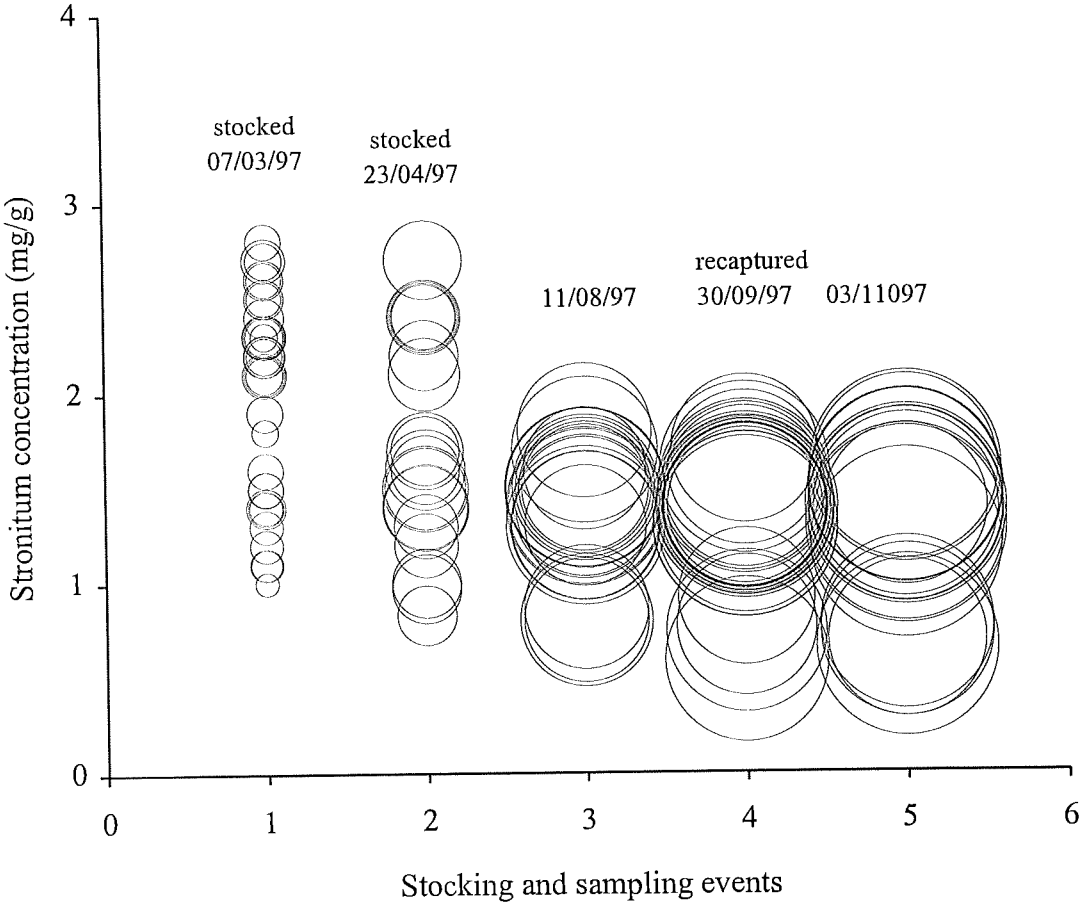
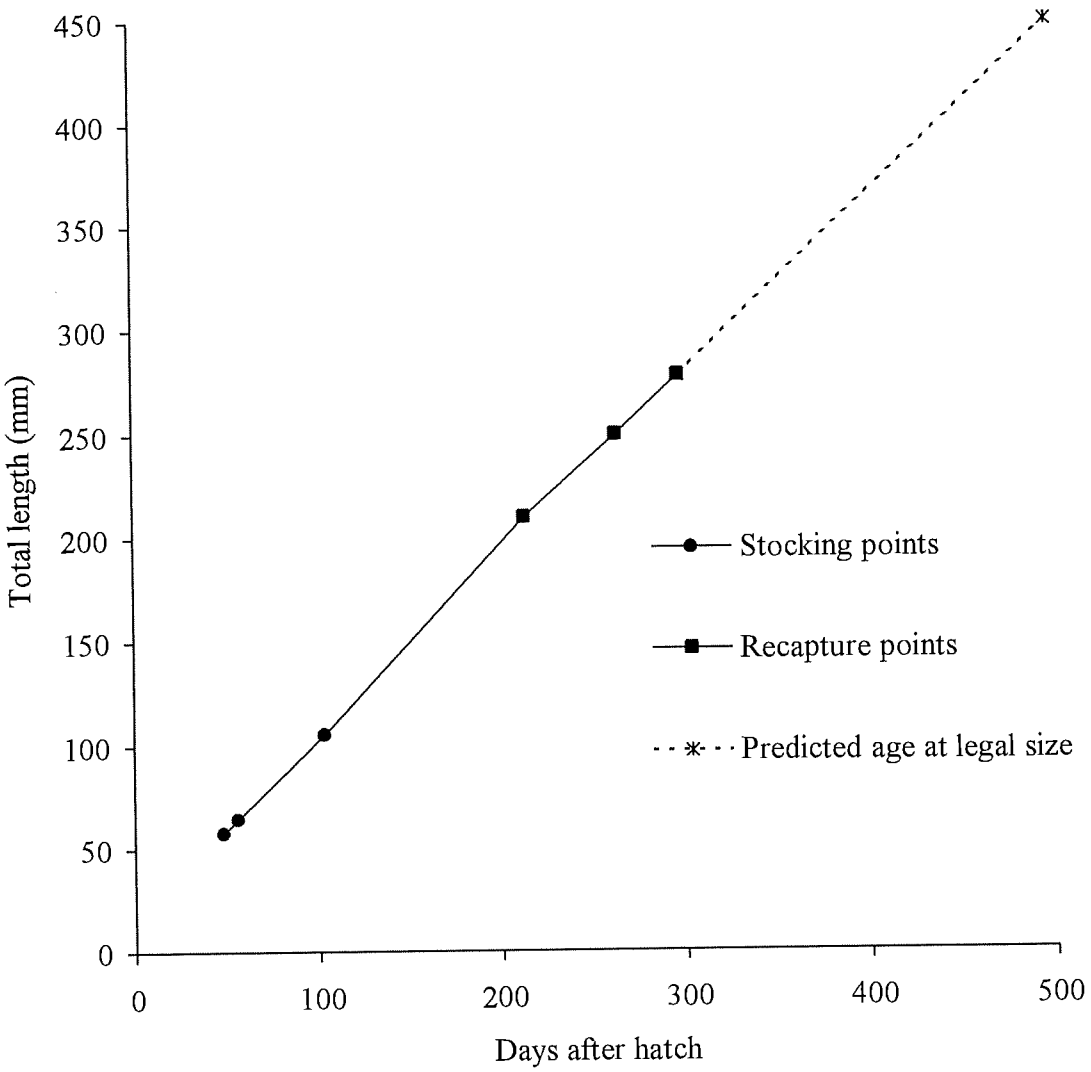


Fig. 9. Change in length of juvenile mulloway recaptured from Smiths Lake. Data are means (n= 17-22 fish).



4.4 Discussion

Approximately 75,000 juvenile mulloway were released into three intermittently opening coastal lagoons during the summers of 1996 and 1997. It is not known whether enhancement of mulloway in Khappinghat Creek and Swan Lake was successful, as post stocking surveys failed to catch any mulloway. Both of these lagoons had opened to the sea for several weeks after stocking of mulloway, therefore it is possible that mulloway escaped from the lagoon. Anecdotal information from NSW Fisheries compliance officers of observations of mulloway swimming through a narrow outlet in Swan Lake supports this theory. Another reason for failure to recapture mulloway in Swan Lake may be that dense benthic macroalgae blooms prevented effective setting of gill nets, and fish simply avoided capture. Alternatively, stocking was not successful.

Stock enhancement of mulloway in Smiths Lake was however very successful. Hundreds of juvenile mulloway were caught incidentally on many occasions by commercial fishers targeting other fish species. Analysis of otoliths of sampled fish for OTC stains demonstrated that the mulloway had been stocked by us in 1997. Strontium was also used to initially mark our artificially propagated juvenile mulloway, however we were unable to detect any similarities in concentration of strontium in captured and initially marked mulloway. We had hoped to possibly distinguish between fish stocked at different times by treating fish with different combinations of OTC and strontium, (OTC + strontium; OTC - strontium), however lack of similarity to recaptured and initially marked fish did not allow this. Although strontium has been used to mark saltwater fish, problems with a reduction in the strontium mark have been caused by dilution due to growth of the fish (Behrens Yamada et al., 1979; Behrens Yamada & Mulligan, 1987). Behrens Yamada & Mulligan (1982) demonstrated that when whole vertebrae of sea run coho salmon, *Oncorhynchus kisutch*, were examined, strontium marked fish could not be detected from controls. However, when central cores of individual vertebrae were studied, strontium marked fish could be detected. We examined whole otoliths; therefore dilution with growth may have reduced our ability to detect marked fish. Processing of individual samples for strontium required specialist equipment and technicians and was expensive ($8.00/otolith). Based on these results we do not recommend strontium for marking of mulloway for stocking programs.

Growth of recaptured mulloway was rapid (approximately 1.0 mm/d) and continual regardless of cooler winter temperatures. This was similar to red drum where juveniles also grow continuously for the first 300 dah without slowing during winter (Matlock, 1984). Observed total length growth rates of red drum in the wild have ranged from 0.4-1.03 mm/d (Matlock & Weaver, 1979; Matlock et al., 1986). The continual growth pattern of the mulloway allowed prediction of approximately 500 dah to reach legal size (450 mm). Commercial fishers

validated this prediction by catching and selling mulloway from Smiths Lake around this time (C. Bramble, pers. comm.).

Preliminary investigation of the gut contents of two size groups (250 and 280 mm TL) of captured mulloway showed that commercial and non commercial prawn and fish species were consumed, and that there was a marked shift in preference from prawns to fish as mulloway increased in size. Comment on the possible reasons for the shift in prey preference, or the possible impacts that the mulloway may have had on the prey or ecosystem in general, is beyond the scope of this project. Clearly, there is a need to examine in detail the environmental aspects of future stock enhancement projects.

These results demonstrate that hatchery reared juvenile mulloway could (1) be produced in large numbers to allow stocking of relatively large (up to 6 km^2) intermittently opening lagoons; (2) be recaptured and identified as stocked fish; (3) grow quickly; and (4) enter the recreational and commercial fishery approximately 500 days after stocking. A number of similarities in the performance of mulloway and red drum stocked into an estuarine environment were demonstrated during this study. Based on this information and the fact that large-scale stock enhancement of juvenile red drum in several bays of southern USA has been extremely successful resulting in the doubling of the historic mean harvest rates in these systems (Rutledge, 1989), there is excellent potential for similar stock enhancement programs in temperate coastal areas of Australia to be successful.

5. Benefits

This project will directly benefit the aquaculture industry, in particular marine hatcheries and prawn farmers who can now produce juveniles for reseeding programs and grow-out operators. It will also provide a cheap source of fingerlings for stocking into sea cages, land based ponds or closed systems such as coastal lagoons. In addition, it may benefit the commercial fishers and recreational anglers of New South Wales. Commercial fishers operating in New South Wales estuaries could, if stock enhancement was completed, benefit from a) having reduced conflict with recreational anglers and b) increased mulloway stocks. Another benefit of being able to stock large numbers of hatchery produced fish will occur in areas which do not normally sustain mulloway populations due to naturally poor larval recruitment e.g. intermittently opening lagoons.

This project therefore constitutes research to enlarge and improve the efficiency of the seafood industry in two main ways. Firstly, it will aid development of a new industry that will a) provide new employment opportunities, b) produce a new aquaculture product, c) assist to replace imports and create opportunities for export. Secondly, it may provide an alternative

management option to the closure of valuable traditional fisheries in estuaries brought on by pressure from recreational anglers.

6. Further Development

Our project demonstrated that large numbers of juvenile mulloway can be propagated, and stock enhancement of mulloway into intermittently opening lagoons is feasible. However it was beyond the scope of the project to determine the environmental and social impacts of stocking large numbers of predatory fish into these lagoons. Clearly, there is a need to investigate these issues associated with the impacts of stocking mulloway, in a co-ordinated fashion which will involve researchers and managers from disciplines such as aquaculture, fisheries and conservation.

7. Conclusions

All objectives for the project were successfully completed.

In excess of 100,000 juvenile mulloway were successfully produced using intensive clearwater, intensive greenwater, and extensive fertilised pond techniques. Intensive hatchery production of mulloway required expensive, dedicated live food and larval fish rearing facilities and a high input of labour from skilled technicians. By contrast, extensive pond rearing was possible in multi-purpose earthen ponds using relatively low input of experienced labour.

Fertilised eggs were obtained by hand-stripping or spontaneous spawning from hormone induced wild and captive, tank-held broodfish. The quality, quantity and reliability of production of eggs were greater for spontaneously spawned fish. Capture of wild fish for hormone induction was also difficult and time consuming. We therefore recommend the establishment of dedicated mulloway broodstock facilities for large-scale production of juvenile mulloway.

Growth and survival of mulloway larvae to metamorphosis was generally higher in extensive ponds than in intensive tanks. Survival of larvae in ponds increased as the age of larvae at stocking was increased. The optimum density for initial stocking of mulloway was similar to that of other sciaenids and ranged from 200,000 to approximately 640,000 larvae/ha.

Regardless of the age or density of stocked larvae, their growth in ponds was approximately 3-5 times faster than in intensive tanks. Despite regular size-grading, cannibalism was prevalent in intensive tanks, and may have contributed to the low survival rate for larvae. Size-grading was not conducted in ponds.

Larval and juvenile mulloway grew well at salinities from 5-35 g/L, however growth and survival was best at low salinities, 5-12.5 g/L. Intensive production trials had been conducted at salinity of 30-35 g/L, and survival may have been better if lower salinities were employed.

Significant mortality in intensive trials was caused by infestation of an ectoparasite, *Amyloodinium* sp.. Long-term exposure to 25 mg/L formalin was not effective in controlling the parasite. Cormorants caused major mortality of juvenile mulloway grown in an un-netted 1.0 ha pond.

There was variation in performance success of juvenile mulloway in ponds stocked at different times. This was probably a result of variations in the management of plankton blooms, which can be difficult as productivity is directly related to uncontrollable environmental factors such as temperature, salinity and light intensity.

A strategy of an initial period of intensive rearing followed by final growout in fertilised ponds may optimise sustainable production of juvenile mulloway.

Approximately 75,000 juvenile mulloway were stocked into three intermittently opening lagoons. Survival of juveniles in one lagoon was demonstrated by capture by commercial fishers of large numbers of mulloway which were identified as hatchery-reared fish by fluorescing OTC stains on their otoliths. Mulloway were not recaptured from two of the lagoons.

Mulloway grew quickly and constantly at approximately 1.0 mm/d total length increment, regardless of winter temperatures. Hatchery-reared mulloway would reach legal size of 450 mm total length in Smiths Lake in approximately 500 dah.

Large numbers of juvenile mulloway were produced and successfully stocked into an intermittently opening lagoon. Many attributes of mulloway production were similar to the red drum, which has been successfully stocked to enhance wild fisheries in several parts of southern USA.

Our results suggest that there is excellent potential for stock enhancement of mulloway into temperate Australian marine and brackish waterways. However, further investigation of all possible impacts of stocking is warranted before this can be progressed.

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9. Appendices

9.1 Appendix 1: Intellectual Property

No patentable inventions or processes have been developed as part of this project. All results will be published in relevant scientific articles and other public domain literature.

9.2 Appendix 2: Staff

The following members of staff were employed on the project:

Mr Stewart Fielder	Principal Investigator
Dr Geoff Allan	Principal Investigator
Mr Bill Bardsley	Technical Officer
Mr Paul Beevers	Technical Officer
Mr Paul Mannix	Technical Officer (temporary)
Mr Craig Knott	Technical Officer (temporary)

9.3 Appendix 3: Fielder, D. S. & W. J. Bardsley. A preliminary study on the effects of salinity on growth and survival of mulloway *Argyrosomus japonicus* larvae and juveniles. Journal of the World Aquaculture Society (in press).

A Preliminary Study on the Effects of Salinity on Growth and Survival of Mulloway Argyrosomus japonicus Larvae and Juveniles.

D. S. FIELDER[1] AND W. BARDSLEY

NSW Fisheries, Port Stephens Research Centre

Taylors Beach Road, Taylors Beach

NSW 2316 Australia

[1] Corresponding Author

NSW Fisheries, Port Stephens Research Centre

Taylors Beach Road, Taylors Beach

NSW 2316 Australia

Two experiments were conducted to determine the effects of salinity on growth and survival of mulloway (Argyrosomus japonicus) larvae and juveniles. First, 6 d old larvae were stocked into different salinities (5, 12.5, 20, 27.5 and 35 ppt) for 14 d. Larvae grew at all salinities, but based on results for growth and survival, the optimum range of salinity for 6 - 20 day old larvae is 5 - 12.5 ppt. During this experiment larvae held in all experimental salinities were infested by a dinoflagellate ectoparasite, Amyloodinium sp.. Degree of infestation was affected by salinity. There were very low infestation rates at 5 ppt (0.2 parasites/larva), infestation increased with salinity to 20 ppt (33.1 parasites/larva), then declined with salinity to 35 ppt (1.5 parasites/larva). For the second experiment, juveniles (6.1±0.1 g/fish) were stocked into different salinities (0.6, 5, 10, 20 and 35 ppt) for 28 d. Juveniles were removed from freshwater, 3 d after transfer, as they did not feed, several fish died and many fish had lost equilibrium. However, when transferred directly to 5 ppt, these stressed fish recovered and behaved normally. Trends in final mean weight and food conversion ratio of juvenile mulloway suggest that fish performed best at 5 ppt. Although salinity (5 to 35 ppt) had no significant ($P>0.05$) effect on growth, survival, or food conversion ratio of juveniles, statistical power of the experiment was low (0.22). Based on these results we recommend that mulloway larvae older than 6 d, be cultured at 5 to 12.5 ppt. Optimum growth of juveniles may also be achieved at low salinities.

The mulloway (Argyrosomus japonicus) is a large, estuarine sciaenid distributed around southern Australia, southern Africa, Pakistan, India, Hong Kong, Korea and Japan. In South Africa and Australia, mulloway (previously described as A. hololepidotus) is a highly desired commercial and recreational species (Griffiths and Heemstra 1995) and there is interest in development of techniques for production of fish for enhancement of wild stocks and aquaculture (Battaglene and Talbot 1994; Fielder and Bardsley 1997).

The Port Stephens Research Centre (32° 45'S, 152° 04'E) was the first hatchery to propagate mulloway artificially (Battaglene and Talbot 1994) using techniques based on those developed for intensive rearing of Australian bass (Macquaria novemaculeata) and snapper (Pagrus auratus) (Battaglene et al. 1989; Battaglene and Talbot 1992). Similar hatchery methods have been used to culture several other species of sciaenids such as red drum (Sciaenops ocellatus) (Holt et al. 1990), and orangemouth corvina (Cynoscion xanthulus) (Prentice and Colura 1984; Prentice et al. 1989).

The biology and ecology of A. japonicus in Australia are not well understood, however, in South Africa it is thought that adults spawn in inshore waters and larvae and juveniles recruit to nearshore areas and estuaries (Wallace 1975; Smale 1985; Smale and Bruton 1985; Beckley 1990). Gray and McDonall (1993) showed that juveniles entered a south eastern Australian estuary in late summer-winter and tended to congregate in areas where salinity was brackish (range 5 to 25 ppt), but relatively large numbers were also caught in areas with salinities of 0 to 2 ppt at the time of sampling. In contrast with other studies (Anon. 1981), few juveniles were caught in the predominantly marine sites, closest to the mouth of the estuary. This information suggests that juvenile mulloway can tolerate a wide range of salinities, however it does not indicate the effect of salinity on growth and survival. This information is necessary to maximize hatchery production, and select the best sites for stock enhancement and culture.

We examined the influence of salinity on growth and survival of mulloway larvae from 6 to 20 days after hatching (dah), and juveniles (6.1 g/fish initial weight) grown for 28 days. We also examined the effect of salinity on the abundance of a dinoflagellate ectoparasite, Amyloodinium sp., which developed during the larval rearing study.

Materials and Methods

General

Fertilized mulloway eggs were obtained using techniques similar to those described by Battaglene and Talbot (1994) for broodstock collection, maintenance, and hormone induction of spawning. On 6 March 1997, a total of 4,800,000 eggs with a fertilization percentage of 34% were obtained after two, 13 kg female mulloway spawned naturally in a 35,000 L seawater (35 ppt; 24±1 C) tank at the Fisheries Research Institute (FRI, Cronulla, New South Wales [NSW], Australia).

Fertilized eggs were road-freighted (4 h) in two, 750 L transporting tanks filled with 1 µm filtered seawater (35 ppt; 24±1 C) from FRI to the Port Stephens Research Centre (PSRC) hatchery. Equal numbers of eggs were then transferred by bucket from the transporting tanks into six, 2,000 L conical-bottomed tanks with black sides and a white bottom. The tanks were housed in a photoperiod controlled room. Each 2,000 L tank was filled with seawater (33 ppt; 23±1 C), which had been treated with sodium hypochlorite solution (12.5% active chlorine) at a rate of 0.2 mL/L and neutralized with sodium thiosulphate after 24 h. The tanks were lightly aerated (200 mL air/min) and water was recirculated through a mechanical and biological filter at 5 L/min. Tanks were siphoned daily to remove unhatched eggs and detritus. Seawater was replaced with sterilized water as required.

Larvae began hatching at 1730 on 6 March 1997 and were kept in the dark until 3 dah. Surface skimmers were supplied to remove surface films and thus encourage swim bladder inflation (Chatain and Ounais-Guschemann 1990; Battaglene and Talbot 1994). From 4 dah,

fluorescent light (Philips White TLA 40W 33QS) was provided at 42±26 lux (mean ± standard deviation [sd]; n=10) on a 14 : 10 h light : dark cycle. A standard feeding regime was used while larvae remained in the 2,000 L tanks (Experiment 1 to 6 dah; Experiment 2 to 11 dah) and for the duration of Experiment 1. The feeding regime was: Rotifers, Brachionus plicatilis, were fed to larvae from 4 dah at a density of 10/mL. Uneaten rotifers were removed from the tank each night by passing recirculated water through a 53 μm filter bag. Rotifer density was returned to 10/mL each morning by adding newly harvested rotifers. Rotifers were nutritionally enriched by feeding Tahitian Isochrysis aff. galbana and Pavlova lutheri (Battaglene and Talbot 1994), and DC DHA Selco (Inve Aquaculture NV, Oeverstraat 7-01200, Baasrode, Belgium) for 24 h before harvest.

At 6 dah, one tank of larvae was harvested and stocked into tanks allocated for Experiment 1. At 11 dah, two tanks were harvested and larvae were transported to a commercial hatchery at Palmers Island, NSW and stocked into a 0.25 ha fertilized earthen pond filled with 20 ppt water for ongrowing, using techniques similar to those described by Battaglene et al. (1992). At 42 dah, 1,000 juveniles were harvested from the pond and transported back to PSRC, where they were held for 83 d in a 10,000 L, flow-through, seawater tank (range 30 to 35 ppt) until used in Experiment 2. Juveniles were fed a 55% protein, 2 to 3 mm crumble diet (Kinta Pty Ltd, Yarrawonga, Victoria, Australia).

Experiments 1 and 2 were conducted in fifteen, 100 L conical-bottomed tanks with black sides and white bottoms. Each tank was part of an independent, recirculating system operated with an internal 200 μm mesh-covered standpipe, an external airlift pump and a biofilter. Biofilters were filled with approximately 1,500 cm^3 of shell-grit with particle surface area of 0.79±0.44 cm^2 (mean ± sd; n=20). The shell-grit was covered with a 1 cm layer of filter wool. Water was pumped through the biofilters at 1 to 2 L/min (60 to 120% volume/h). Fluorescent lighting (Philips White TLA 40W 33QS) was provided overhead on a 14 : 10 h light : dark

cycle and intensity of 85±19 lux (mean ± sd; n=10). Tanks were siphoned daily to remove detritus and drained water was replaced. All water was passed through a 1 μm filter before being added to the experimental tanks. Approximately 50 to 75% of the total water volume in each tank was drained and replaced every seven days.

Experimental Salinity, Dilution Schedule and Water Quality

During both experiments, salinity treatments (n=3 replicate tanks) were imposed to randomly selected tanks. The salinity in the control treatments was maintained at 33.8 and 34.5 ppt for Experiments 1 and 2 respectively, whereas salinities in the other treatments were slowly lowered by adding groundwater (0.6 ppt salinity, 66 mg/L total hardness and 148 mg/L total alkalinity).

For Experiment 1 (Exp. 1), salinities were lowered to 27.5, 20, 12.5, and 5 ppt in 3.75, 7.0, 8.5 and 11 h respectively. For Experiment 2 (Exp. 2), salinities were lowered to 20, 10, 5, and 0.6 ppt in 5.75, 13, 15, and 20 h respectively.

During both experiments, salinity, water temperature (Exp. 1, range 23.6 to 24.0 C; Exp. 2, range 17.5 to 22.6 C), dissolved oxygen (>6.0 mg/L), and pH (between 8.3 and 8.9) were measured daily using an Horiba U-10 meter (Horiba Ltd, Kyoto, Japan). Total ammonia-nitrogen (Exp. 1, <1.0 mg/L; Exp. 2, <0.6 mg/L) was measured at least on every third day with a rapid test kit (E. Merck, Model 1.08024, 64271, Darmstadt, Germany). Fresh groundwater was added as required to adjust for evaporation to maintain treatment salinities.

Experiment 1: Effect of Salinity on Growth and Survival of Larvae

At 6 dah, larvae were drain harvested from a 2,000 L tank into an immersed, 53 μm mesh concentrating bag. A random sample (n=10) of live larvae showed that they had inflated swimbladders and had attained a mean total length (TL) of 3.15±0.19 mm . Larvae were mixed gently with an airstone, and 18, 2 L aliquots were taken in plastic buckets. One bucket

of larvae was poured into each of the 15 experimental tanks filled with treated seawater (33.8 ppt; 24±1 C). Larvae in the three extra buckets were counted with an infra-red fish counter (Jensorter, Inc., Model FC-24, 20225 Harvest Lane, Bend, Oregon 97701, USA) to estimate initial number of stocked larvae (2270±61; mean ± standard error [s.e.]). After 24 h, the salinity dilution schedules were imposed.

Every two days, a random sample (n=10) of larvae was taken from each tank and fixed in 2.5% buffered gluteraldehyde solution, which causes negligible shrinkage of preserved fish larvae (Oozeki and Hirano 1988). To estimate growth, fixed larvae were measured (total length; TL in mm) to the nearest 0.1 mm using an occular micrometer under a dissecting microscope. As measuring progressed, it became apparent that many larvae had an ectoparasitic infestation of a species of Amyloodinium. The degree of infestation was estimated at each sampling by counting the number of Amyloodinium sp. on one side of sampled larvae (n=5) from each treatment tank. After 14 d, larvae were harvested and counted to estimate survival.

Experiment 2: Effect of Salinity on Growth and Survival of Juveniles

Before stocking, juveniles were anaesthetised with 30 mg/L of benzocaine, weighed individually, and stocked into tanks filled with treated seawater (34.5 ppt; 17±1 C) at a density of seven fish per tank of 6.2±0.1 g/fish. The salinity dilution schedules were imposed after 24 h. Two additional experimental tanks were maintained with spare fish. Each tank was stocked with ten fish (7.0±2.8 g) and diluted from 34.5 ppt to 10 and 20 ppt respectively.

All dead fish were removed from the tanks as they were noticed, and weighed. To maintain a constant number of fish in each tank, replacement fish of similar weight to the dead fish were pelvic finclipped for identification, weighed, and returned to the tank. To reduce transfer shock, replacement fish were added directly from the spare tank with salinity most similar to

that of the treatment tank. That is, spare fish were replaced from 10 ppt into 5 and 10 ppt, and from 20 ppt into 20 and 35 ppt. Fish were fed to satiation daily by hand at 0900 and 1500 h. Feed consumption was recorded daily. Weight gain was recorded every 14 d. After 28 d, fish were harvested and the percentage survival, mean fish weight, mean adjusted biomass gain = [final total biomass + weight of dead fish] - [initial total biomass + weight of replacement fish], and food conversion ration (FCR) = [weight of food fed/adjusted biomass gain] were calculated from each tank.

Statistical Analyses

Homogeneity of variances was assessed using Cochran's test (Winer 1971), and where necessary, data were transformed (Exp. 1, number of Amyloodinium sp. by log ($\underline{x}$ + 1); Exp. 2, FCR by log ($\underline{x}$)) to satisfy the assumption of homogeneity of variance. Cochran's tests after transformation were not significant. Both experiments were designed for analysis using single factor analysis of variance (ANOVA). Where significant differences ($P < 0.05$) were found, means were compared by the Student-Newman-Keuls test (SNK). Where significant differences were not found, statistical power analysis was performed using *a posteriori* methods on ANOVA of final mean weight of juvenile mulloway (Exp. 2) (Searcy-Bernal 1994). Statistical analyses were conducted using Statgraphics Version 5.0 (STSC Inc., Rockville, Maryland, USA).

Results

Experiment 1: Effect of Salinity on Growth and Survival of Larvae

Salinity had a significant effect ($P<0.05$) on growth and survival of mulloway larvae (Table 1). Larvae grew at all treatment salinities (Fig. 1), but multiple comparison of final treatment means (Table 1) failed to clearly identify the optimum salinity for larvae growth. However, the mean total length was greatest for larvae grown at 5 ppt which was 9.2, 16.4 and 24.6 % longer than larvae grown at 20, 12.5 and 27.5, and 35 ppt, respectively. Survival was highest

at 12.5 ppt (59.9%), followed by 5 and 27.5 ppt which did not differ, and lowest for 20 and 35 ppt (9.9%) which did not differ (Table 1).

There was a significant difference ($P<0.05$) in the number of Amyloodinium sp. found on larvae at the end of the experiment (Table 1). Amyloodinium sp. occurred at all salinities and were first observed on 12 dah larvae grown in 20, 27.5 and 35 ppt (6 days after stocking). In salinities of 5 and 12.5 ppt, Amyloodinium sp. were not observed on larvae until 14 dah (8 days after stocking). The number of Amyloodinium sp. remained low at 5, 12.5 and 35 ppt, but showed a steady and rapid increase at 27.5 and 20 ppt.

Experiment 2: Effect of Salinity on Growth and Survival of Juveniles

Mulloway held in the freshwater (0.6 ppt) treatment were actively swimming for 2 d following salinity dilution, but did not start feeding. After 3 d, three fish died and many others displayed symptoms of stress; they were dark in colour, and many had lost equilibrium and were swimming upside down. At this time the freshwater treatment was terminated and all fish were removed from the freshwater and placed directly into 5 ppt. No more fish died, and they regained equilibrium and began feeding. Growth and food consumption data from the freshwater treatment were not included in statistical analysis of data.

A total of 15 other fish died during the experiment; three fish in each of the 5, 20 and 35 ppt salinities, and six fish in the 10 ppt. The replacement fish were not included in estimation of final mean weight. Amyloodinium sp. did not infest juvenile mulloway during this study.

Juvenile mulloway grown in 5 ppt for 28 d had the greatest final mean weight (12.1 ± 1.0 g) which was 10.0, 6.1 and 28.7 % heavier than fish grown in 10, 20, and 35 ppt respectively. The FCR (1.1 ± 0.1) was also lowest for juvenile fish grown in 5 ppt, and was 1.5, 2.5 and 1.7 times lower than 10, 20 and 35 ppt respectively (Table 2). Although there were no significant differences ($P>0.05$) for final mean weight, weight gain, survival, food consumption and FCR of juvenile mulloway grown in salinities from 5 to 35 ppt (Table 2), statistical power analysis

on ANOVA of final mean weight of juvenile mulloway showed that power of this experiment was low (0.22).

Discussion

Infestation by Amyloodinium ocellatum has been reported to cause major mortality during culture of marine fish larvae, juveniles and adults (Lawler 1977) including sea bream (Sparus aurata) (Paperna 1984) and red drum (Johnson 1990; Sandifer et al. 1993). Paperna (1984) showed that tolerance of A. ocellatum to salinity was highest at 24 to 25 C and that division of the reproductive tomont stage occurred between 1 and 78 ppt. However, results of six infection experiments showed in general, that effective reproduction of tomonts and infestation of postlarval S. aurata occurred only between 12 and 50 ppt, but the existence of populations of A. ocellatum with different salinity tolerances was likely (Paperna 1984). The Amyloodinium infective agent was not specifically identified in our study, but results showed some similarities with A. ocellatum. However infestation at 35 ppt was low, suggesting that a different or less tolerant species of Amyloodinium was present. It appears that the optimum salinity at 24 C for infectivity of the Amyloodinium sp. during our study was 20 ppt.

Based on results for growth and survival, lower salinities were better for mulloway larvae 6 - 20 dah. These preliminary data suggest that the optimum salinity range may be around 5 - 12 ppt. These results may help explain the relative paucity of mulloway captured in marine sites in the Hawkesbury River by Gray and McDonall (1993). Red drum, another euryhaline sciaenid, also grew well as 6 mm larvae at 5 ppt (Crocker et al. 1981). Holt (1990) found that although salinities of 25 - 30 ppt are required for development of yolk-sac to first feeding red drum larvae, older larvae acclimated to a wide range of salinities.

Juvenile mulloway were able to survive in fresh water for 2 d, but did not feed. After 3 d some fish died, while others lost equilibrium and swam upside down. When fish were transferred directly from freshwater to 5 ppt, they recovered and commenced normal feeding

and swimming behaviour. Similar behaviour was observed when European bass (Dicentrarchus labrax) were transferred to freshwater (0.5 ppt), including loss of appetite, heavy mortality within a few days, and like mulloway, exhibited complete recovery when returned to 5 ppt (Dendrinos and Thorpe 1985). Our results also support Gray and McDonall (1993) who suggested that juvenile mulloway prefer water with some marine influence, but are able to survive in freshwater, for at least short periods of time.

The inability of mulloway to survive and grow for long periods of time in freshwater differs from that observed for some other sciaenids. Red drum are found naturally in freshwater (Holt et al. 1981; Crocker et al. 1981) and have been successfully stocked into freshwater impoundments in the USA (Lasswell et al. 1977). Crocker et al. (1981) showed that red drum grew and survived well in freshwater with high specific conductance and osmolality. Orangemouth corvina have also been successfully transferred from seawater to freshwater and cultured for six months, however growth in freshwater was markedly lower than in seawater (Prentice 1985).

Following the growth experiment with juveniles, statistical analysis of data for weight gain, biomass gain, survival, food consumption and FCR did not indicate effect, of salinity treatments from 5 - 35 ppt were significant ($P>0.05$). However, the statistical power of this experiment (Searcy-Bernal 1994) was very low (0.22 for mean final weight) as the high variability within treatments meant that replication was inadequate. With such low power, it would be imprudent to accept the null hypothesis that salinity from 5 - 35 ppt had no effect on fish performance. Trends in the data suggested that 5 ppt was better for juveniles and further research is warrantd to establish salinity optima. As juveniles were cultured in salinity of 20 - 35 ppt before use in the experiment, it is unlikely that the apparent preference for lower salinity was due to early acclimation.

Based on growth performance, survival and prevalence of Amyloodinium sp., we recommend the culture of mulloway larvae, older than 6 dah, at 5 to 12.5 ppt. Juvenile mulloway grew well at all treatment salinities from 5 to 35 ppt. Although the optimum salinity for growth was not determined due to low statistical power of the experiment, good growth and food conversion efficiency of juveniles also occured at 5 ppt. Manipulation of salinity has been used successfully to treat a variety of infectious fish diseases (Munday 1996). Consequently, exposure of mulloway juveniles to freshwater for up to 2 d may be an effective method to treat infestation of Amyloodinium sp.

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Figure Legends

Figure 1. Total length of mulloway Argyrosomus japonicus larvae grown in different salinities for 14 d. Points represent the means ± standard errors (n=3 tanks). (Exp. 1)

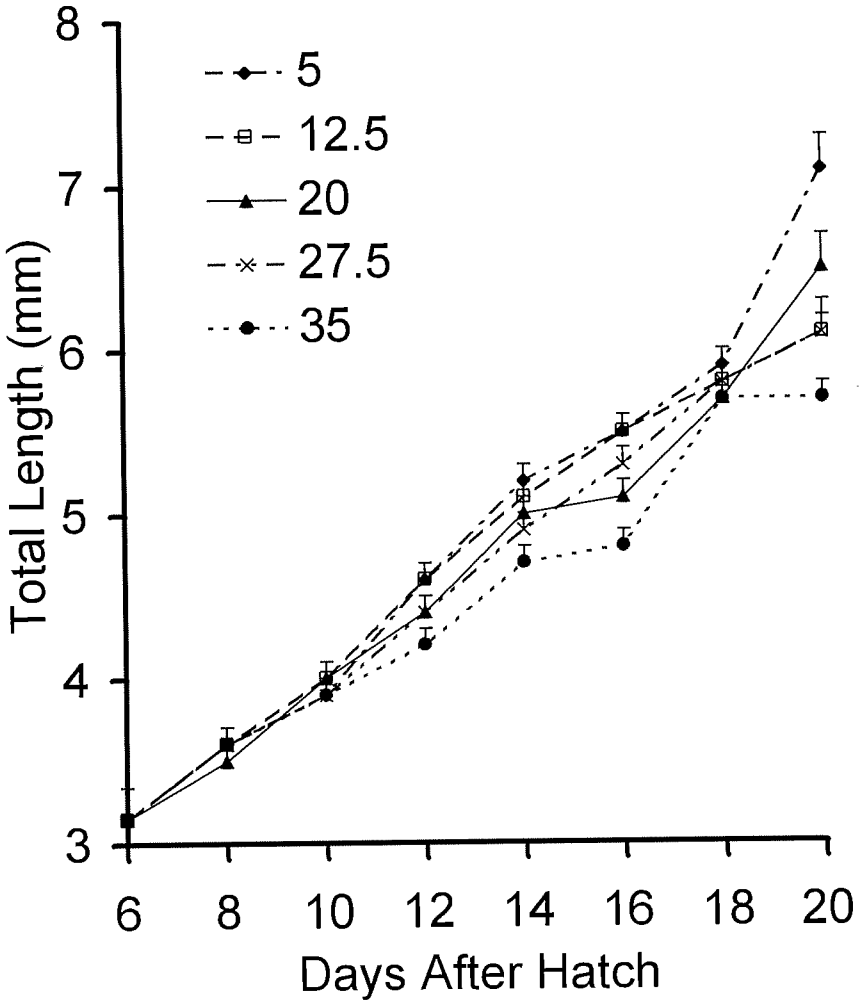
8
7
6
5
4
3
Total Length (mm)
5
12.5
20
27.5
35
6
8
10
12
14
16
18
20
Days After Hatch

Table 1. Final percent survival, total length and Amyloodinium sp. infestation of mulloway Argyrosomus japonicus larvae grown in different salinities from day 6 to day 20 [x]. (Exp. 1)

Salinity	Percent survival	Total length (mm)	Number of Amyloodinium sp. [y]
5	41.7±7.4 [b]	7.1±0.2 [a]	0.2±0.1 [a]
12.5	59.9±2.7 [a]	6.1±0.2 [b]	2.6±1.4 [b]
20	11.1±5.6 [c]	6.5±0.2 [ab]	33.1±7.6 [d]
27.5	29.1±4.9 [b]	6.1±0.1 [b]	8.5±1.2 [c]
35	9.9±1.9 [c]	5.7±0.1 [b]	1.5±0.3 [b]

[x] Data are means ± standard errors for 3 replicate tanks. Within columns, means with a common superscript letter do not differ significantly ($P>0.05$).

[y] Number on one side of larvae (n=5 larvae).

Table 2. Growth performance, survival and food conversion of juvenile mulloway Argyrosomus japonicus grown in different salinities for 28 days. [a] (Exp. 2)

Salinity	Initial weight (g)	Final weight (g)	Adjusted biomass gain [b] (g)	Percent survival [c]	Food input [d] (g)	FCR [e]
0.6 [f]	6.1±0.1	———	———	———	———	———
5	6.3±0.1	12.1±1.0	38.9±6.9	85.7±8.4	40.8±3.6	1.1±0.1
10	6.0±0.3	11.0±1.2	30.6±11.1	71.3±8.4	39.6±3.0	1.6±0.4
20	6.2±0.1	11.4±0.9	30.3±12.6	85.7±14.3	43.5±4.5	2.7±1.6
35	6.1±0.3	9.4±0.8	20.0±5.1	85.7±8.4	34.5±2.2	1.9±0.3

[a] Data are means ± standard errors for 3 replicate tanks. Means in each column are not significantly different ($P>0.05$).

[b] Adjusted biomass gain = [final total biomass + weight of mortalities] - [initial total biomass + weight of replacement fish].

[c] Percent survival of original stocked fish

[d] Mean total feed consumed per tank expressed as grams dry weight.

[e] Food conversion ratio = weight of food added/adjusted biomass gain.

[f] Several fish died and many fish lost equilibrium within 2 to 3 d of stocking and were removed from the treatment tanks.

9.4 Appendix 4: A precursory assessment of the nutrient and toxicant status of lagoons chosen for stocking with juvenile mulloway

Appendix 4:

A precursory assessment of the nutrient and toxicant status of lagoons chosen for stocking with juvenile mulloway.

Introduction

Basic sampling for organochlorines and trace metals was carried out in the three lagoons that were stocked with juvenile mulloway. Prior to stocking, analyses on sediment, water, fish and decapod samples were completed for Khappinghat Creek and Swan Lake. Several recaptured mulloway were analysed from Smiths Lake,.

The sampling regime was not scaled to assess the risk associated with consumption of either stocked or native fish that may have been potentially contaminated by any previous land usage. Sampling was designed to indicate the general environmental background of nutrients and toxicants into which fish were released, and to highlight whether further investigation is warranted.

Methods

Samples of water, sediment, fish and invertebrates were taken from Khappinghat Creek and Swan Lake. Four sample sites were chosen per lagoon for water and sediment samples. Tissue samples were obtained from one site. Sample sites were selected to provide maximum coverage of the lagoons' environs. Sampling of Smiths Lake was restricted to the analysis of several mulloway captured on 30/09/97. All samples were placed directly on ice, and frozen as soon as possible after collection. Samples were processed by Australian Water Technologies, Sydney.

Water was collected in 500 mL glass Schott bottles for nutrient analysis. Bottles had been washed with hexane and rinsed with distilled water. Samples were analysed for total phosphorous, filtered total phosphorous, total uncombined ammonia, oxidised nitrogen and total kjeldahl nitrogen.

Total phosphorous was determined by digestion of samples in alkaline conditions in an autoclave at 120 °C with persulphate digestion mix. Ascorbic acid reduction then allowed reading of phosphorous concentration using flow injection analysis (FIA).

Samples analysed for filtered total phosphorous were passed through a 0.45 um filter and digested with persulphate. Ascorbic acid reduction then allowed reading of phosphorous concentration using FIA.

Total uncombined ammonia samples were filtered to 0.45 um. Concentrations were determined using the salicylate modification of the phenate method.

The automated cadmium method was used to determine oxidised nitrogen concentrations.

Total kjeldahl nitrogen samples were digested in potassium sulphate, sulphuric acid and mercuric oxide. Concentrations were determined using the salicylate modification of the automated phenate method.

Sediment samples were taken from the same sites as water samples and analysed for trace metals and organochlorines. Each sample site provided two 60 mL samples. The first sample was taken in a sterilised plastic jar, and the other in a hexane-washed glass Schott bottle. Samples were taken with a 150 mm pvc sediment grab pressed into the lagoon bottom. A hexane-washed spoon was used to scoop the surface layer of fine silt directly into the jars. Aluminium foil was placed over the mouth of the jar before sealing. The grab was rinsed clean with distilled water between samples.

Trace metal concentrations were determined by inductively coupled plasma mass spectrophotometry (ICP-MS). Approximately 5 g of wet sediment sample was accurately weighed and transferred to a teflon vessel. Nitric acid and hydrogen peroxide were added and the vessel placed in a microwave for digestion. The cooled sample was diluted to 250 mL and analysed by ICP-MS. Mercury was determined by digesting the equivalent of 0.5 g dry weight of sample in sulphuric acid, potassium permanganate and potassium peroxydisulphate. Excess potassium permanganate was reduced with hydroxylamine hydrochloric acid and the sample diluted to 100 mL. Mercury in solution was reduced to solid with tin chloride, stripped by nitrite and transported to the measuring cell of the atomic absorption spectrophotometry. Trace metal concentrated were reported as mean ± standard deviation. In cases where analytes were below detectable limits, the detectable limit was used in calculations.

Organochlorine concentrations were determined using gas chromatography mass spectrophotometry (GC-MS). Dewatered salts and organic extracts were analysed from a known weight of dried sludge.

Predatory fish were targeted to provide tissue samples. Fish over 150 mm were either angled or gill netted from each lagoon. Six fish (4 bream and 2 queenfish) were taken from Khappinghat Creek. Four bream were sampled from Swan Lake. Five mulloway were taken from Smith's Lake. Each fish provided four samples (1 fillet into a glass jar, 1 fillet into a plastic jar, half the liver into a glass jar, half the liver into a plastic jar). Each fish was filleted with a clean knife on an aluminium foil covered cutting board. The fillet was skinned before placement into the respective jar. Both lobes of the liver were excised and halved between bottles. The knife was thoroughly cleaned and the cutting board foil was changed between fish. Organochlorine levels in flesh were determined using the same method as for sediment samples.

Invertebrate samples were only obtained from the northern lagoon, Khappinghat Creek. *Acetes spp.* were caught with dip nets from around submerged logs and weed beds. The samples were placed directly into plastic bags and onto an ice slurry. Invertebrate flesh samples were analysed for organochlorines and trace metals using the same methods as for fish flesh.

Results and Discussion

With one exception, organochlorine levels were lower than detectable limits, and were not significant. Consequently, results are presented in Table 1 as the assessed organochlorine type, and their minimum detectable limits. Dieldrin contained in the sediment of Khappinghat Creek was the only sample to register above the detectable limit. However, dieldrin concentration ranged from 2 to 5 ug/kg, which is below guidelines for potential biological effects.

Trace metals are a natural part of the marine environment, and are commonly found in low concentrations in marine sediments. Several trace metals, such as copper and zinc, are biologically essential. Anthropogenic inputs into the marine environment may increase trace metal concentrations. Contaminants may be introduced through atmospheric drop out, freshwater run off from industrial land, and domestic waste and sewage discharge. Despite a low background level of sedimentary contaminants, top chain predators tend to bioaccumulate available concentrations of trace metals.

Trace metal concentrations in tissue samples are shown in Table 4. The values for trace metals in sediment are calculated on a wet weight basis. Reference materials present concentrations on dry weight basis. A factor of twice the wet weight concentration is generally applied to enable comparison with dry weight standards (Dr P. Gibbs, NSW Fisheries, 1998).

Concentrations of trace metals in the sediments of both Khappinghat Creek and Swan Lake were predominantly below "pristine" levels (Sadiq 1992). The only exception was the concentration of lead in Swan Lake (5.54 mg/kg), which marginally exceeded the pristine value (5 mg/kg), but was lower than the Ontario guidelines (120 mg/kg) (Persaud *et al.*, 1993). Pristine values were not available for zinc, however sediment concentrations were below Ontario guidelines. No recommended values for selenium concentrations were available.

Trace metal concentrations generally were below the Maximum Residue Levels (MRLs) for edible tissue samples in the flesh samples taken from Khappinghat Creek and Swan Lake. Concentrations of arsenic and selenium in Khappinghat Creek exceeded MRLs set by the National Food Authority (Table 2). The MRL for arsenic was similarly exceeded in flesh of fish from Swan Lake.

It is likely that the excess concentrations of arsenic in fish flesh samples from Khappinghat Creek and Swan Lake are spurious, as the MRL quoted is for inorganic arsenic. Our results are presented as total arsenic. In the Sydney region, <1% of arsenic in fish is inorganic (Gibbs & Miskiewicz, 1995). Therefore, it is highly unlikely that the value for inorganic arsenic would exceed MRLs.

Selenium is coaccumulated with mercury, principally through the food chain. Selenium is believed to provide protection against mercury toxicity (Palmisano *et al.*, 1995). This may help explain why the concentration of selenium in flesh of fish captured in Khappinghat Creek was slightly higher than the MRL.

Trace metal concentrations in the flesh of mulloway recaptured from Smiths Lake were all below MRLs.

Toxicants are not evenly distributed throughout body tissues. Concentrations of trace metals are typically higher in the liver than the flesh of sampled fish, due to the metabolic function of the livers in blood purification (Thompson, 1990). Although concentrations in the liver were comparable to biologically expected values, in several instances they exceed the MRLs cited for edible tissue. Lead was the only analyte from livers of Khappinghat Creek fish which was lower than MRLs. In Swan Lake, lead and zinc were lower than MRLs. In Smiths Lake, only selenium and arsenic concentrations exceeded MRLs. It is unlikely that concentrations of trace metals in the liver samples which exceeded MRLs are relevant, as the MRL guidelines are established for edible tissue, and the consumption of fish livers is uncommon.

Concentrations of copper, arsenic and selenium in *Acetes spp.* collected from Khappinghat Creek exceeded MRLs. Reasons for the excess concentrations of arsenic and selenium may also be similar to the corresponding excesses in the fish flesh samples. Decapod crustaceans can regulate copper concentrations well above ambient levels. A possible explanation for the minor excess of copper displayed in our study, is that blood of crustaceans is haemocyanin (copper) based, rather than haemoglobin (iron) based (Maher, 1985). Copper concentrations in prawns are found routinely at much higher concentrations than displayed in Khappinghat Creek. It is thought that copper is detoxified by binding with metalothioneins, and subsequently accumulated (Rainbow *et al.*, 1990). Although the accumulation of copper in this fashion results in high apparent concentrations, the metal is bound and has no biological consequence.

Water samples were taken to determine nutrient levels in the lagoons at the time of stocking. There are no rigid guidelines on permissible nutrient levels, as comparison between waterways is difficult. Individual waterways have differing abilities to cope with nutrient loadings, according to light intensities, turbidity, temperature, substrates, and water chemistry. Extensive site-specific sampling would be required to establish nutrient levels with the potential to induce eutrophication. The values obtained in this study (Table 3) are within indicative levels above which problems have occurred (ANZECC, 1992).

Analysis of sediment, water and resident fauna of Smiths Lake, Swan Lake and Khappinghat Creek, suggest that juvenile mulloway were released into healthy waterways, with negligible toxicant status. These results suggest that further sampling would be unnecessary.

Table 1. Detection limits for organochlorines tested for in flesh and sediment samples from Khappinghat Creek, Swan Lake and Smiths Lake.

Organochlorine	Detection Limit (ug/kg)	Organochlorine	Detection Limit (ug/kg)
alpha BHCa	<1	Heptachlor epoxide	<1
beta BHCa	<1	Lindane (gBHC)	<1
delta BHCa	<1	4,4-DDE	<1
Methoxychlora	<1	4,4-DDD	<1
Endosulphan (alpha) a	<1	4,4-DDT	<1
Endosulfan (beta)a	<1	Heptachlor	<1
Endrina	<1	Hexachlorobenzene	<1
Dieldrin	<1	Total Chlordane	<5
Aldrin	<1	Total PCBb	<10

a Analytes only tested for in Khappinghat Creek samples
b Analytes not tested for in Khappinghat Creek samples

Table 2. Maximum Residual Limits for trace metals in edible tissue samples, as set by the National Food Authority

Heavy metals	Maximum Residue Limit (mg/kg)
Total mercury	0.5
Total lead	1.5
Total copper	10
Total cadmium	0.2
Total zinc	150
Total arsenic	1.0 (inorganic)
Total selenium	1.0

Table 3. Average nutrient levels in water sampled (n=4) from Khappinghat Creek and Swan Lake

	Swan Lake (mg/L)	Khappinghat Creek (mg/L)
Filtered oxidised nitrogen	0.02±0.02	-
Total uncombined ammonia	0.07±0.02	0.09±0.08
Total kjeldahl nitrogen	0.58±0.05	0.91±0.04
Total phosphorous	0.03±0.02	0.04±0.01
Filtered total phosphorous	0.02±0.02	0.04±0.01

Table 4. Trace metal concentrations in samples taken from intermittently opening lagoons stocked with juvenile mulloway.

Analyte (mg/kg)	Khappinghat Creek				Swan Lake			Smiths Lake	
	Sediment[1] (n=4)	Flesh (n=6)	Liver (n=6)	Acetes	Sediment[1] (n=4)	Flesh (n=4)	Liver (n=4)	Flesh (n=5)	Liver (n=4)
Mercury	0.001±0.00[d]	0.137±0.035[b]	-	<0.100	0.007±0.008[a]	0.054±0.017	-	0.011±0.0	0.104±0.045
Lead	1.40±0.75	0.06±0.02	0.14±0.02	0.33	2.77±3.73	0.04±0.02[b]	0.20±0.08	0.02±0.00	0.14±0.10
Copper	1.17±0.95	0.40±0.19	45.09±36.43	12.1	1.00±1.36[c]	0.28±0.15	13.90±6.01	0.16±0.08	3.663±0.395
Cadmium	0.01±0.01[b]	0.01±0.00[e]	0.61±0.41	0.02	0.02±0.02	0.02±0.01[a]	0.25±0.06	0.01±0.00	0.03±0.01
Zinc	6.3±4.6	6.1±0.7	251.9±145.9	10.8	4.1±4.7[a]	5.1±1.4	91.6±23.8	3.4±1.5[e]	28.0±2.3
Arsenic[2]	4.60±2.97	1.6±0.82	4.54±1.60	1.62	1.19±1.66	1.25±0.30	4.27±1.43	0.69±0.43	1.61±0.32
Selenium	0.20±0.11	1.18±0.26	7.18±2.17	1.25	0.23±0.28[b]	0.52±0.08	4.45±0.85	1.00±0.59	3.84±0.26

[1] sediment concentrations are for wet weight of sample.

[2] values are for combined organic and inorganic arsenic

[a,b,c,d,e] number of samples under minimum detectable limits: [a] =1, [b] =2, [c] =3, [d] =4, [e] =5. Where values were below detectable limits, the detectable limits were used to calculate averages and standard deviations.

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9.5 Appendix 5: Gut contents of mulloway recaptured from Smiths Lake

Gut content of individual mulloway recaptured from Smith's Lake. Mulloway captured on 30/09/97 had mean total length and weight of 249.5±12.5 mm and 163.6±36.3 g, respectively. Mulloway captured on 03/11/97 had mean total length and weight of 278.1±17.5 mm and 232.0±43.8 g, respectively.

Date of Recapture	Gut content
	1 Hardyhead; 1 unknown fish (backbone); 1 *Peneaus plebejus*
30/09/97 (n=24 fish)	1 unknown fish
	1 Penaeid; 1 unknown fish
	1 Penaeid; 1 Hardyhead
	1 Penaeid; 1 fish fragment
	1 Penaeid; small bits of fish fragments
	1 *Peneaus plebejus*; 1 Penaeid
	2 unknown fish
	2 Penaeids; 1 unknown fish (backbone); 1 isopod
	2 Penaeids; 1 unknown fish; 1 polychaete fragment
	1 Penaeid
	1 unknown fish; 1 Penaeid
	2 Penaeids; 1 *Peneaus plebejus*
	1 *Gerres subfasciatus*
	1 Penaeid
	1 Penaeid
	2 Penaeids; 2 *Peneaus* plebejus; 1 Hardyhead
	1 Penaeid
	1 Penaeid; 1 *Peneaus plebejus*
	1 Penaeid; 1 isopod (Family Cirolanidae ?)
	2 Penaeid; 1 *Peneaus plebejus*
	1 *Peneaus* plebejus; 1 Hardyhead
	2 *Metapenaeus macleayi*; 4 unknown fish
	1 *Metapenaeus spp*; 1 unknown fish
03/11/97 (n=15 fish)	1 Hardyhead
	1 Hardyhead; 1 unknown fish (backbone)
	1 unknown fish
	3 unknown fish
	1 Decapod prawn
	1 unknown fish (backbone)
	Fish fragments ?
	2 Hardyheads; 1 unknown fish
	1 unknown fish
	1 Penaeid
	1 *Penaeus plebjus*
	1 Hardyhead
	Fish fragments
	3 Hardyheads
	1 unknown fish (backbone)

9.6 Appendix 6: Fielder, D. S. & W. J. Bardsley, 1997. Production techniques and stock enhancement of mulloway. TAFE National Fishing Centre Conference Proceedings, Grafton, December 6-7, 1997.

Production Techniques and Stock Enhancement of Mulloway

by

Stewart Fielder and Bill Bardsley

NSW Fisheries
Port Stephens Research Centre
Taylors Beach Road
Taylors Beach NSW 2315

Introduction

NSW Fisheries has been assessing the potential for hatchery production of mulloway *Argyrosomus japonicus* for aquaculture to determine whether wild catches can be enhanced by reseeding, and an industry developed for cultured mulloway. In 1990 the Fisheries Research Institute (FRI) started a marine fish breeding program at its Cronulla laboratories and the Port Stephens Research Centre (PSRC). Mulloway was selected as a potential aquaculture candidate because it is a widely distributed temperate species, and receives high market prices. It also produces large quantities of eggs, can tolerate a wide range of salinities, and importantly, grows quickly (Battaglene and Bell, 1991; Gray and McDonall, 1993; Battaglene and Talbot, 1994).

Mulloway is an economically important species targeted by commercial and recreational fishers in all states except Tasmania. Like many other estuarine and in-shore fisheries, catches of mulloway in NSW have declined. In 1980/81 approximately 250 tonnes were caught compared with 150 tonnes in 1990/91 (NSW Fisheries unpublished data). In NSW this trend has led to increased restrictions on the minimum size limit and the introduction of bag limits for amateur fishers. Conflict between commercial and recreational fishers has resulted from the belief that the large by-catch of juvenile mulloway, taken by prawn trawlers operating in estuaries, such as the Clarence River, is partially responsible for the decline in mulloway catches. A reduction in natural populations may also be caused by an event at a critical life-history stage, such as failure for juveniles to recruit to intermittently opening coastal lagoons. A perceived solution to these problems is to release hatchery produced fish into estuaries and lagoons, at the end of the prawn trawling season to compensate for removal or poor recruitment of large numbers of juvenile mulloway.

Reseeding or enhancement of wild Australian marine fish stocks to date, has received little attention; however, it is common in Japan and the USA. For example, the stocking of red drum, *Sciaenops ocellatus* in estuaries in the USA has been highly successful (Rutledge, 1989). Both mulloway and red drum belong to the same family Sciaenidae. To be economically viable the reseeded species must have a low post-reseeding mortality, a fast growth rate to market size, and a high unit value. The unit value can be measured in a number of ways. First, directly in terms of return/kg received on the open market by commercial fishers for human consumption, or second, in terms of the return/kg generated by expenditure associated with catching the fish, as occurs with recreational fisheries. Recreational anglers spend a great deal money in the pursuit of fish e.g. fishing tackle, bait, boats, camping equipment, fuel etc., thus

significantly increasing the value/kg of fish (Talbot, pers.comm., 1997). Results of our research into development of techniques for the culture of mulloway, indicate that this species may satisfy all of these requirements.

Broodstock management

The first major bottleneck for production of mulloway juveniles, was a reliable supply of high quality eggs. Technology is developing for management of broodstock and has involved capture and hormone induction of wild spawners, as well as maintenance of captive broodstock in land-based tanks.

Mulloway spawn naturally near Sydney during the summer months of January to March. Initially wild broodstock were collected for hormone induction during these months by baited line or gillnet from inshore reefs and estuaries; however, production of high quality eggs was not reliable for several reasons. First, it proved difficult to keep many fish in good health after capture due to associated stress, external damage, and gill embolisms (gas bubbles in the bloodstream) caused by removing fish quickly from deep water(>3m). Mortality of captured fish during transport to the hatchery was common. Second, it was often difficult to synchronise capture of viable male and female fish. Third, eggs were hand-stripped from the female fish. This technique relies on prediction of ovulation followed by anaesthesia and handling, often on several occasions, of the female fish. Eggs can be underripe or overripe, resulting in poor egg fertilisation and hatching rates. Handling damage to the fish can also occur.

As a consequence of these problems, a program was established to develop techniques for land-based management of wild-caught broodstock in environmentally controlled tanks. Fourteen fish (1:1;M:F) were held in a 30 000 L tank under an artificial light and temperature (phototherm) regime designed to emulate ambient conditions, and 5 fish (3:2;M:F) were held in a 17 000 L tank under repeated truncated (120 day) phototherm regimes. In the ambient tank, male fish began spermiation and most female fish matured (eggs >500 μm) after 12 months. Natural spawning did not occur but female fish were successfully induced to spawn in January and March 1997, after injection with 1000IU/kg hCG . However, fertilisation rates were variable (range 0 - 65%). In the truncated phototherm tank (365 day seasonal pattern was compressed to 120 days), fish matured 'out of season' in October 1996 and April 1997 following exposure to repeated, 120 day phototherm regimes. Similar to the ambient tank, fish did not spawn naturally but were induced to spawn following injection of hCG. Fertilisation rates of eggs were however very low (<1%).

Maintenance of mulloway broodstock in tanks compared to reliance on wild-caught fish, has significantly increased our ability to induce spawning, however reliable production of high quality eggs still requires improvement. Alternative truncated phototherm regimes and the use of first generation hatchery reared broodstock need to be investigated.

Larval Rearing

Large numbers of mulloway juveniles have been successfully reared at PSRC using intensive hatchery techniques and at Searle Aquaculture using extensive fertilised pond techniques.

Intensive hatchery techniques involve either the use of indoor, 2000 L, clearwater, recirculation tanks or outdoor, 10 000 L, greenwater, flow-through tanks. Newly hatched larvae are stocked at 50 to 100/L, directly into the tanks and start feeding after 3 days at temperature of 23 °C. Both techniques require the production of live foods, rotifers *Brachionus plicatilis* and brine shrimp *Artemia* sp. A typical feeding regime involved the feeding of rotifers at 10/ml from about day 3 to 15 and brine shrimp at 1-5/ml from day 10 to 25. Prior to feeding to the larvae, rotifers and artemia were nutritionally enriched for 24h with the microalgae, Tahitian *Isochrysis* aff. *galbana* and *Pavlova lutheri*, and DHA Super Selco. Artificial feed (ML250, Fukui &Co, Japan) was provided and readily accepted by the larvae from about day 20.

Our clearwater tank system operates using recirculation by pumping water through a series of mechanical and biological filters. The tanks are situated indoors, and temperature and light can be completely controlled. The greenwater tanks are situated outdoors and operated similarly to the method described by Palmer *et al* (1992) for rearing of barramundi, where a bloom of cultured marine microalgae is maintained in the larvae tank by addition of fertilisers. The algal bloom reduces the ammonia concentration, provides a low-light (low stress) environment, and may also provide some antibacterial properties. The tanks were mostly static, with minimal water exchange of about 10%/day.

Mulloway juveniles become cannibalistic around 20 days after hatch. To reduce the incidence of cannibalism in the intensive cultures, regular (bi-weekly) size grading was necessary. This was labour intensive and involved drain harvesting the fish and sorting them using a floating, bar grader.

Survival to day 30, (15 - 20 mm total length) has been variable but about 10-15% has been achieved on several occasions. Major mortality of larvae and juveniles in both clearwater and greenwater cultures has been associated with outbreaks of the dinoflagellate, *Amyloodinium* sp.

Extensive larval rearing techniques involve production of natural zooplankton blooms in earthen ponds, by addition of fertilisers (Rutledge, 1989; Rutledge and Rimmer, 1991). There is often very little or no water exchange. Mulloway juveniles have been successfully reared in 0.025, 0.25 and 1.0 ha ponds. An experiment was conducted in January 1996, which investigated the effect of age of stocking on growth and survival of mulloway larvae. Larvae were reared in an intensive clearwater hatchery and transferred to each of 2 replicate, 0.025 ha ponds at 3, 7, 15 and 21 days after hatch. Results showed that there was a significant effect of age of stocking on growth and survival. Generally, survival increased as age of stocking was increased and ranged from 22% for 3 days after hatch to 76% for 21 days after hatch. The average growth rate was about 1.6 mm/day.

In January 1997, 250 000 and 450 000 newly hatched (4 days old) larvae were stocked into a 0.25 and 1.0 ha pond respectively. The 0.25 ha pond was partially harvested at 30 , 38 and 77 days after stocking. A total of 41 500 fingerlings were harvested (16.5% survival), and ranged in size from 2-12g (70 - 100mm length). Unfortunately a major influx of cormorants, resulted in complete mortality of fingerlings in the 1.0 ha pond.

The above results show that mulloway fingerlings can be successfully reared using a variety of intensive and extensive techniques. However, there are pros and cons for each technique. Intensive clearwater techniques offer the greatest degree of control, and therefore predictability, over the larval rearing conditions but also require the greatest input of labour (live food production, vacuuming, water quality monitoring, etc.) and sophisticated infrastructure for operation. Our intensive greenwater techniques are outdoors and therefore are prone to environmental variability, such as fluctuating temperature, light, and rain (affecting salinity). Maintenance of algal blooms can consequently be problematic. However, the operating labour and infrastructure inputs are less than the clearwater system (e.g. tank maintenance is reduced). Size-grading is necessary for both techniques.

Extensive pond growout offers the least amount of control over rearing conditions. Production of live food is reliant on sunlight, temperature and salinity. Timing of stocking and reliable fingerling production can therefore be compromised if environmental conditions are not conducive to plankton growth. However, successful pond production requires the least amount of labour input (e.g. grading is not necessary), facilities do not need to be highly-sophisticated, and larvae growth rates can be very rapid.

Our research to date suggests, that significant benefits may be gained by employing a combination of intensive and extensive techniques in order to control the reliability of fingerling production, maximise growth rates, reduce labour costs, and minimise the cost of fingerling production.

Stock Enhancement

The first assessment of the potential for enhancement of mulloway in intermittently opening coastal lagoons was started by NSW Fisheries in January 1996. Mulloway fingerlings ranging in size from 2 - 12g (70 -100 mm respectively) were produced in the summers of 1996 and 1997. Approximately 25 000 fish were stocked into one(1) NSW lagoon in 1996 and to each of two (2) NSW lagoons in 1997. All fingerlings were "tagged" with oxytetracycline (OTC) and strontium chloride prior to release. Mulloway have not been recaptured in two (2) lagoons. However, large numbers of juvenile mulloway have been recaptured in one (1) of the stocked lagoons. By early July 1997 (4 months after stocking), a professional fisher was catching and releasing hundreds of mulloway while haul netting in the lagoon.
Three samples (n=20) of captured mulloway have been taken to date and individual length/weights recorded and both sagittal otoliths extracted for identification of OTC and strontium marks.

Results show that a large proportion of the captured mulloway juveniles have otoliths marked with OTC. For example, Thirteen of the twenty otoliths examined (65%) from mulloway captured on 11 August 1997, displayed a positive mark. 'Control' otoliths (n=20) which were extracted from tagged mulloway that had been held in tanks at PSRC, displayed 80% with a positive OTC mark. Identification of OTC marks requires delicate transverse sectioning through the core of the otolith. Consequently, it is possible to miss the core which results in failure to identify the OTC mark. We are waiting on results of strontium analysis, which may confirm a higher percentage of captured mulloway with a hatchery origin.

Average growth rates were excellent despite the mulloway being stocked before winter. Based on the assumption that all captured mulloway were hatchery-reared, average growth (total length) rate was estimated at approximately 1.0 mm/day. It is therefore possible to assume that legal-size (450 mm TL) mulloway will be present after 13 months from stocking. This period may potentially be shorter if increased growth rates are realized during the
oncoming summer period. With the assistance of the professional fisher, monitoring of the mulloway stock in lagoon will be continued.

These exciting results highlight the excellent potential for production of mulloway for large-scale stock enhancement and aquaculture projects.

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