



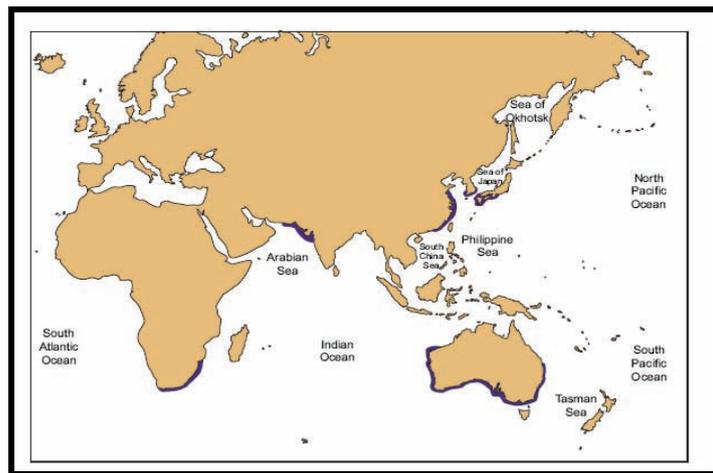
## **Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish**

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## 2. MULLOWAY



**FIGURE 40:** Global distribution of mullet (Source: Silberschneider and Gray, 2005)

## 2.1 Appearance, Distribution and Movement

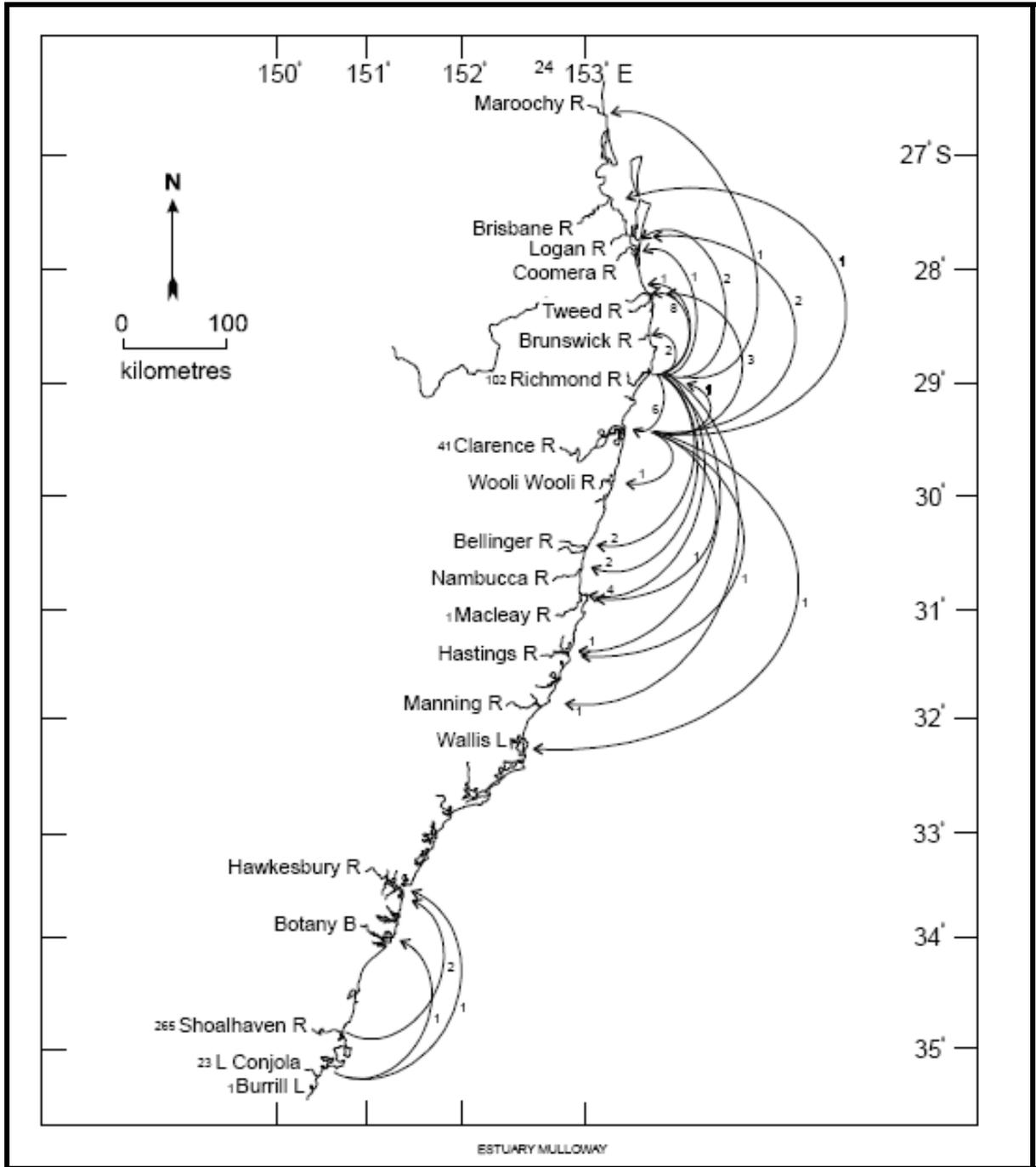
(Based on Silberschneider and Gray, 2005. and on supplementary sources as cited throughout the text)

Mulloway (*Argyrosomus japonicus*) is a member of the family Sciaenidae, commonly referred to as croakers and drums. Sciaenids are mostly demersal fishes found in fresh, estuarine and coastal marine waters in subtropical to temperate regions of the Atlantic, Indian and Pacific Oceans. Mulloway is a near-shore coastal (<100 m depth) species that also occurs in estuaries. Its distribution includes Pacific and Indian Ocean waters surrounding Australia, Africa, India, Pakistan, China, Korea and Japan (Fig. 40). In Australia, it is distributed along the eastern, southern and western seaboard from the Burnett River in Queensland to North West Cape in Western Australia. Although these fish are particularly common in South Australia, especially around the mouth of the Murray River (Lakes Alexandrina and Albert, the Coorong Lagoon) and adjacent coast through to western Victoria, they are much less abundant between Melbourne and southern New South Wales and have rarely been reported from Bass Strait.

In Africa, mulloway are found along the south-east coast from the Cape of Good Hope to southern Mozambique. In the northern Indian Ocean, they occur off Pakistan and the northwest coast of India. In the Northern Pacific they have been reported from Hong Kong, northwards along the Chinese coast, to southern Korea and Japan. It is an esteemed angling fish in Australia and South Africa and is a highly regarded food fish and important commercially-exploited species throughout its distribution

Tag and release research show that while some mulloway, especially juveniles, are relatively sedentary, others move significant distances along a coastline and from one estuary to another. For example, in a South African study, 83% of the 263 recaptures (primarily juvenile and sub adult fish < 120 cm) were found < 10 km from where they were originally tagged even though these fish recorded long periods of liberty (> 1400 days). Only 15 fish were recaptured >30 km from the initial tag location.

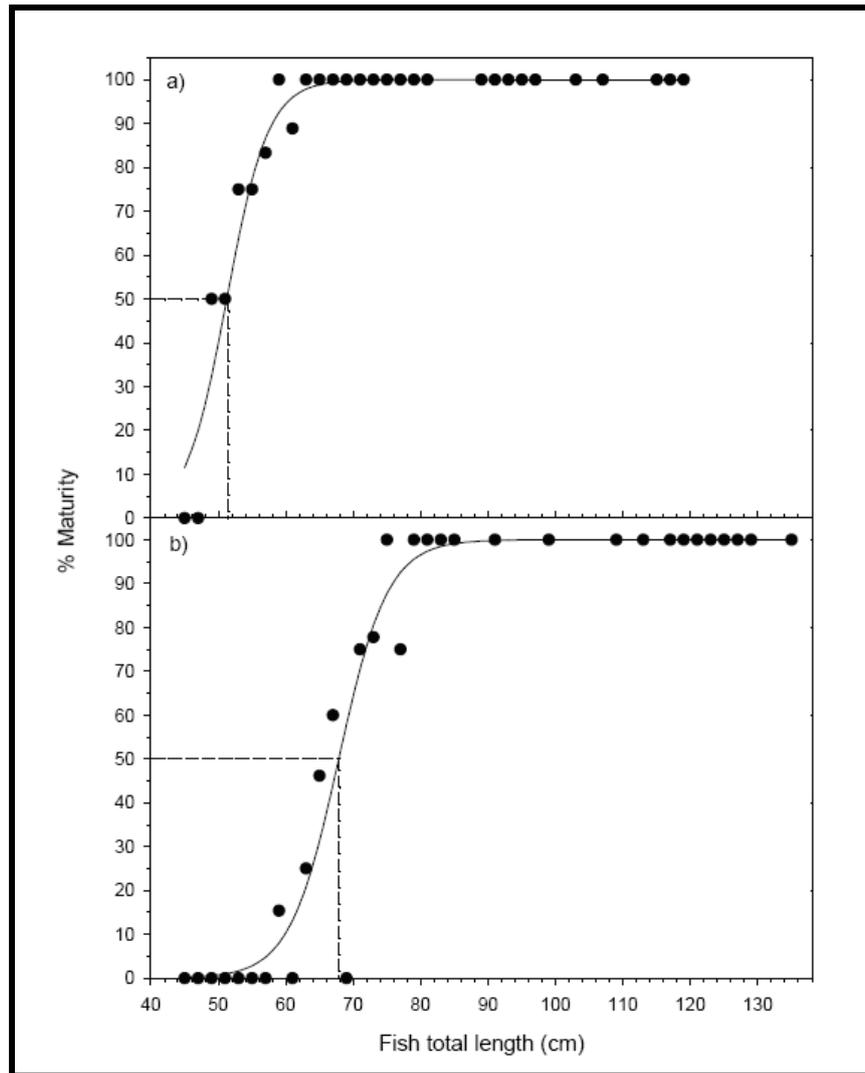
Similarly, in a tag and recapture study in NSW, 83% of the mulloway were recaptured in the estuary where they were originally tagged while 13.5% of recaptures were of fish that had moved to another estuary. The greatest distance migrated was approximately 400 km (Fig. 41). The longest period at liberty was 1954 days, with the fish recaptured approximately 375 km south of where it was tagged. Similar observations have been made of tagged mulloway in South Australia and South Africa.



**FIGURE 41:** Map showing locations of release and recapture of tagged mulloway in New South Wales, (Source: Silberschneider & Gray, 2005)

## 2.2 Breeding and Early Life History

Average size and age at which mulloway attain sexual maturity in NSW is estimated at 51 cm and at 2+ years for males (Fig 42a) and 68 cm at 3+ years for females (Fig 42b). In South and Western Australia, mulloway do not become sexually mature until they are approximately 70 - 80 cm (approx. 4 kg) and 5 to 6 years old. This again contrasts with male and female mulloway in South Africa reported as maturing at average lengths and ages of 92 and 107 cm and at 5+ and 7+ years, respectively.



**FIGURE 42:** Size and sexual maturity of mulloway from New South Wales (Source: Silberschneider and Gray, 2005).

As with the size and age at maturity, the spawning season of mulloay varies between geographic regions and with latitude and is probably related to water temperature and oceanography. For example, in southern Africa, spawning occurs from August to November (winter to spring) in the northern KwaZulu region (30 - 31°S), and from October to January (summer) in the southern and south-east Cape regions (33 - 35°S). Similarly, along the West Australian coast, fish with ripe gonads occur in September and October in Shark Bay (26°S), whereas in the Swan River (32°S) they occur 3 months later between December and January. In South Australia, mulloay spawn from late spring to late summer (November to February). Spawning in central NSW (around 35°S) appears to take place in late summer and autumn (January to April) but possibly year round in northern NSW.

Hall (1984) suggested spawning may take place near the mouths of estuaries as large fish (80-150 cm TL) in spawning condition have been caught in the mouth of the Murray River in South Australia. He further postulated that freshwater outflow during summer may promote aggregations of spawning fish near the mouths of estuaries as peak freshwater discharge generally coincided with, or just preceded, the spawning season. The spring/summer-spawning season in South Africa also coincides with the highest periods of rainfall and river discharge in that region. Which suggests that mulloay may have adapted a river discharge-spawning relationship as an evolutionary tactic to enhance recruitment of juveniles to estuaries.

No estimates of the fecundity of wild mulloay have been reported, but hatchery fish in NSW of around 10 kg are reported to spawn approximately 1 million eggs with spawning being group synchronous.

The eggs of mulloay are pelagic, approximately  $0.938 \pm 0.024$  mm in diameter and under laboratory conditions, hatch in 28–30 hours (at 23°C) after spawning, with the larvae being 2.2–2.3 mm TL upon hatching. Eggs have been collected near the surface in coastal waters off south-eastern Africa and larvae (up to 10 mm TL) have been caught in estuarine and coastal waters (out to 100 m depth contour) off south-eastern Australia between February and April. During daytime sampling in coastal waters of NSW, mulloay larvae were caught in subsurface waters, with greatest concentrations below 30 m depth. Similarly, most larvae captured in towed plankton nets in a coastal embayment (Botany Bay) in NSW, were close to the substratum, suggesting that larval mulloay may prefer deeper parts of the water column.

Small (< 30 cm TL) juveniles are found in estuaries and near-shore coastal environments, including surf zones. Some ambiguity exists however, concerning the timing, age and length that individuals recruit to estuaries. Mulloay recruit to estuaries in South Africa at 2-3 cm TL approximately 4 weeks after hatching. This latter study in South Africa, and the fact that larvae and small juveniles (2-10 cm TL) have been caught in estuaries in NSW, suggests that small mulloay are present in estuaries from a very early age, but are probably not susceptible to capture in most common research sampling methods until they reach a larger length.

In estuaries, juveniles (including early post-settlement stages) that have a wide salinity tolerance may favour deeper waters rather than shallow littoral fringes where most sampling for juvenile fishes has traditionally taken place. For example, in a study in two estuaries in northern NSW most small mulloay (2 -40 cm TL) were captured by trawling in the deeper waters of the main river channels, particularly where prawn abundances were high. None were caught in shallow waters or small tributaries (using small seine nets). Further, relatively few small juveniles have been caught along the shallow (<2 m depth) vegetated (e.g. seagrass and mangrove) and non-vegetated fringes of estuaries, despite the extensive sampling of these habitats in south-eastern Australia and southern Africa. Unlike many other scianenid species, mulloay do not apparently depend on shallow vegetated habitats as a juvenile nursery habitat.

Juveniles occur in estuaries, embayments and near-shore coastal environments. The horizontal distribution of mullet in estuaries can vary substantially and is probably linked to environmental factors including salinity, freshwater flows, turbidity and life history stage. Fish <15 cm predominantly recruit to the upper regions of estuaries where salinities are <5 ppt. In the Hawkesbury River New South Wales, most juvenile fish (10–20 cm TL) were found to occur at locations in an estuary where the salinity was 15 to 20 ppt. However, some juvenile fish were caught in upstream locations where salinity was <5 ppt and also near the estuary mouth where salinity was >25 ppt. Juveniles were also found to be more prevalent in turbid versus non-turbid estuaries in South Africa which suggests that juveniles may be more abundant in estuaries with significant freshwater flows. This may also be true in NSW where juveniles appear to be more prevalent in the deeper riverine type estuaries compared to the shallower barrier (coastal lagoon) estuaries.

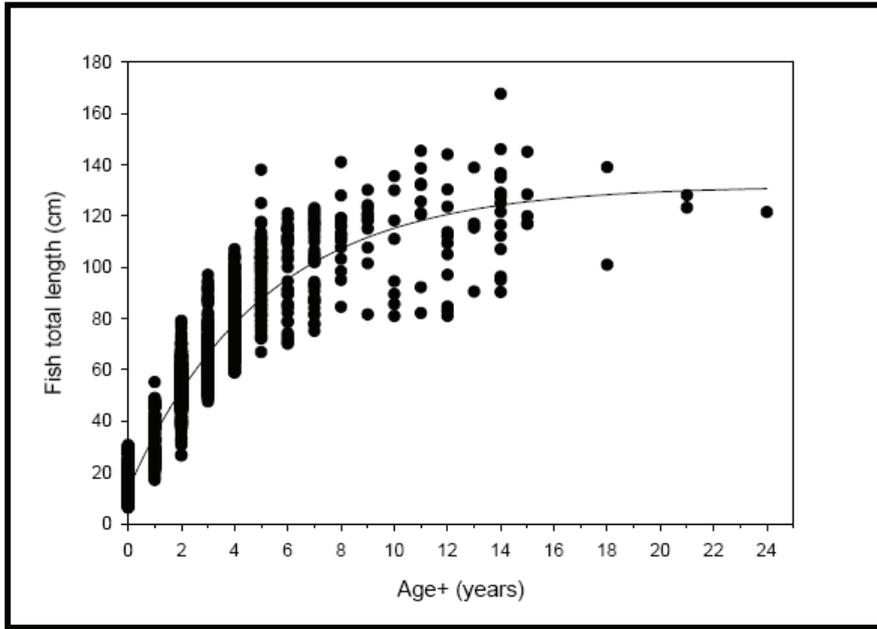
Sub-adult and adult mullet occur in estuarine and ocean water. In estuaries, larger juveniles and sub-adult fish (>40 cm TL) appear to be more abundant in the lower reaches where salinities are nearer to seawater. The distribution of these larger individuals may be related to particular hydro-graphic conditions. For example, larger fish have been reported to move from estuaries to the ocean in Western Australia in winter when estuarine salinity levels dropped. Large individuals are caught around the mouths of estuaries, in surf zones and around rocky reefs and ridges in offshore waters.

### **2.3 Food and Feeding**

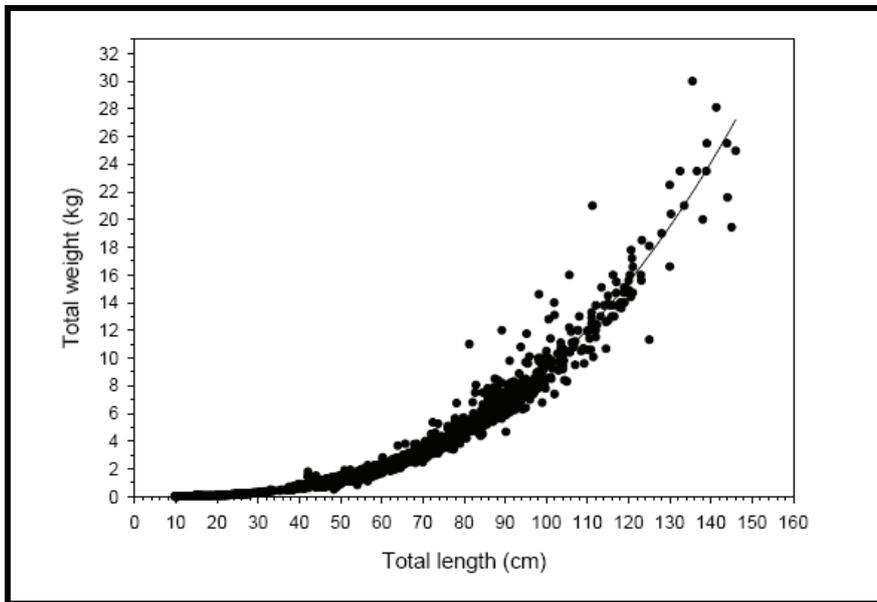
Mullet has a relatively large mouth with caniniform teeth, sharp gill rakers and a short intestine with a large distensible stomach. It is regarded as a benthic carnivore but can apparently feed throughout the water column. The importance of different dietary components has varied between studies and for different life history stages. Overall, crustaceans, notably penaeid, mysid and alpheid shrimp, and small teleost fish have been the primary dietary items observed in the stomachs of juvenile mullet. Crustaceans accounted for between 14 and 81% of the reported diet of juveniles. The importance of crustaceans in the diet of mullet appears to decrease with increasing fish size, resulting in fish and squid being the prey of greater relative importance in larger mullet.

### **2.4 Growth, Longevity and Mortality**

Growth of mullet varies greatly between different geographic regions. In South Africa, Mullet grow to a large size and are relatively long lived, with the maximum reported length being 181 cm TL, weight of 75 kg and age of 42 years. Growth of both sexes is initially rapid and similar for the first 2 years, after which the rate of growth declines with females growing faster to attain an overall greater length (165–170 cm) and age (42 years) than males (140– 45 cm and age 30 years) (Fig. 43). Although the rates of growth differed between sexes, the length/weight relationships for males and females did not differ significantly (Fig. 44). Tag recapture based growth rate data for mullet in Australia show that juvenile Mullet grow rapidly especially from January to March. Fish 2 years old average 46 cm in total length and 1.5 kg in weight; for 5-year-old fish the corresponding sizes are 80 cm and 8 kg. However these data also illustrate a variation in growth rate between individuals.



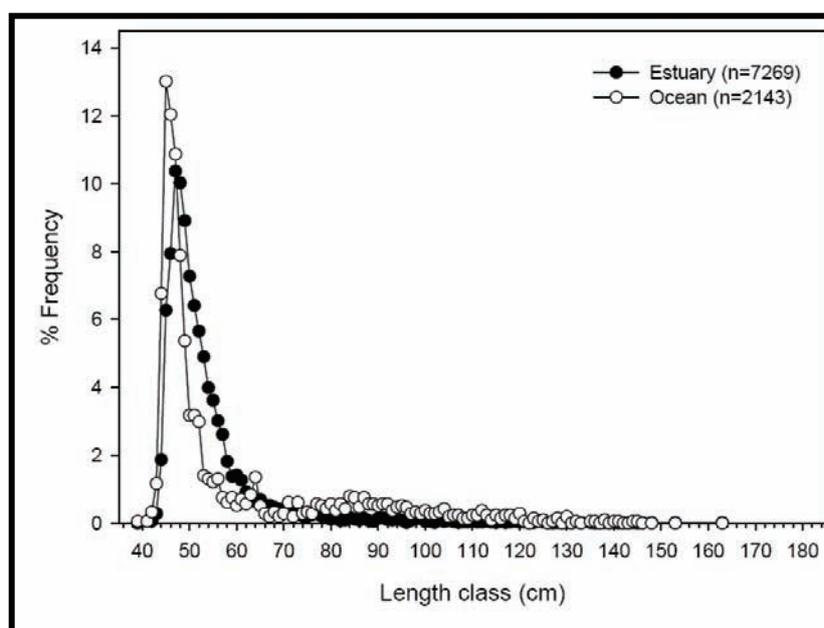
**FIGURE 43:** Length data with fitted growth curve for mulloway in New South Wales. Lengths are presented as total length (TL). (Source: Silberschneider and Gray, 2005).



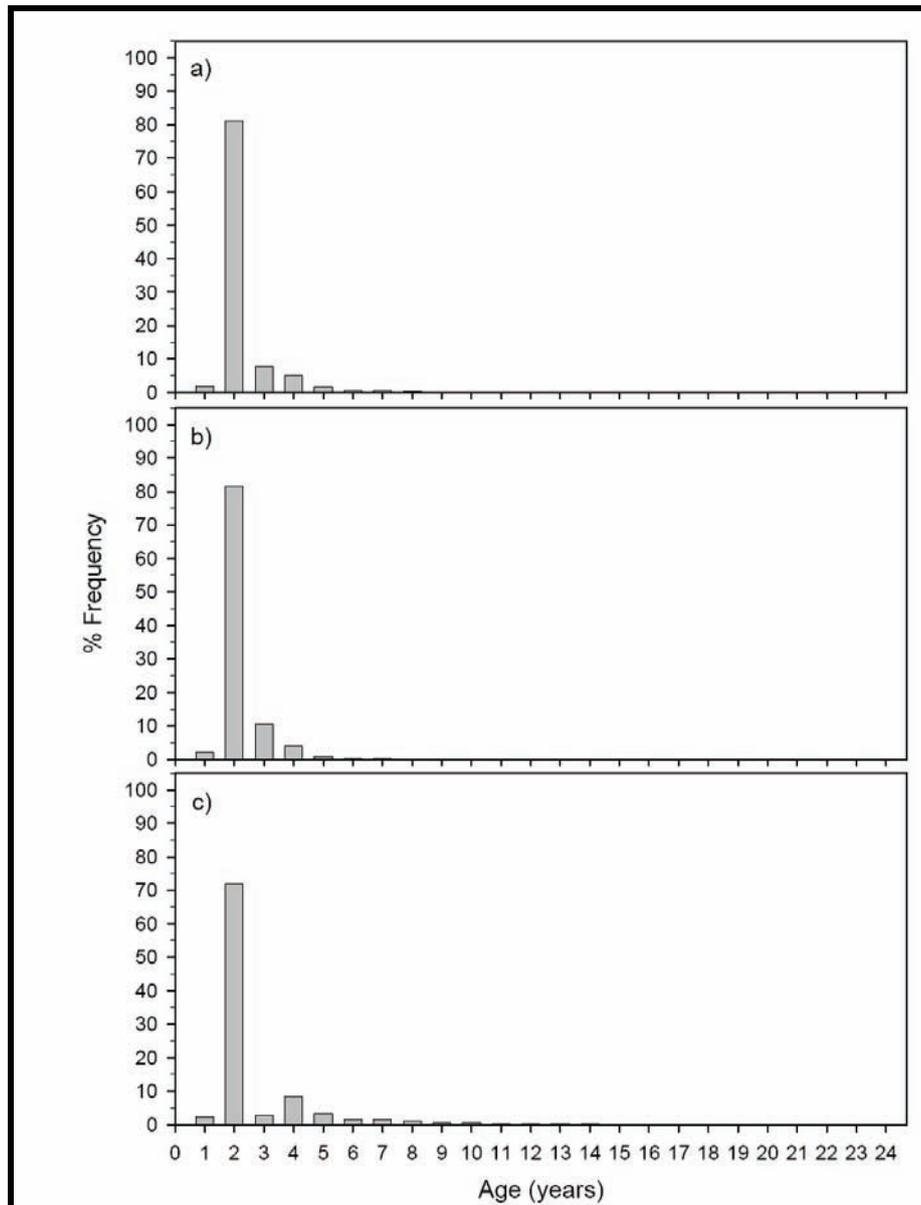
**FIGURE 44:** Length-weight relationship of mulloway sampled in NSW (n = 2865). (Source: Silberschneider and Gray, 2005).

## 2.5 Natural and Fishing Mortality

The estimated length and age compositions of the estuarine and coastal catches of mulloway were very similar, being dominated by fish very close to the minimum legal length of 45 cm and age of 2 years (Fig. 45). The relatively small proportion of fish aged >2 years in landings, despite having the potential to live for more than 40 years, is indicative of a fishery that is heavily and probably over exploited (Fig. 46). Estimated mortality due to fishing is 3 to 6 times greater than estimated natural mortality, a situation that is likely to be unsustainable. Estimates of total mortality (0.45 to 0.7) is high and indicates that between 36 and 50% of mulloway die each year.



**FIGURE 45:** Length composition of sampled estuarine and ocean commercial catches of mulloway (pooled across regions). (Source: Silberschneider & Gray, 2005)



**FIGURE 46:** Estimated age compositions of a) the total commercial catch (n = 2605), b) the estuarine catch (n = 1681), and c) ocean retained commercial catches (n = 381) of mulloway in NSW 2003 to 2005. (Source: Silberschneider & Gray, 2005)

## 2.6 Hatchery Protocols - Mulloway

### 2.6.1 Broodstock husbandry

#### Introduction

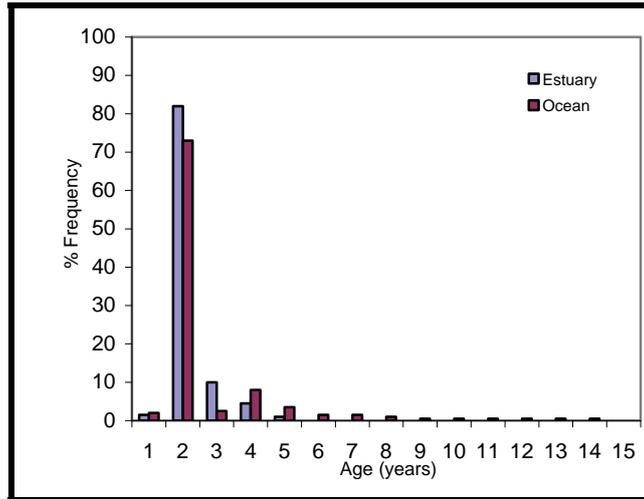
Mulloway has many attributes beneficial for aquaculture. It is a widely distributed temperate species, commanding high prices. It is highly fecund, euryhaline and, most importantly, grows quickly.

I&I NSW has been assessing the potential of the hatchery production of mulloway since 1990. The first successful hatchery production was achieved in 1992 using fertilised eggs sourced from captive broodstock line-caught from the wild 18 to 24 months earlier (Battaglione and Talbot, 1994). From 1992 to 1996 experimental batches of mulloway fingerlings produced at the Port Stephens Fisheries Institute (PSFI) were used for trial commercial farming in earthen ponds and sea-cages. Intensive indoor clear-water hatchery rearing techniques employed require dedicated, controlled environment facilities, high input of labour by skilled technicians and relied totally on artificial propagation of live rotifers and brine shrimp as a food source. Although up to 100,000 Mulloway fingerlings were produced per year using these techniques, production was expensive and not well suited to generating fingerlings at a low enough price or on a scale sufficiently large for seeding depleted or recruitment limited natural populations.

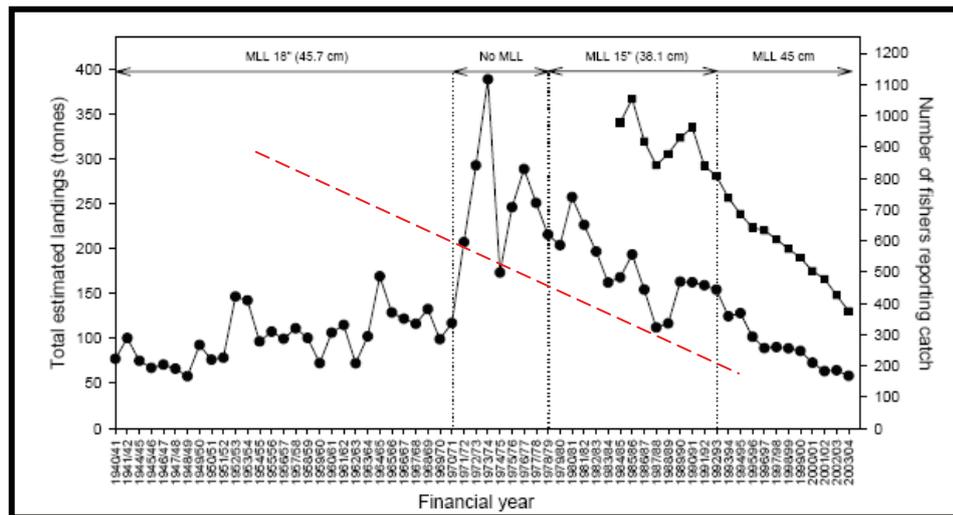
Research to develop extensive green water production of fingerlings was initiated in 1995 (Fielder, Bardsley and Allan, 1999). This work was prompted by previous success achieved with other marine fisheries reseeding programs based on extensive pond production and release of fingerlings. The latter included those involving the closely related sciaenid species red drum *Sciaenops ocellatus* in the USA (Rutledge, 1989) and barramundi (*Lates calcarifer*) (Rutledge and Rimmer, 1991) in Queensland, which have very similar life histories and breeding requirements to mulloway.

As previously discussed, the age and size structure of wild mulloway populations in NSW are dominated by fish less than 2 years old (Fig. 47). Estimated mortality due to fishing is between 36 and 50 %, and being 3 to 6 times greater than that of natural mortality is likely to be unsustainable as reflected by declining annual catches (Fig. 48). This trend in NSW 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 trawling in estuaries, is partially responsible for the decline in mulloway catches. However it is also widely acknowledged that entry of post-larval and early juvenile mulloway into more than 30 estuaries throughout NSW, is in many cases, restricted by intermittent blocking of entrances by natural shifts of sediments. A suggested way of combating these recruitment limitation factors was to release fingerlings at the end of the prawn trawling season, inside the entrances of estuaries whether blocked or not. This further strengthened the imperative to develop extensive low cost fingerling production technology.



**FIGURE 47:** Size and age frequency composition of commercial catches of mullet in NSW (pooled across regions). (Source :Silberschneider & Gray, 2005)



**FIGURE 48:** Commercial catch data for mullet in NSW 1940/41 to 2003/04

### 2.6.2 Acquisition of ripe broodstock

Fertilised mullet eggs can be obtained by artificial induction (hormonal injection) of ripe wild adults immediately following capture or by the much more reliable method of acquiring them from captive broodstock that are either wild collected or derived from previous generations of hatchery reared stock.

### 2.6.3 Capture and stripping of ripe wild broodstock<sup>2</sup> (Not recommended)

Ripe (late pre-spawning condition) wild adults can be collected by gill netting of spawning aggregations that form around headlands often in proximity to the mouths of estuaries and coastal lakes especially following protracted rainfall and freshwater runoff. In South and Western Australia Mulloway do not become sexually mature until they are approximately 70-80 cm (approx. 4 kg) and 5 to 6 years old. Male and female mulloway in South Africa mature at larger average lengths and ages of 92 cm/7kg and 107 cm/11kg and at 5+ and 7+ years respectively. By contrast the size and age at which mulloway attain sexual maturity in NSW is much smaller averaging 51 cm/2kg and 2+ years old for males and 68 cm/3.5kg and 3+ years for females.

The natural breeding season of mulloway also varies markedly between geographic regions and with latitude and is probably related to water temperature and oceanography. For example, in southern Africa, spawning in mulloway (known locally as “cob”) occurs from August to November (winter to spring) in the northern KwaZulu region (30-31°S), and from October to January (summer) in the southern and south-east Cape regions (33- 5°S). Similarly, along the West Australian coast, fish with ripe gonads occur in September and October in Shark Bay (26°S), whereas in the Swan River (32°S) they to occur 3 months later between December and January. In South Australia, mulloway spawn from late spring to late summer (November to February). In NSW spawning of mulloway is protracted and but possibly year round in the far north of the state. In central NSW (around 35°S) it is restricted to late summer and autumn (January to April).

Inaugural attempts by I&I NSW (then NSW Fisheries) in the early 1990’s to initiate hatchery rearing of mulloway were based on the capture and immediate hormone induced spawning of wild broodstock. The fish were caught using baited lines or gillnets from shallow inshore reefs and ocean beaches. However, these operations failed to consistently yield large numbers of high quality eggs for several reasons:

- Large (8 to 25 kg) fish in relatively deep areas were targeted and these, as discussed above, proved difficult to capture and hold in good health through to spawning. Particular problems were high capture-induced physiological stress and physical trauma plus gill embolisms (gas bubbles in the bloodstream) in the case of fish hooked and rapidly line hauled to the surface from depths as little as 3 m. As a result, mortality of captured fish during transport back to the hatchery was common.
- It was often difficult to synchronise capture of ripe male and female fish.
- As the latency period between administration of hormones and ovulation was unknown, accessing eggs for evidence of ovulation and hence the correct time to strip, imposed the need of multiple handling operations.
- Yields of eggs stripped from ripe wild stock were about a third the number (average of 1.25 million eggs per 12-15 kg female ) obtainable from equivalent size captive stock (4.25 million eggs per fish). Moreover hormone injected captive females commonly spawn over 3 consecutive nights. Eggs and fertilisation rates are more than four times (mean±s.d. 50±35%; range 0-95%) that of manually stripped wild caught counterparts. (mean±s.d. 11±10%; range 0-20%) Fielder, Bardsley and Allan, 1999)

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<sup>2</sup> It is cautioned that all other activities involving the capture and retention of broodstock especially during seasonal and/or area fishing closures are of course subject to procurement of collection permits from relevant fisheries authorities.

#### 2.6.4 *Use of captive broodstock as a source of fertilised eggs (Recommended)*

As a consequence of difficulties in reliably sourcing quality fertilised eggs, and hence viable larvae, from recently captured wild broodstock, a program was established for land-based management of captive broodstock in environmentally controlled conditions by I&I NSW (then NSW Fisheries) in 1992. The following account of techniques developed for mullocky is largely based on information sourced from Battaglione and Talbot, 1994 and Fielder, Bardsley and Allan, 1999. Supplementary information relating to the propagation of snapper and black bream but also relevant to mullocky has been sourced from Partridge et al, 2003.

#### 2.6.5 *Methods of capture*

##### *Gill netting*

In New South Wales, almost 100% of the commercial mullocky catch is by gill netting and although netting is not the preferred method of broodstock collection, it may be the only practical means in areas where the stocks are scarce and difficult to catch with hook and line. As the length of entrapment in nets has a direct influence on the subsequent health of the fish, nets used for collecting broodstock should be continuously attended and gilled fish immediately cut free of entangling mesh and transferred to floating pens, live wells aboard tender boats or equivalent large vehicle mounted transportation tanks to recover. Fish captured by hook and line should be lifted, by the fishing line and placed directly into floating holding pens constructed of soft, square mesh (non entangling) netting. Such stockpiling and holding facilities require high continuous rates of water exchange to minimise post capture stress of fish prior to their earliest transportation to hatchery holding facilities.

Fish that are left entangled in nets or held under highly confined conditions for extended periods will succumb to stress and quickly lose condition. Energy normally used for growth and reproduction is instead used to power stress responses, such as increased respiration and/or attempts to escape. Gonads begin to break down (atresia) affecting the fish's short-term reproductive capacity. Such fish typically display obvious external damage including loss of scales and deep bruising and/or lacerations. Less obvious, although potentially just as serious damage inflicted by netting, is loss of the mucous layer. The mucous layer coats the entire body and affords protection from fungi, bacteria and some ecto-parasites of the skin and gills. Mucous is continually secreted and sloughed off, taking potential pathogens with it. Although removal of the mucous as a result of abrasion against nets and handling has no immediate apparent consequences, it exposes the fish to infectious agents in the water.

##### *Capture by line and hook*

In contrast to netting, the most serious potential damage inflicted by line fishing is hook punctures in the mouth area that generally heal quickly and without infection. Only fish that are hooked in the mouth should be retained. Long-nose pliers can be used to grasp the shank of the hook for removal. Barbed hooks are preferred: whilst barbless hooks cause less damage and are easy to remove, it is better to land the fish and have a little extra difficulty removing a barbed hook than to lose the fish. As mullocky are usually caught in shallow water (<10 m deep), venting of swim bladder gas is not required. However as previously discussed air embolism can be induced in fish fast hauled to the surface from depths as shallow as 3m. Fish captured by hook and line should be lifted, by the fishing line and placed directly into floating holding pens constructed of soft, square mesh (non entangling) netting. Likewise gill netted fish should be cradle lifted into holding pens before being cut free of entangling mesh. Alternative short term holding and/or stockpiling equipment include smooth walled live-wells aboard tender boats and equivalent vehicle mounted transporter tanks.

Preferred size and age of wild collected stock at the time of capture is recently matured 2 to 4 kg , two to three year old fish (Figs. 43 and 45). Principal advantages of this class of fish are that they are far more numerous and hence procurable than larger older classes, much easier and faster to land, handle and transport back to the hatchery and can be held in larger numbers. The latter helps broaden the gene pool / genetic diversity of offspring to be used to rebuild or enhance depleted fisheries stocks.

#### 2.6.6 *Transportation of wild collected stock back to the hatchery*

Newly captured mulloway brood stock, whether line caught or netted, should be transported as soon as possible to the hatchery holding facility in an aerated live well or equivalent road transportation tank large enough for the fish to swim and maintain normal orientation without contacting surfaces. Such provisions translate to minimum linear dimensions of at least 1 metre and a volume of at least 500 L for fish up to 5 kg and of 1000L for larger fish of 6 to 15 kg. Aeration or oxygenation and resultant water movement must be provided to maintain DO at or near an optimal level of 100% saturation and to generate continuous lateral currents to assist and encourage swimming and maintenance of normal orientation by the fish. Aeration and water movement can be generated either by oil-less compressor driven directly off the vehicle's engine, or indirectly by vehicle's electrical alternator or alternatively by an independent petrol driven generator (Fig. 49). Another alternative is use of a 12 volt bilge pump or a battery operated aerator. Pure compressed oxygen may also be provided as an alternative or backup to air compressors. For a more comprehensive review on transporting live fish see Rimmer and Franklin (1997); 'Development of Improved Techniques for Transport of Live Fish.'



**FIGURE 49:** The PSFI fish transport tank with compressed oxygen bottle.

On arrival at the hatchery, the fish should be immediately acclimated to the broodstock tank conditions by gradually adding the hatchery water to the transportation tank over a period of 1 to 2 hours. The addition of new hatchery water in this way should continue until the temperature, pH and salinity of the water in the transport tank are the same as those in the broodstock holding tank. Before being transferred to a holding tank, the fish should be treated to reduce the possibility of introduction of disease to the hatchery. Mulloway broodstock are generally held in a quarantine tank of 10,000 L for 2-4 weeks.

Experience has shown that although mullet are robust fish, scale and mucous loss at capture can have serious health consequences. Such damage needs careful attention to prevent disease outbreaks. Prophylactic bathing in water dosed with 50-100 mg/L oxytetracycline and/or a series of 200 ppm formalin baths is advisable when first introducing new fish to hatchery. Such treatment should be administered in holding/quarantine tanks where the fish should be held for up to a month under regular observation for signs of infectious disease and to fully recover from post-capture trauma and stress prior to mixing with resident broodstock.

#### 2.6.7 *Broodstock holding and conditioning facilities*

Mullet broodstock in the range 2 to 15 kg should be held in tanks at least 20 m<sup>3</sup> in volume without sharp corners i.e. that are either round or preferably cylindro-conical and in the range 20 to 50 m<sup>3</sup> (Figs. 50 and 51). Supplementary stock can also be held in out door ponds up to 500,000 L (Fig. 52). There are several reasons for preferential use of cylindro-conical broodstock tanks:

- Using slow rotational currents, uneaten food and faeces will accumulate in the central bottom region where it can be easily removed by periodically opening the bottom drain.
- The hydrodynamics of cylindro-conical tanks promote efficient mixing of the water and hence the maintenance of homogenous conditions.
- A cylindro conical design ensures that any fertilised eggs in the surface waters of the tank are efficiently delivered to the egg collectors mounted in the overflow outlets. Pelagic spawning marine fish such as mullet release eggs that are positively buoyant. As already discussed in relation to Australian bass, (see Chapter 1), water level in mullet broodstock tanks is best set by an overflow opening, or pipe, which directs the water to an egg collector. Any viable buoyant eggs are thus automatically and continuously skimmed from the surface of the tank and collected in an egg net, set within the egg collection vessel. A water flow rate equivalent to approximately eight tank volumes per day in a cylindro-conical broodstock tank will ensure that all of the eggs are collected within 12 hours of spawning.



**FIGURE 50:** Broodstock room at the PSFI.



**FIGURE 51:** Representative broodstock tank used to hold mullet, bass, yellowtail kingfish and snapper at PSFI.



**FIGURE 52:** Outdoor ponds used for holding supplementary broodstock at PSFI.

### 2.6.8 *Management of captive broodstock*

#### *Stocking rates, sizes and ages and sex ratio*

A conservative stocking density of 2 - 4 kg of fish / m<sup>3</sup> should be adopted to reduce captive stress on wild collected mulloway broodstock. Thus 20, 50, and 100 m<sup>3</sup> tanks should be stocked with a maximum combined broodstock biomasses of no more than 80, 200 and 400 kg, respectively. Male to female sex ratio should be maintained at about 1:1.

As discussed above, mulloway in NSW first mature and spawn from an age and size averaging 51 cm/2 kg and 2+ years old for males and 68 cm/3.5 kg and 3+ years for females. The threshold size and age at which 100% of mulloway have attained sexual maturity are, in the case of males, 65 cm/3kg and 3+ years old and in the case females, 80 cm/5kg and 5 years old. As wild collected mulloway broodstock generally require 1 to 2 years of domestication before coming into regular breeding condition, and as the bulk of wild populations comprise 0+ to 2+ year old stock (Fig. 46) the most opportune age of capture for males is as 2+ year olds and for females as 3+ year olds. It is recommended stock intended to produce fingerlings for seeding enhancement of depleted or recruitment limited wild populations should comprise at least 25 gender pairs. It is also recommended that individual captive broodstock be replaced by new wild sourced counterparts after a maximum of 3 or 4 breeding seasons to help further ensure that the genetic diversity of wild stocks are not compromised by large-scale stocking with hatchery produced fingerlings. This translates to the replacement of 20-30% fish each year and an overall average age and size of captive broodstock of 5 to 7 years old and 8 to 10 kg respectively.

### *Physiochemical conditions*

As mature mullet are lower estuarine and inshore coastal fish, salinity should be maintained at  $30 \pm 2$  ‰ which has been found optimum for reproductive performance. Critical water quality parameters in broodstock tanks should be maintained at all times within ranges provided in Table 6. This can be achieved either by the provision of good quality flow-through water for hatcheries with year-round access to unpolluted coastal seawater, or with the use of a recirculating seawater systems. The I&I NSW hatchery at PSFI is located on an estuary where locally available seawater fluctuates in salinity from 20 to 35 ‰ and is subject on occasions to high level runoff from local acid sulphates soils that can reduce pH to levels as low as 5. Accordingly, primary mullet broodstock conditioning facilities at PSFI are operated as closed recirculating systems as described for yellowtail kingfish in Chapter 3.

#### *2.6.9 Food and feeding*

The diet fed to broodstock should not be limiting in terms of quantity, quality or variety. Extensive research with a range of marine fish species, has shown that the nutritional content of the diet fed to pre-spawning broodstock has a significant effect on the number, size and quality of eggs spawned and the subsequent viability of the larvae produced. Of specific importance are the highly unsaturated fatty acids, particularly DHA and EPA, vitamins A, C and E, and carotenoids such as astaxanthin. Broodstock should be fed a broad, locally sourced fresh or freshly frozen diet that includes uncooked (green) prawns, squid, high oil containing clupeid fish such as sardines, whitebait, or pilchards, prepared in bite size pieces, plus shucked flesh of marine bivalves (mussels, clams scallops or oysters) in a ratio range of 1-2:1-2:1-2:1-2:1-2:1-2.

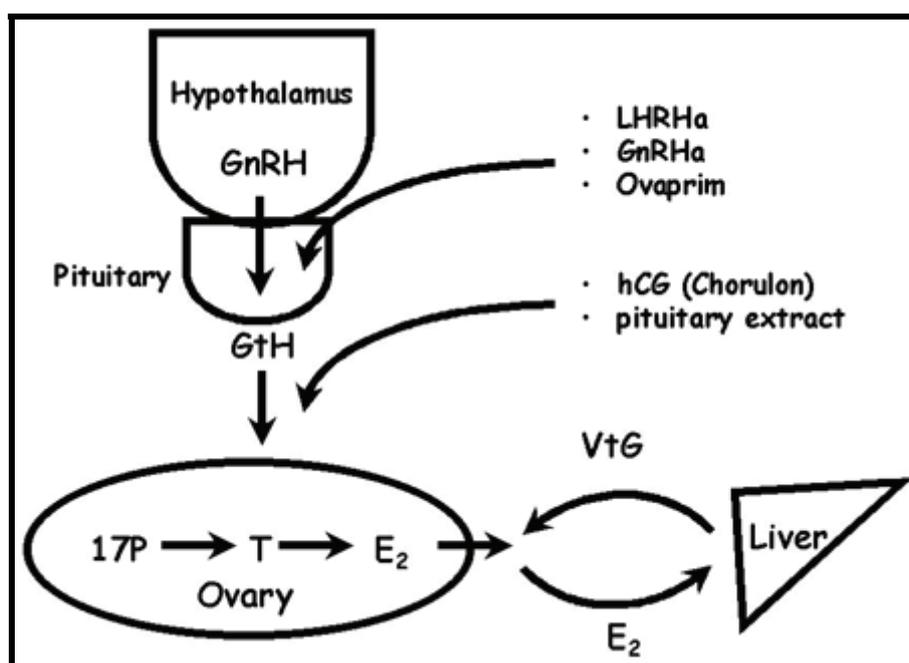
Mullet broodstock should be fed to satiation 5 to 7 days a week at an approximate rate of 1% of their body weight per day but more food will be consumed during the warmer phases of controlled thermo-photo cycles described below. Close observation of the fish, in particular feed intake, will quickly determine the optimum feeding rate for each group of fish and will also provide a good indication of fish health, as decreased appetite is often the first indicator of stress and/or disease. Records of feed composition, intake and behaviour are vital for efficient broodstock management (Appendix 6.4: Hatchery data sheets).

Surplus feed and faeces should be removed daily by vacuuming the tank bottom and opening the drain valve. The sides of the tank should be wiped weekly with a sponge or broom to remove any biofouling that may accumulate on these surfaces.

## 2.7 Seasonal and Controlled Year-Round Induction of Breeding

(based on Battaglene and Talbot 1994 and Fielder, Bardsley and Allan 1999)

There are two main approaches to inducing ovulation in fish; treatment with gonadotropin (GtH) or analogues of gonadotropin-releasing hormones (GnRHa). GtH mimics the action of the fish's natural gonadotropins, which stimulate the production of reproductive steroids in the ovary (17P, T, E<sub>2</sub>) and therefore induce maturation and ovulation (Fig. 53). The most common hormone used for this approach is human chorionic gonadotropin (hCG), which is injected in a saline solution or distilled water either into the muscle or peritoneal cavity of the fish. HCG can be purchased as Chorulon<sup>®</sup> (Intervet). Preparations of GnRHa stimulate the natural secretion of the fish's own gonadotropin (Fig. 53). In some species, but not mulloway, GnRHa may need to be administered in conjunction with a dopamine antagonist (eg domperidone) since the release of GtH is often down-regulated by dopamine. The two most common forms of GnRHa are Ovaprim<sup>®</sup> and Luteinising Hormone Releasing Hormone analogue (LHRHa). Ovaprim<sup>®</sup> differs from LHRHa in that it contains domperidone. Both hormones may be administered as for hCG, although LHRHa can also be administered as a slow release pellet placed into the body cavity of the fish. All of the hormones outlined above are available under prescription from most major chemical or veterinary supply companies.



**FIGURE 53:** Schematic diagram of the reproductive-endocrine pathway in teleost fish indicating the action of various hormone treatments. (Source: Partridge et al., 2003).

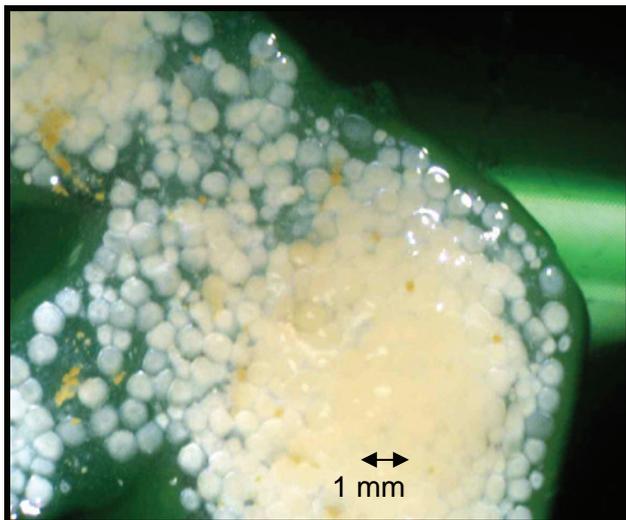
For mulloway, all wild collected broodstock held in the hatchery under ambient conditions (natural photo-therms) will come into breeding condition within 1 to 2 years. Males begin spermiation and most females attain pre-spawning condition (eggs >500 micron) during the warmer months of the year (January to March in central NSW).

First generation (F1) hatchery reared mullet broodstock at the PSFI ovulate and spawn naturally i.e. without the need to be injected with exogenous hormones. If F1 or subsequent generation hatchery reared broodstock are not available, induction of ovulation via administration of exogenous hormones as described above will be required. As can be seen in Figure 53, the exogenous hormone hCG acts lower down the reproductive-endocrine pathway and therefore has a more immediate effect on the ovary. For this reason, hCG is often used when inducing newly caught wild broodstock to ovulate. Eggs of female mullet must be greater than 500  $\mu\text{m}$  in diameter (Fig. 54).

HCG induction can also be used to increase milt volume and motility in males. For mullet, hCG is effective at dosages of 1000 international units (IU)/kg for females and between 500 and 1000 IU/kg for males. As described for Australian bass, (see Chapter 1), a single dose of hCG is injected into the peritoneal cavity of the anaesthetised fish. The peak fecundity and fertilisation of eggs from stripped wild fish occurs 30 to 35 hours after induction, and successful repeat stripping is possible. Wild fish induced with hCG at PSFI have been repeatedly stripped up to seven times post injection (Battaglione, 1995).

Fish that first spawn naturally 30 to 35 hours after hCG injection, commonly repeat spawn over 3 consecutive nights. Fertilisation rates of spawned eggs vary over a range of from 30 to 95%. Hormonally induced females that ovulate but fail to spawn need to be strip spawned as do companion males. Stripping yields lower numbers of eggs with “in vitro” fertilised eggs yielding more variable fertilisation rates in the range 0–70%.

At PSFI, the alternative use of LHRH-a allows female mullet with oocytes as small as 400  $\mu\text{m}$  in diameter to ovulate successfully. Induction of females with oocytes above 500  $\mu\text{m}$  however, generally provides more reliable spawning and higher fertilisation rates. LHRH-a is injected in the form of a single slow-release cholesterol-based pellet at doses between 20 and 50  $\mu\text{g}/\text{kg}$  (see Appendix 6.3 for details on the manufacture and implantation of cholesterol-based LHRH-a pellets). The pellet is injected into the dorsal musculature of the fish under anaesthesia. Approximately 7-10 days after implantation the fish then begin to spawn serially for up to several weeks.



**FIGURE 54:** Microscopic view of an ovarian sample. (Source: Partridge et al., 2003).

Hatchery-reared broodstock exposed to ambient temperatures and photoperiods are generally expected to exhibit a similar spawning season to that of wild fish. The duration of the spawning season can be extended slightly by heating the tanks at either end of the season; so that optimum temperatures are reached earlier and maintained for longer than in the wild.

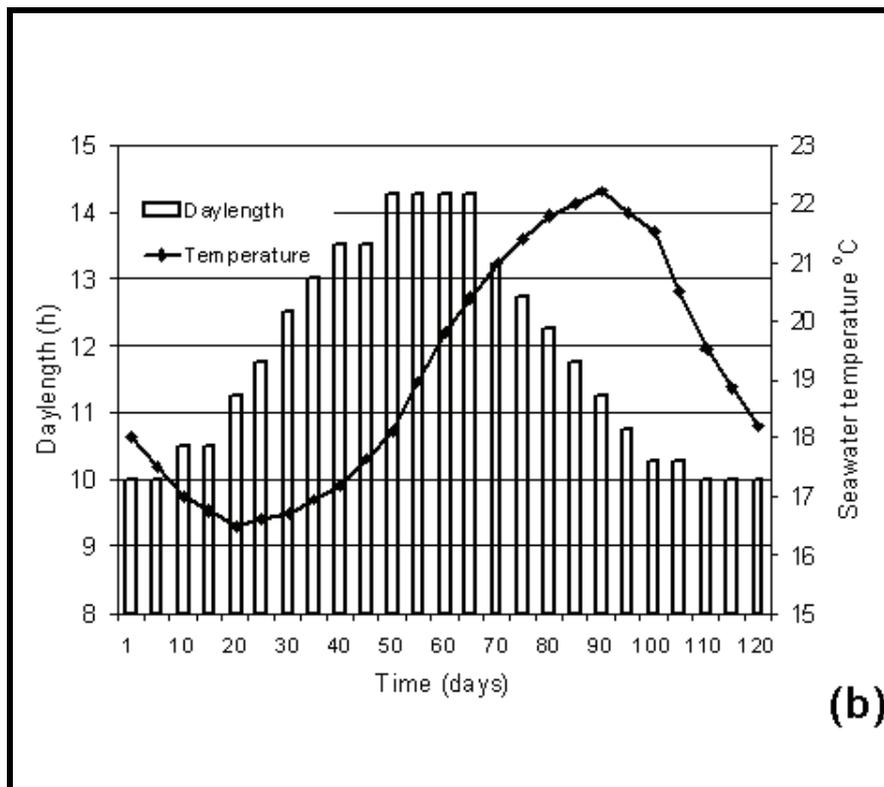
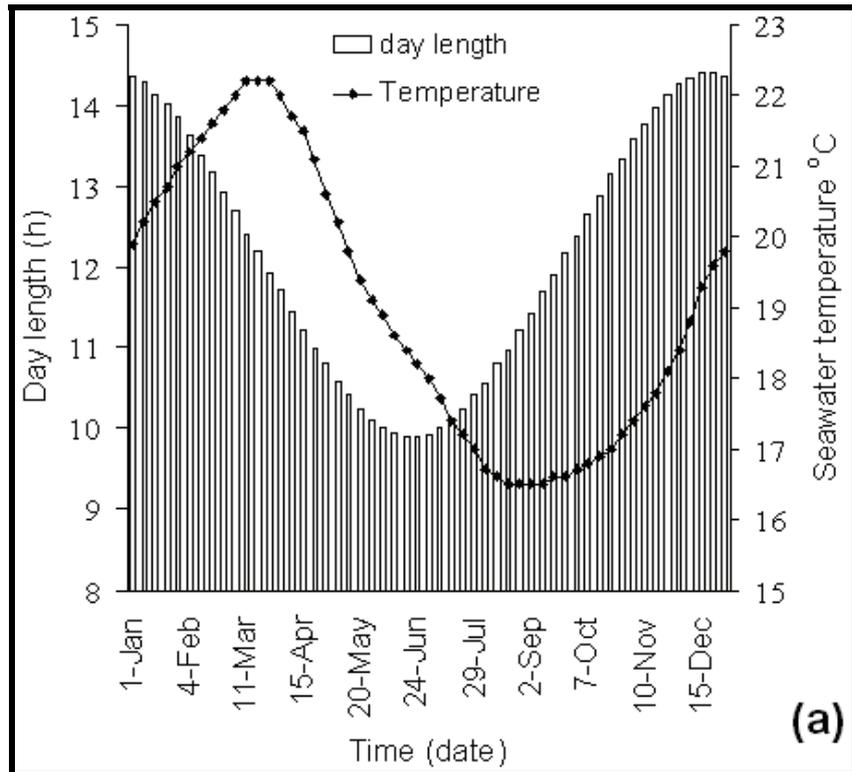
Use of photo-thermal conditioning units enables out-of-season hatchery production of mullet. Broodstock held in such systems are maintained on artificial photoperiod and temperature regimes that simulate natural seasonal cycles. Fish can be 'programmed' to mature (or spawn) out of season by offsetting the summer-winter cycle or to spawn more than once per year by compressing more than one summer-winter cycle into one year. The gradual seasonal changes that occur in nature (Fig. 55a) can be compressed into a cycle as brief as 120 days, allowing up to three cycles, and hence three spawning seasons per controlled system per year (see Fig. 55b and Table 5). By operating say 3 multiple photo-therm conditioning units out of synchrony (e.g. set at staggered intervals of 0, +40 days and +80 days) it is possible to produce eggs and larvae in most months. Benefits conferred are higher, more efficient and versatile hatchery output. However, in common with counterparts held under ambient conditions, wild collected mullet conditioned in phototherms still require administration of exogenous hormones to induce ovulation and spawning.

At PSFI, use of 2- independent, recirculating conditioning units (Fig. 56) both operating as truncated 120 day seasonal cycles but offset from one another by 60 days (maximum asynchrony) enables the year-round production of commercial numbers of mullet or snapper seed stock..

At the PSFI, hormone induction is no longer used to trigger final maturation and spawning of captive F1 fish. Raising the water temperature from 16 to 22°C within 24-48h provides the final cue to initiate spontaneous spawning.

**TABLE 5:** Compressed seasonal photoperiod and temperature regime used in the phototherm rooms at PSFI. This regimen is suitable for mullo way and YTK as well as snapper for which it was originally designed.

<b>Date</b>	<b>Day-length</b>	<b>Light ON</b>	<b>Light OFF</b>	<b>Temperature °C</b>	<b>Day</b>
1-Jan	10.5	6:45	17:15	16	1
6-Jan	11.25	6:15	17:30	16.5	5
11-Jan	11.75	6:00	17:45	16.5	10
16-Jan	12.5	5:30	18:00	16.7	15
21-Jan	13	5:15	18:15	16.95	20
26-Jan	13.5	5:00	18:30	17.2	25
31-Jan	13.5	5:00	18:30	17.65	30
5-Feb	14.25	4:45	19:00	18.1	35
10-Feb	14.25	4:45	19:00	18.95	40
15-Feb	14.25	4:45	19:00	19.8	45
20-Feb	14.25	4:45	19:00	20.4	50
25-Feb	13.25	5:15	18:30	21	55
2-Mar	12.75	5:30	18:15	21.4	60
7-Mar	12.25	5:45	18:00	21.8	65
12-Mar	11.75	6:00	17:45	22	70
17-Mar	11.25	6:15	17:30	22.2	75
22-Mar	10.75	6:30	17:15	21.85	80
27-Mar	10.25	6:45	17:00	21.5	85
1-Apr	10.25	6:45	17:00	20.5	90
6-Apr	10	7:00	17:00	19.5	95
11-Apr	10	7:00	17:00	18.85	100
16-Apr	10	7:00	17:00	18.2	105
21-Apr	10	7:00	17:00	16	110
26-Apr	10	7:00	17:00	16	115
1-May	10.5	6:45	17:15	16	120



**FIGURE 55:** Natural (a) and compressed (b) temperature and photoperiod regime suitable for inducing breeding in broodstock mullet and YTK as well as in snapper for which it was originally designed.

### 2.7.2 Design, operation and performance of broodstock photo-therm rooms

There is one broodstock conditioning facility at PSFI. The shed measures 30 m x 30 m with a roof height of 14 m. Ten 22,000-L tanks measuring 4.0 meters in diameter and 1.8 m in height are held in the shed. Each tank is provided with adequate space for easy access to the broodstock tank, sump and bio-filter to facilitate ease of feeding, siphoning and back-flushing filters. Pumps and mechanical filters servicing each tank can be inside or outside the photo-therm room depending on available space.

Each tank is operated at approximately 22,000 L water volume, holds between 10 and 20 fish and is aerated via a single 12 mm airline and ceramic air-stone. As shown in Figures 51 and 56, a single 80 mm PVC pipe is positioned approximately 100 mm below the top of the tank and allow water to overflow from the broodstock tank into a 500 L sump. Each sump is fitted with an internal, fully immersed 500 µm mesh net bag to harvest eggs. Water then flows into a self-cleaning Hydrotech drum filter fitted with a 25 µm mesh to remove suspended solids. Filtered water then flows into 1000-L biological filter filled with 300 kg of plastic bee-cell media. The biofilter is heavily aerated from a perforated 12 mm polypipe air ring to provide oxygen to the nitrifying bacteria and to encourage sloughing of built-up bacterial slime. The water is then pumped through an outdoor heater/chiller unit and then returned to the main broodstock tank. Each tank is covered with a solid, fibreglass lid with a removable manhole to allow access from the top for feeding, vacuuming and entry of staff to the tank if fish handling is required. Artificial light is supplied from a 40W fluorescent tube situated on the lid and operated with a simple time switch. A viewing window is positioned 2/3 of the way up each tank. These are uncovered to allow easy observation of fish. Three tanks of mulloway broodstock are maintained at PSFI.

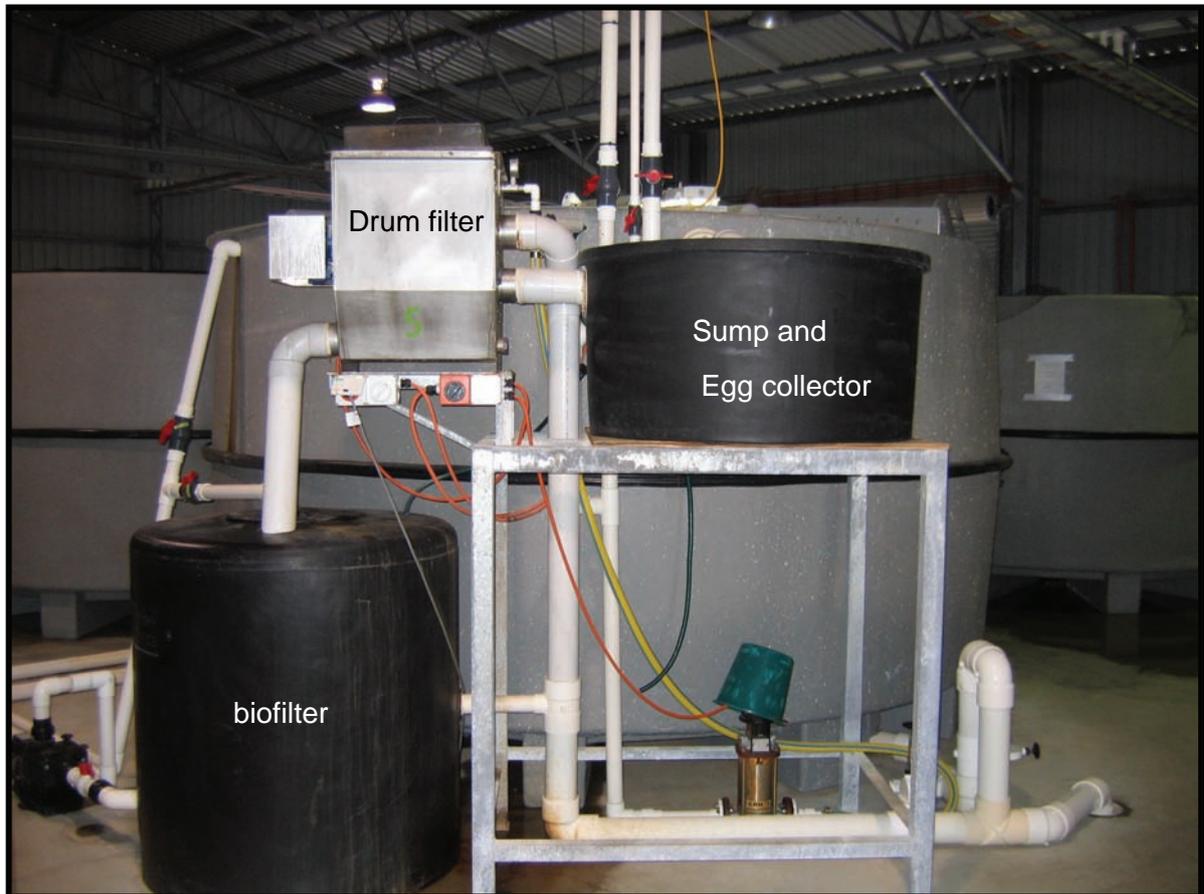
At PSFI, the incoming (exchange) estuarine supply water is filtered to 10 µm (nominal) with *Quiptron*® cartridge filters and is delivered into the broodstock tank. Approximately 2000L (10%) of new filtered seawater is exchanged each day in each tank. The room also has an additional estuarine supply line to allow rapid re-filling of tanks. A 25mm freshwater line and hose is supplied for easy cleaning of the room and equipment.

The water in each broodstock tank is recycled approximately every 2 hours. Incoming estuarine water is supplied continuously. Bacterial blooms and accumulated suspended detritus can rapidly cloud recirculated seawater. Accordingly, clarity of the tank water can be a good indicator of blockages or failures of the filters. In an enclosed recirculated system, exchange is needed principally to maintain nitrate concentrations at low levels. Nitrate is the end product of the nitrification process that occurs in the bio-filter and therefore nitrate concentrations can only be kept at low levels in these particular recirculating tank systems by constant exchanging of water at a net rate of 5 to 10% of the tank volume daily.

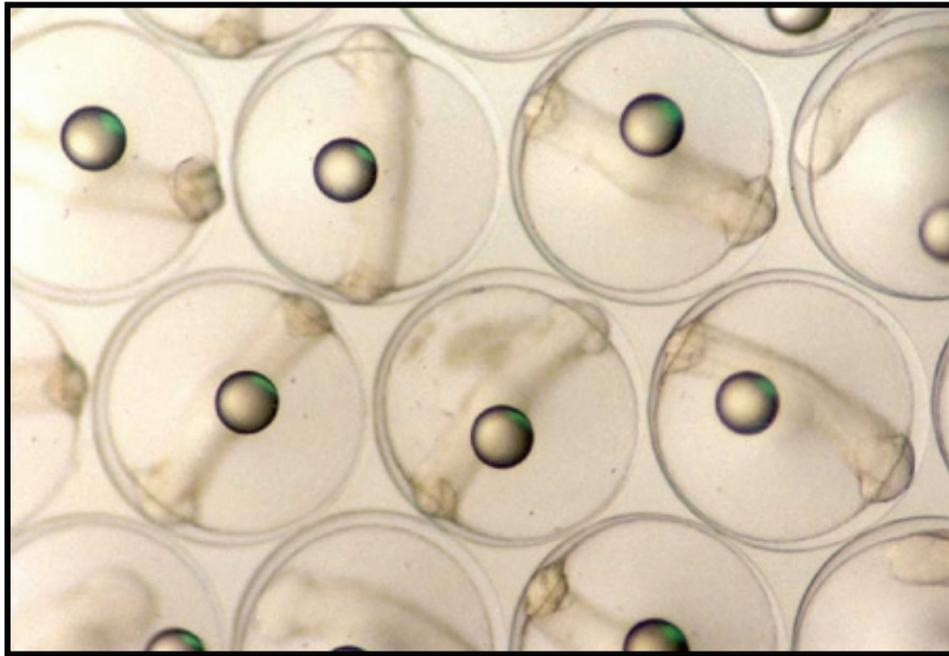
Production of eggs (Fig. 57) from domesticated mulloway broodstock began at the PSFI in 1998 however, consistent reliable production of large numbers of eggs was not achieved until 2002. The demand for mulloway eggs for research and production has been intermittent from 2002 to present, however, F1 mulloway have successfully spawned spontaneously without fail usually 2-3 days after a temperature spike from 16 to 22°C.

### 2.7.3 Egg harvesting counting and incubation

As for Australian Bass previously described in Chapter 1.



**FIGURE 56:** Controlled environment recirculation tank for broodstock maintenance at PSFI. Note the plumbing from the pump goes to an outdoor heater/chiller unit and then returns to the tank.



**FIGURE 57:** Mulloway eggs have a mean diameter of approximately  $938 \pm 24 \mu\text{m}$  with a single oil globule with mean diameter of  $270 \pm 30 \mu\text{m}$ .

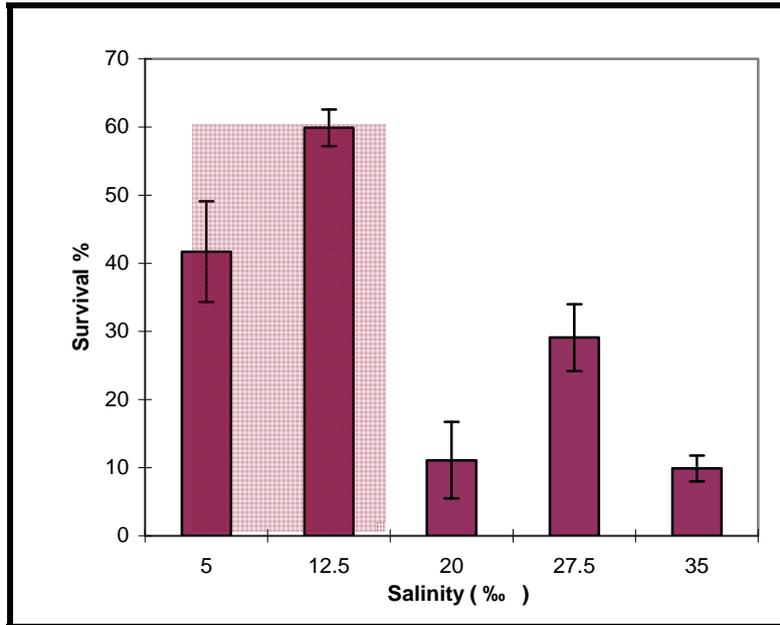
## 2.8 Larviculture

### 2.8.1 Introduction and background

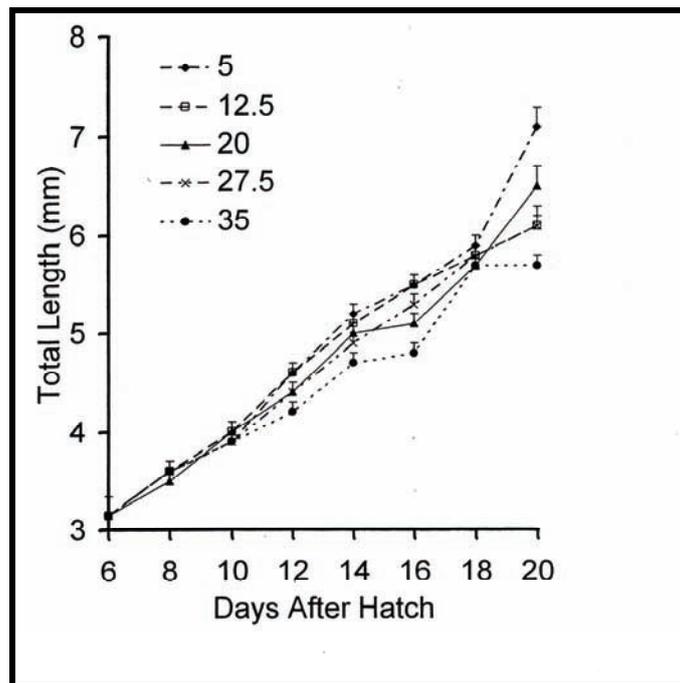
Intensive clear-water hatchery methods were initially used by I&I NSW (formerly NSW Department of Primary Industries, formerly NSW Fisheries) to produce mulloway fingerlings and thence to assess its potential for farming and fisheries enhancement. However intensive hatchery production is expensive requiring both dedicated live food production and larval fish rearing facilities and a high input of labour from skilled technicians. To address this issue research was undertaken by I&I NSW (Fielder, Bardsley and Allan, 1999) with support funding from the FRDC (Project no. 95/145) to compare the utility of intensive clear water production of fingerlings with two alternative methods, namely semi-intensive green water culture in large outdoor tanks and extensive rearing in multi-purpose earthen or lined ponds using a relatively low input of experienced labour. Equipment and operating protocols for the three alternative systems are described below.

At hatch, mulloway larvae have a mean  $\pm$  s.d total length (hereafter referred to as length) of  $2.25 \pm 0.09$  mm with a yolk sac of  $0.88 \pm 0.08$  mm and oil globules of  $0.27 + 0.03$  mm. Initial swim bladder inflation, exhaustion of yolk reserves and hence need for exogenous feeding occurs on day 3 or 4 after hatch.

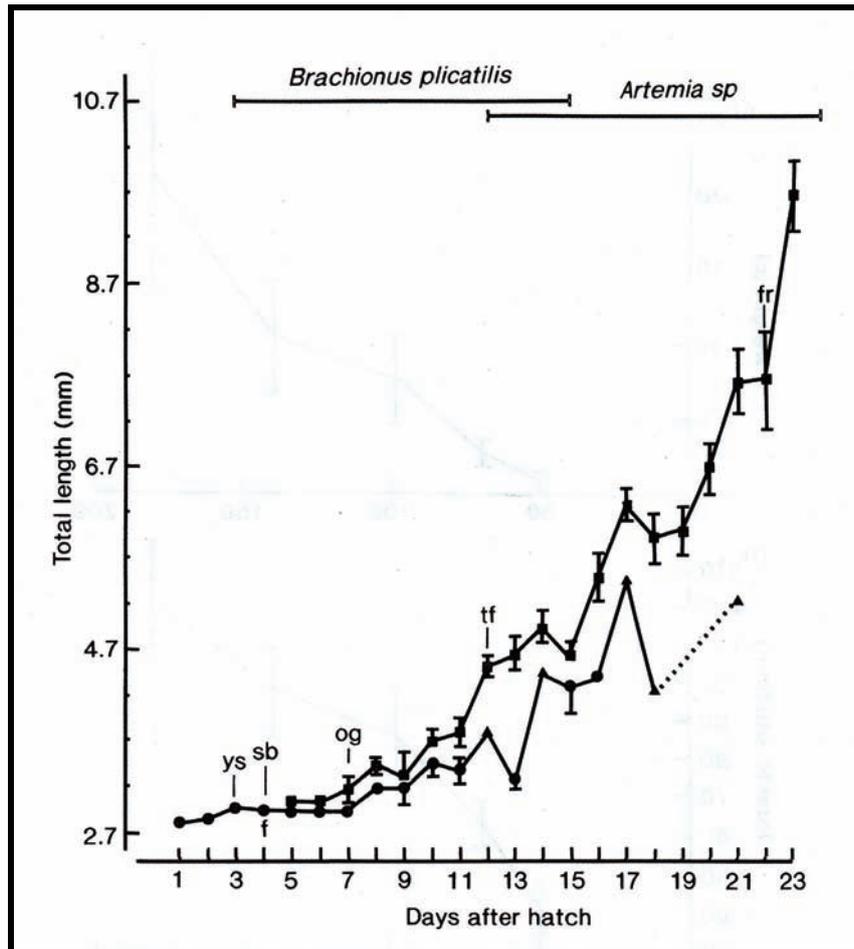
Optimum salinity for larviculture, is in the range 5-10 ‰ (Fielder, Bardsley and Allan, 1999). This low range is particularly important in sustaining high rates of survival (Fig. 58) and to a lesser degree growth (Fig. 59). At optimum rearing temperatures of 20 to 24°C, metamorphosis of intensive hatchery reared mulloway larvae first occurs around 23 days after hatch at a TL of about 12 mm and is completed around 34 dah by which time larvae are in the range 15-26 mm. Larvae with functional swim bladders (generally  $> 70\%$  by day 11) grow faster than those without (Fig. 60) and cannibalise the latter smaller fish from about day 18 onwards.



**FIGURE 58:** Effect of salinity on the survival rate (means  $\pm$  se) of 20 dah mulloway larvae at  $23 \pm 1^\circ\text{C}$ . Shaded area is recommended salinity band. (Source: Fielder, Bardsley and Allan, 1999).



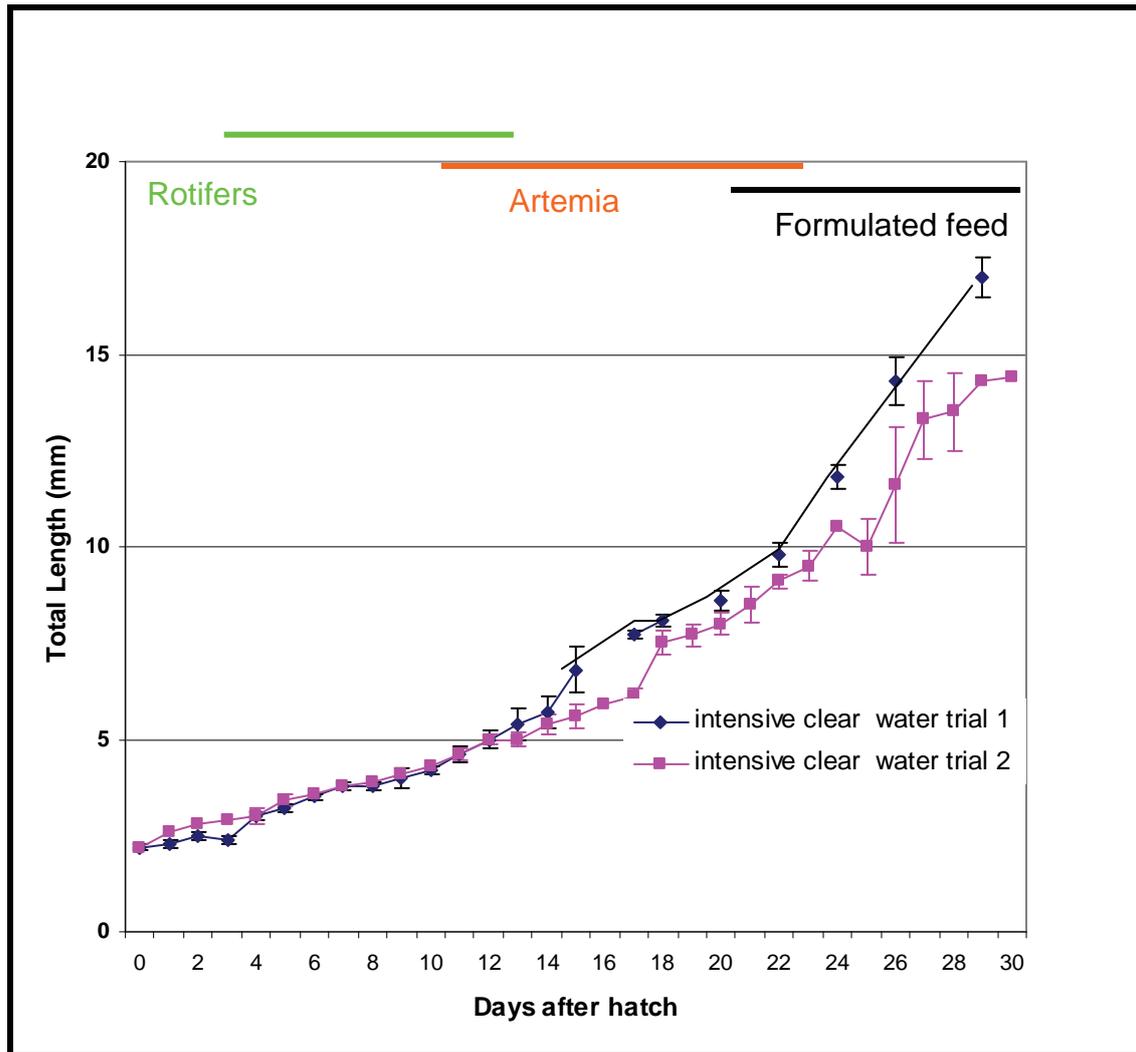
**FIGURE 59:** Effect of salinity on the growth (means  $\pm$  sd) of 20 dah mulloway larvae at  $23 \pm 1^\circ\text{C}$ . (Source: Fielder, Bardsley and Allan, 1999).



**FIGURE 60:** Growth and development of larval mulloway with (■) and without (●) swim bladders. Feeding regimes and important stages of larval development are indicated. f = feeding started; fr = fin rays present; og = oil globule absorption; sb = initial swim bladder inflation; tf = tail flexion; ys = yolk-sac absorption. Data are mean  $\pm$  s.e. (Source : Battaglene and Talbot, 1994).

### 2.8.2 Intensive indoor clear-water larviculture at the PSFC

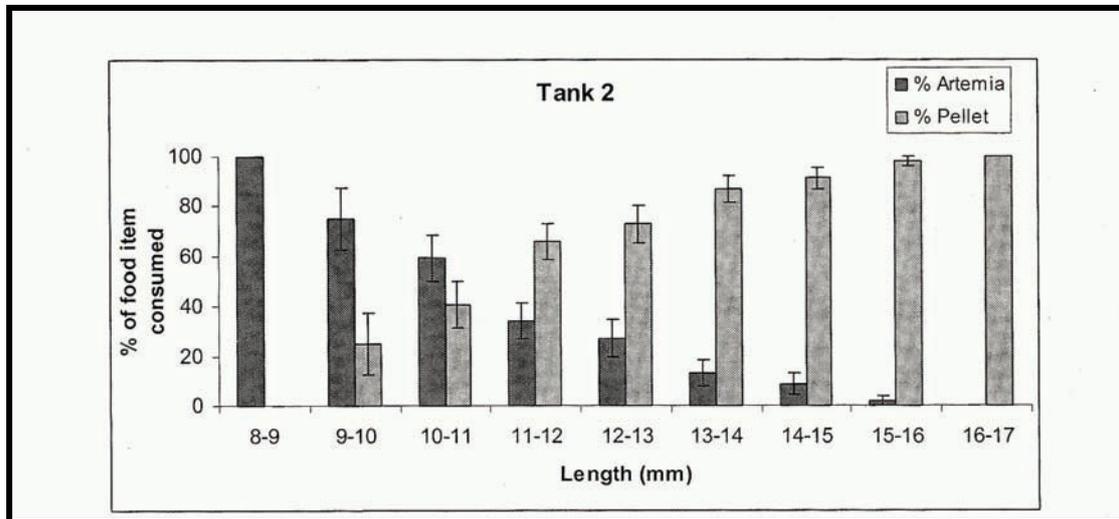
Intensive indoor clear-water hatchery rearing equipment and operating protocols for mulloway at the PSFI are in most respects the same as already described within this manual for rearing Australian bass (see Chapter 1; pp 38-50) and for rearing snapper as described in **Chapter 6, pp 49-58 of Partridge et al. (2003)**. Yolk-sac larvae should be initially stocked at 50–100 per litre with and expected yield of 10- 20% of fully weaned post metamorphic 14-18mm juveniles at about 30 dah.



**FIGURE 61:** Growth of mulloway larvae in 2000L clear water recirculation tanks. Data are mean Total Length  $\pm$  s.d. (trial 1: n=3 tanks into 20 dah and n=2 tanks 20-30 dah; trial 1: n=2 tanks throughout). (Source: Fielder, Bardsley and Allan, 1999).

As with Australian bass, feeding of mulloway larvae at about 10/mL is commenced and maintained with large strain rotifers (Fig. 61) between days 3 and 16. The rotifers are nutritionally fortified overnight with *Isochrysis galbana* and *Pavlova lutheri* as well as Algamac 3050®. As with snapper and Australian bass larvae, optimised feeding and food conversion efficiency are promoted using a 12h:12h light to dark regime until swimbladder inflation has occurred. After swimbladder inflation, photoperiod should be increased to 18h:6h light to dark regime to promote optimal growth (Ballagh et al., 2010). At 10 to 12 days after hatch and a mean length of about 5.2mm the rotifer diet is supplemented with on-grown *Artemia* metanauplii at 1/mL (Ballagh et al., 2010). The *Artemia* are also nutritionally boosted with the micro-algae *Isochrysis galbana* and *Pavlova lutheri* and with Algamac 3050® (see detailed procedures for enrichment of rotifers and *Artemia* in Chapter 8 of Partridge et al., 2003). Feeding of recently hatched *Artemia* nauplii to mulloway larvae is discouraged as nutrient deficiencies have been found to promote high mortality in pre-metamorphic larvae.

Once reaching a length of about 11 mm, coincident with metamorphosis (transition from larvae to juveniles), mullet larvae can be weaned off *Artemia* metanauplii onto formulated feeds. Weaning involves learning and behavioural changes and as illustrated in Fig. 61, it is best to phase in pellet feeds and to progressively reduce quantities of *Artemia* metanauplii from a mean length of about 10 mm over an age span of 22 to 28 days after hatch (Fig. 62) (Ballagh et al., 2010).



**FIGURE 62:** Percentages (mean  $\pm$  s.e.) of *Artemia* and artificial particulate diet consumed by mullet larvae (Source: Ballagh et al. 2010).

Suitable weaning diets include a Japanese diet, Otohime®, the European diet, INVE Proton®, or a Thai sourced diet, INVE NRD 4/6 crumble. Artificial feeds initially offered should range in particle size from 200 – 400  $\mu\text{m}$ . This size can be increased within about 5 days to 400-600  $\mu\text{m}$  and over the next 7 days up to 1400  $\mu\text{m}$  using blends of feed grades comprising progressively larger mean particle sizes. Adding formulated feed regularly in small amounts spread evenly across the entire tank surface, maximises the larvae’s opportunity to encounter the artificial food particles and therefore to ‘learn’ to recognise them as food. Feeding every hour during daylight hours is preferable. However as this method relies on an excess of food available in the water column, uneaten food will accumulate on the tank bottom, and must be siphoned off daily.

Over the a weaning period of about a week *Artemia* feeding levels should be progressively reduced from 1.0 to 0.8 to 0.6 and finally to 0.3/ml for several additional days or until all fish are exclusively feeding on the artificial diet. As a guide, by the time *Artemia* have been completely withdrawn, approximately 5-10 g of food per 1,000 larvae should be offered per day. Once mullet larvae are fully weaned, expensive imported diets cited above can be replaced by a much cheaper Australian diet (*Skretting* 0.6 mm crumble or similar). Initially, a combination of Otohime® and crumble are offered. Once acclimated to the crumble, the Otohime® is phased out. The juvenile fish should be feeding exclusively on *Skretting* or similar crumble by an age of approximately 50 dah. Once the larvae are consuming the artificial diet, automatic feeders can be employed to feed part of the daily ration.

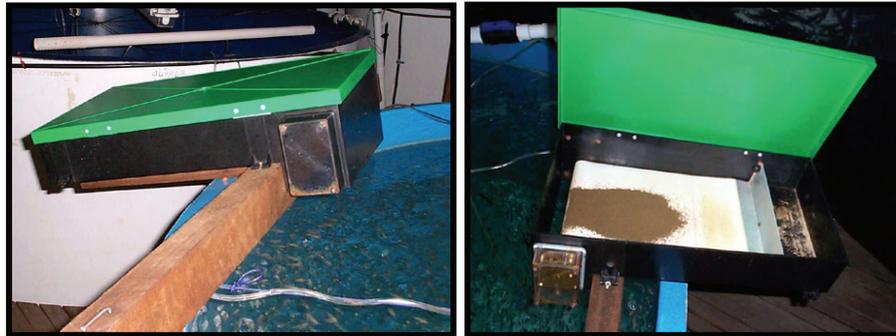
Belt feeders are used for this purpose (Fig. 63), usually from around Day 30 and the use of two or more feeders eliminates the tendency for larger fish to congregate under a single feeder and out-compete the smaller fish for food. Auto-feeders should not be used solely; no more than 80% of the daily ration should be fed automatically. Hand-feeding the remainder of the daily ration allows observation to be made on the fish growth performance and health. Results of recent investigations (Fig. 64) however suggest that once fully weaned, twice daily feeding in combination with a 18h light:6 h dark regimen is at least as good as continuous feeding in terms of growth and food conversion efficiency (Ballagh et al., 2010). Results of these investigations have also shown that juvenile mullet grow faster and more efficiently under medium light intensity ( $\approx 130$  lux) than at low ( $\approx 0.3$  lux) or high ( $\approx 900$  lux) light intensities and that being gregarious and exhibiting strong schooling behaviour, they grow faster and more efficiently at medium densities of 500 to 1000 fish/m<sup>3</sup> than at a low density of 250/m<sup>3</sup> (Fig. 65; Fielder et al., 2010). The optimal rearing parameters and feeding schedule for mullet larval rearing used at PSFI is summarized in Table 6.

**Table 6:** The optimal rearing parameters and feeding schedule for mullet larval rearing at PSFI.

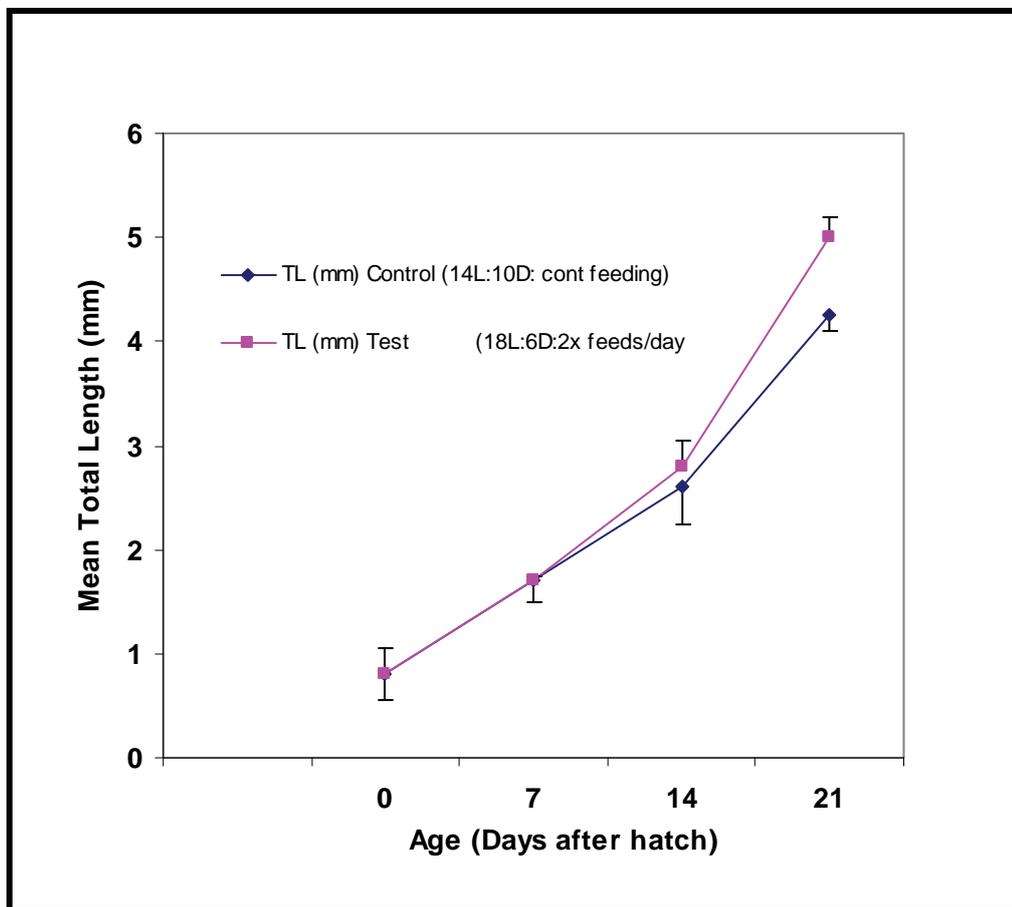
<b>Species:</b> Mullet ( <i>Agyrosomus japonicus</i> )			
<b>Parameter</b>	<b>Target</b>	<b>Dah</b>	<b>Adjustment</b>
<b>pH</b>	7.6 - 8.2	0+	
<b>Dissolved Oxygen (mg/l)</b>	>6.00	0+	Use compressed oxygen diffuser to maintain saturation level
<b>Temperature (°C)</b>	22	0+	Increase post SB inflation
<b>Salinity (ppt)</b>	5 to 35	0+	5-12.5 ppt optimal
<b>Water Exchange (%/day)</b>	100 - 200	0+	Increase exchange as larvae develop
<b>Surface Skimmer (hrs/day)</b>	24	4+	Monitor skimmer to ensure larvae at water surface are not affected
<b>Photo-period (L:D)</b>	(12:12) (18:06)	(0+) (6+)	Increase post SB inflation
<b>Light Intensity (Lux)</b>	225-400	0+	Start with light at lower intensity
<b>Green-water (cells/ml)</b>	$1.4 \times 10^6$	0+	Pro-Aqua* concentrate $57 \times 10^9$ per ml
<b>Rotifer (R/ml)</b>	20.0 - 5.0	4+	Initial 20/mL until feeding and then increase frequency of reduced concentration (e.g. 4x5/ml/d).
<b>Artemia (A/ml)</b>	0.2 - 2.0	12+	0.2/mL until weaned, then increase concentration and frequency. Start at 5.4 mm TL
<b>Weaning Diet size (<math>\mu\text{m}</math>)</b>	200 - 800	22+	Commence weaning at 10.5 mm TL

\*Algae concentrate used Rotifer Diet-3600 (*Nannochloroosis/Tetraselmis* blend) from Reed Mariculture Instant Algae, imported via Proaqua Australia. <http://www.proaqua.net.au>

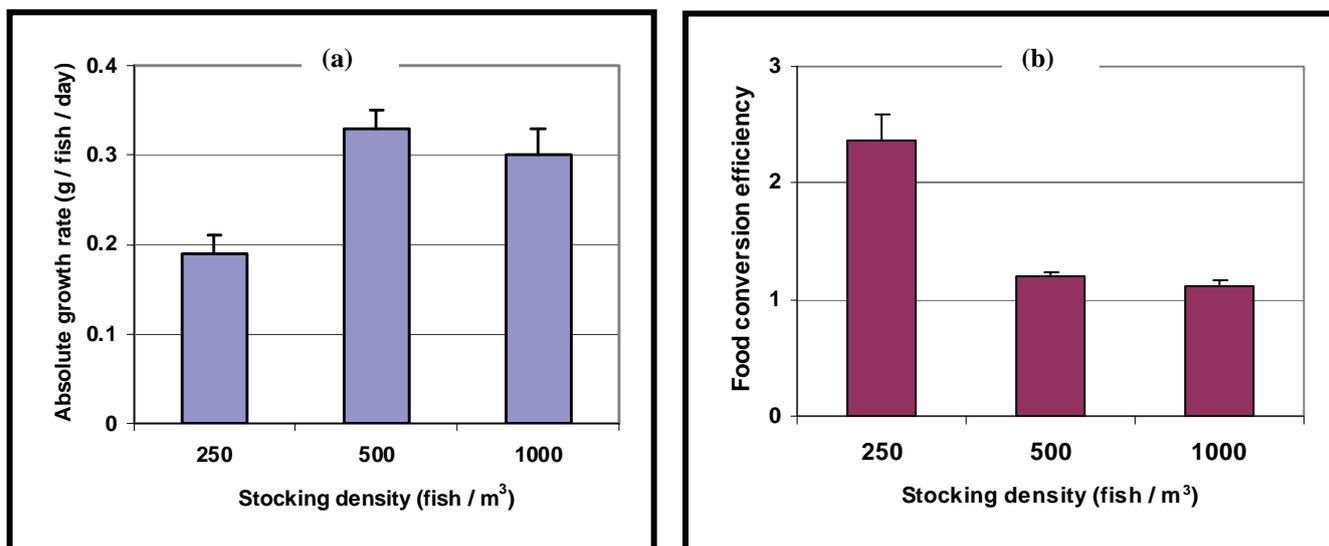
NB. One lux is equal to 1.46 milliwatts (0.00146 watts) Full daylight at noon  $\approx 100,000$  lux  $\approx 10,000$  foot candle  $\approx 500 \mu\text{mol/m}^2/\text{sec}$  (microeinstens/square metre/second)



**FIGURE 63:** Belt feeder used to dispense formulated feeds during weaning and beyond. Source: Partridge et al., 2003.



**FIGURE 64:** Mean  $\pm$  sd live-weight of juvenile mullet grown under 2 regimes, the first (Control) involving continuous feeding and a 14h light : 10h dark cycle and the other “test regimen” involving twice daily feeding coupled with an 18h light :6 h dark regimen. (Source: Fielder et al., 2010).



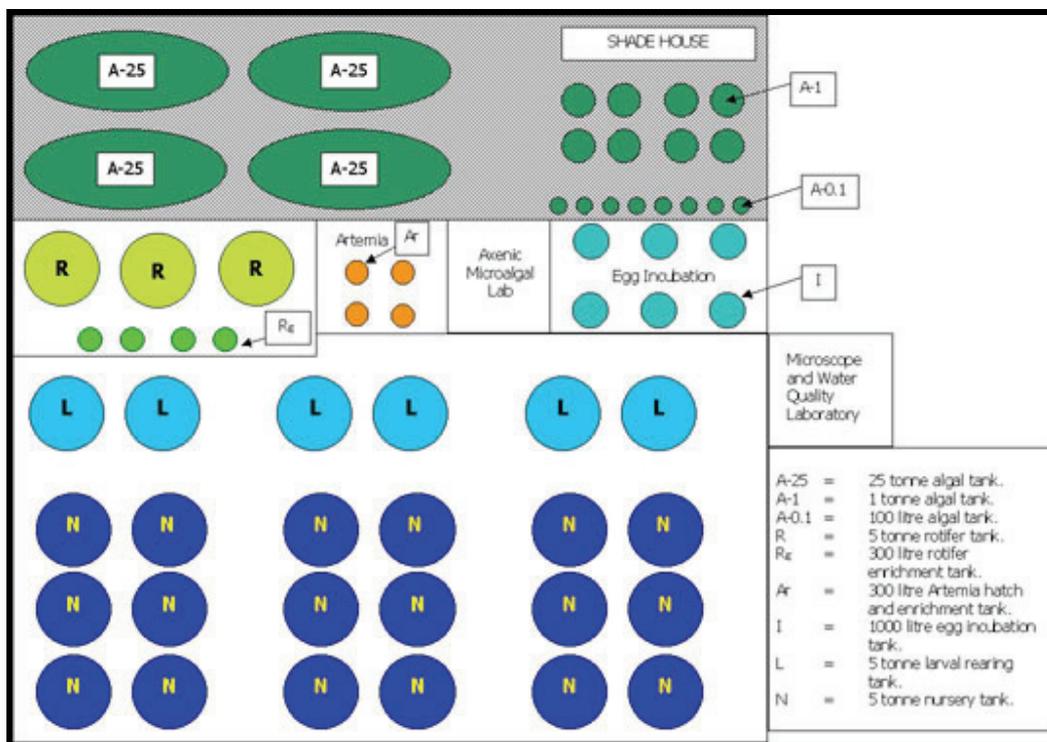
**FIGURE 65:** Effect of stocking density on: a) absolute growth rate (mean  $\pm$  sd) and b) food conversion efficiency, (Food Conversion Ratio [FCR] = weight of dry food/wet weight gain of fish), of juvenile mulloway (initial mean weight per fish  $17 \pm 3.5$  g, final mean weight 17 to 29 g) over 37 days. Stocking density in terms of biomass ranged from  $4.0 - 5.75$  kg/m<sup>3</sup> for fish stocked at 250 /m<sup>3</sup>;  $8-14$  kg/m<sup>3</sup> for fish stocked at 500 /m<sup>3</sup> and  $16 - 27$  kg/m<sup>3</sup> for fish stocked at 1000/m<sup>3</sup>.

### 2.8.3 Semi-intensive greenwater larviculture of mullet

#### System design and general operating specifications

Greenwater hatchery technology for mullet is very similar to that applied to snapper and black bream. The following account is largely based on techniques described for snapper and black bream in Chapter 6, pages 42 to 49 of Partridge et al., 2003. Cost effective green-water larviculture of mullet can either be conducted indoors or in outdoor green houses. Outdoor greenhouse culture in southern Australia is effective in summer under ambient temperatures of 20-30°C, Light: Dark regimens of 14h: 10h and average light intensities up to 30,000 lux. In winter, natural light intensities and photoperiods are insufficient and supplementary artificial lighting is required. Best sources are metal halide or fluorescent lights that emit spectral profiles conducive to photosynthesis at surface intensities around 9,000 lux.

Alternative indoor green-water larviculture systems must be housed in controlled environment rooms set at a 14L:10D lighting regimen at a surface light intensity of 9,000 -12000 lux and at temperatures in the optimum range of 22-26°C. Automated dimmable floodlights that provide sunrise and sunset effects should be used to minimise stress associated with the abrupt changes in light intensity when metal halide or fluorescent lights are switched on and off. Figure 66 is a schematic layout of a hatchery with the potential to produce 1 million, 2 gram juvenile mullet or snapper per year. The production cycle is based on stocking 100,000, 2 day old larvae into each of 2 x 5000 L tanks every 21 days. At an age of 42 days after hatch, metamorphosed juveniles are graded and transferred into 6 x 5 tonne nursery tanks (N in Fig. 66). The juveniles can be held and graded within these tanks for a maximum of 60 days, by which time they should weigh approximately 2 grams.



**FIGURE 66:** Schematic layout of greenwater marine fish larval rearing unit. (Source: Partridge et al., 2003).

The following operating criteria have been assumed:

- A continuous supply of viable fertilised eggs is available. This could be achieved through the use of controlled environment broodstock tanks, operating out-of-phase, or through naturally spawning broodstock.
- Maximum algal requirement; 6,000 litres per day (from A-25).
- Routine maximum rotifer demand;  $450 \times 10^6$  per day.
- Peak rotifer demand;  $800 \times 10^6$  per day (on each day a new tank is stocked)
- Maximum enriched *Artemia* requirement;  $80 \times 10^6$  per day.
- Survival during the larval period; 40%.
- Survival during the nursery period; 80% (from Day 42 to 2 g).
- Approximate water flow rate required;  $30 \text{ m}^3$  per hour.

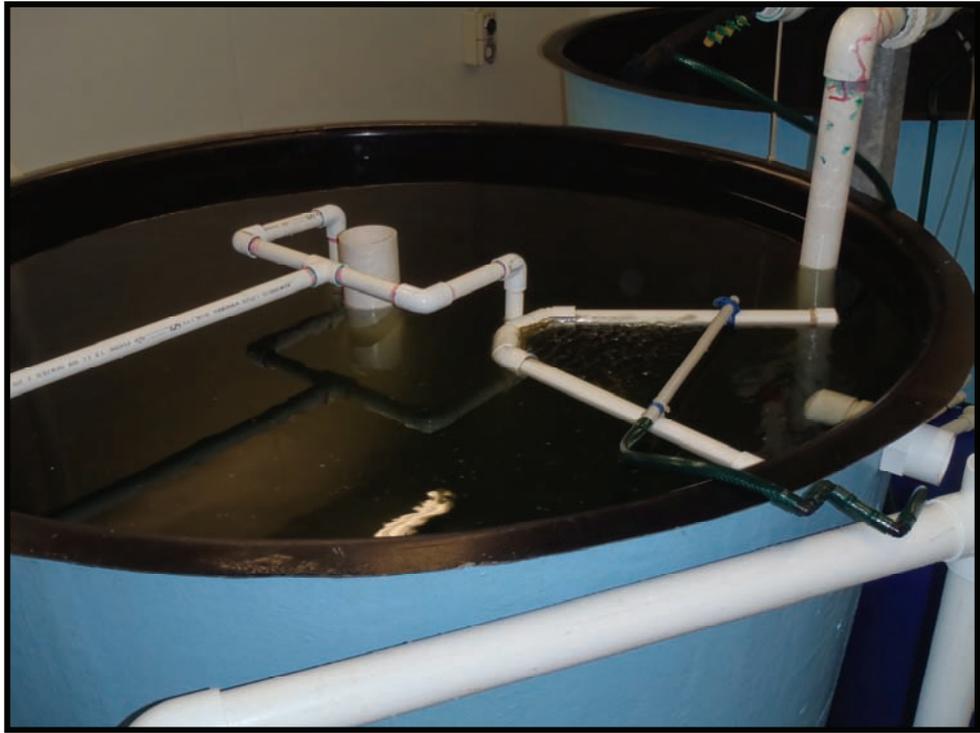
Types of rearing vessels successfully used for green water culture of mulloway have included 2,000 to 50,000 litre cylindro-conical tanks 2.4-7.5 metres in diameter with a bottom slope of 0-5° (Figs. 67 and 68). The bottom of the tanks should be white and the inner wall dark blue or black. The dark walls enhance the visibility of the prey to the larvae while the white bottom facilitates observation of the larvae and the bottom of the tank during brief clear-water interludes.

Aeration is supplied to the larviculture tank via large-bubble diffusers (pore size  $65 \mu\text{m}$  producing bubbles 4–5 mm in diameter), suspended approximately 150 mm above the tank floor. Nine diffusers are required in a 5,000-L tank, with each diffuser delivering about 400 mL of air per minute. This level of aeration is low enough not to generate undue turbulence in the water column which may hinder feeding but sufficient to maintain adequate levels of dissolved oxygen.

#### *Source water and pre-treatment*

If saline water is sourced from a bore or well and is low in dissolved oxygen, it should be introduced into the tank through a degassing column. These columns comprise a pipe (usually PVC) filled with a high-surface-area inert (food grade) plastic or ceramic media. As the water cascades over the media, the air-water interface is increased, enabling traces of toxic gases such as  $\text{H}_2\text{S}$  and excessive levels of carbon dioxide to diffuse into the air and facilitating diffusion of oxygen from the air.

If source water contains high loads of undesirable bacteria, it is advisable to pre-filter through successive 10, 5 and  $1\mu\text{m}$  filters and finally to disinfect it. A cheap and simple disinfection method is to fill larviculture tanks, adjust salinity to 30 ‰ and disinfect by adding 200 mg/L of liquid sodium hypochlorite (pool chlorine; 100-125 g/L active chlorine) on the day before stocking. The following day, residual active chlorine is neutralised by adding 200 g/L sodium thiosulphate solution in equal volume to the added chlorine solution whilst applying heavy aeration. Alternatively ozonation incorporated with protein fractionation can be used effectively to disinfect and remove dissolved organic compounds from influent seawater.



**FIGURE 67:** 2000-L larval rearing tanks at PSFI.



**FIGURE 68:** 10,000-L larval rearing tanks at PSFI. Tanks are covered with translucent, polyethylene sheeting.

### *Micro algae management and monitoring*

Once residual chlorine has been neutralised or residual ozone dissipated and other residual oxidants removed with activated carbon, microalgae can be safely added. Additions of the green microalgae *Nannochloropsis oculata* are made daily to indoor rearing tanks from the day prior to larval stocking at optimum densities of between 500,000 and 1,000,000 cells/mL. This is achieved by adding 60-100 L of bulk bag or tank algae culture per 1000L of larviculture tank volume (See chapter 8, pages 77 to 80 and Videos 10 and 11 of Partridge et al., 2003 for a step by step account of micro-algae culture equipment and practises). Algae is pumped through a 25 µm filter bag to baffle the flow and prevent disruption of sediment from the tank bottom. The culture water displaced by the incoming algae overflows through a submerged outlet screened with 350 µm mesh.

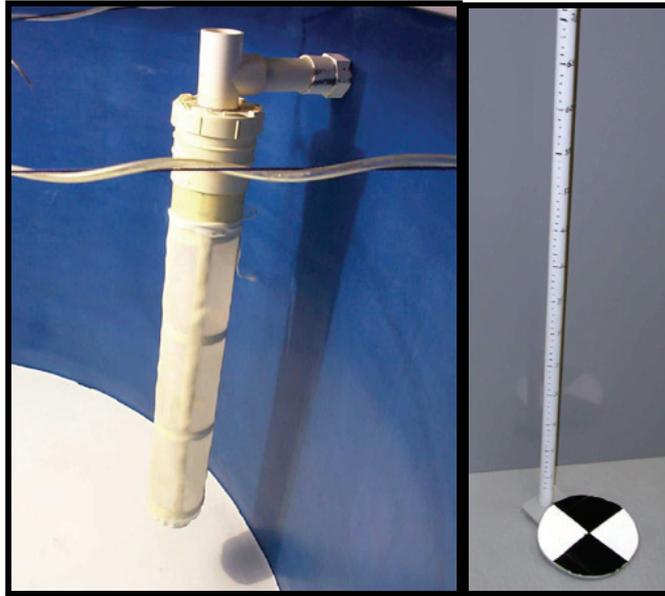
Outdoor green-housed larval cultures which generally receive more light, may not require daily additions of microalgae. In such systems, algae is only added when necessary to maintain the required cell density. Alternatively, frozen concentrates of *Nannochloropsis* can be used to provide greenwater at approximately 20mL of concentrate per 1000 L.

Algal cell densities within larval tanks are most easily estimated using a secchi disc, which measures turbidity. As shown in Figure 69, a secchi disc is a solid disc, approximately 200 mm in diameter, with alternating black and white quadrants. The disc is attached to a stick with labelled depth graduations every 5 cm. The secchi depth is that depth at which the black and white quadrants can no longer be distinguished. Secchi depth in the range of 40 to 60 cm will yield the required cell density of *Nannochloropsis oculata*. It should be noted, however, that the accuracy of such depth readings is dependent on several factors including light intensity and the concentration of other suspended particulates in the water column. Secchi depth readings therefore need to be calibrated periodically against algal suspensions of known cell concentration (see Chapter 8 pp 77-80 and Appendix 12 of Partridge et al., 2003 for detailed procedures).

Secchi depth measurements are made and recorded on larval batch data sheets three times daily:

- in the morning, prior to addition of algae
- approximately 1 hour after algae addition, and
- last thing in the afternoon

Natural blooms of brown algae may occur in the semi-intensive rearing tanks, beginning usually on day 6 or 7 after hatch. Two species of heterotrophic dinoflagellates responsible for these blooms have been tentatively identified as *Gymnodinium* sp. and *Prorocentrum* sp. These species appear to have no adverse effects on the culture and may in fact be beneficial, due to their heterotrophic nature and the fact they contain high levels of the essential fatty acid, DHA.

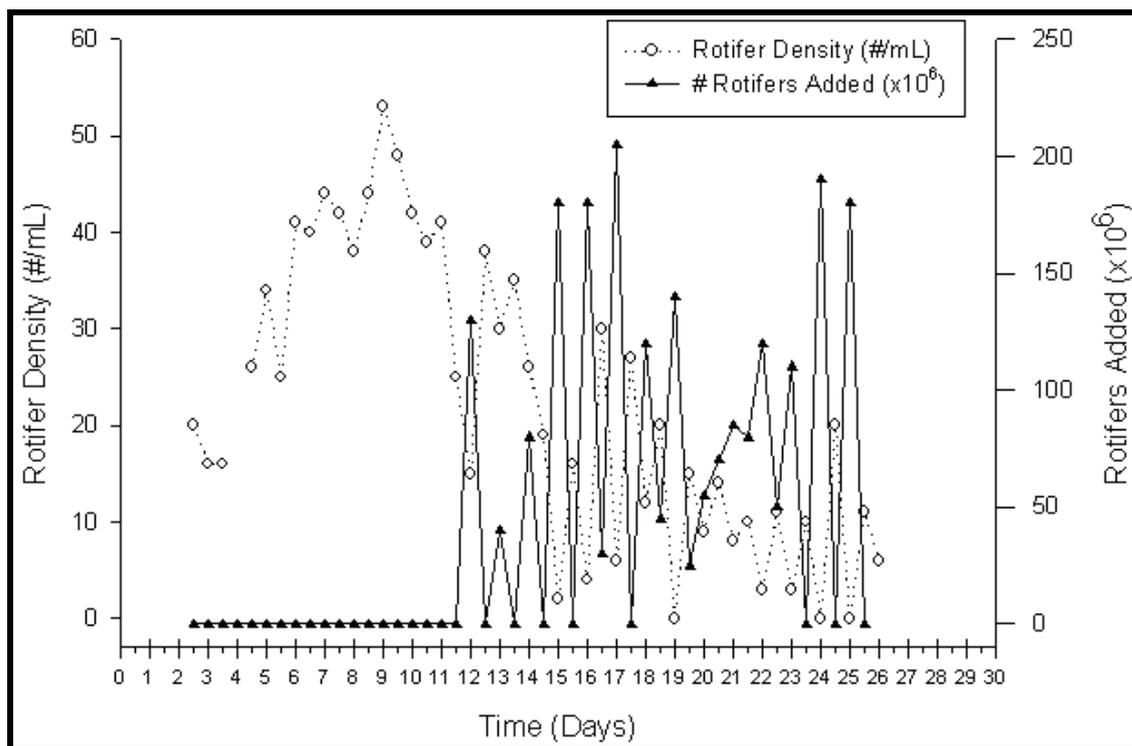


**FIGURE 69:** Cylindrical screen outlet and Secchi Disc used to assess microalgal cell density. (Source: Partridge et al., 2003).

#### 2.8.4 Rotifer feeding phase

Although mulloway larvae do not start feeding until day 3 after hatch, algae and rotifers are introduced to the culture tank on day 1 after hatch at a density of 10 large strain or 20 small strain rotifers/mL. This allows time for the rotifers to adapt to the culture conditions before being preyed upon by the larvae. If the rotifer density in the tank does not begin to increase by day 4 after hatch, an additional 10 large strain or 20 small strain rotifers per mL may need to be added to ensure a sufficient starting population. On day 2 after hatch the larvae are transferred from the incubation tanks into the larval rearing tanks at about 10/litre. (see Egg Production, Collection and Handling). Rotifer density is determined twice daily by counting at least five, 1 mL samples under a dissecting microscope. Alternatively, by holding a hollow 1 mL graduated glass tube (pipette) against a dark background under sufficiently strong light, rotifers can be counted by eye. Samples are taken from various locations around the tank and close to the air-stones to ensure the rotifers are uniformly distributed. During the early stages of the culture the rotifer density increases until a peak is reached between Days 8 and 14. When the consumption of rotifers by the larvae exceeds the daily rotifer production capacity their density begins to decline. When the density of rotifers drops below 40/mL small strain or 20/mL large strain additional rotifers are added to maintain this density, which is then maintained until water flow to the tank is commenced.

An example of rotifer densities and additions for a semi-intensive mulloway culture is shown in Fig. 70 (as per Fig. 38, Partridge et al., 2003). Although this example is fairly typical of mulloway, snapper and black-bream culture, the exact timing and magnitude of the rotifer peak and the periodicity of additions will vary depending on factors such as larval density, temperature and the quality and quantity of the microalgae added to the culture. Peaks up to 70 rotifers/mL are typical. Once the larvae begin feeding heavily, rotifer numbers are rapidly depleted and further additions need to be made to the culture tank twice daily. When rearing 50,000 larvae, the peak demand for rotifers is approximately  $200 \times 10^6$  (200 million) rotifers per day. Additional rotifers added to the culture are enriched on a combination of microalgae and artificial enrichment products (see Chapter 8, Live foods - Rotifer enrichment of Partridge et al., 2003)



**FIGURE 70:** Rotifer densities and additions made to a semi-intensive snapper culture. (Source: Partridge et al., 2003).

### 2.8.5 *Artemia* feeding phase

When average total length of larvae reaches 5 to 6 mm TL on two consecutive days, feeding on enriched *Artemia* meta-nauplii commences. Larvae reach this length between Days 12 and 14. On the first day of *Artemia* feeding, a single feed of 0.2 *Artemia*/mL is offered in the morning. The larvae adapt quickly to the new feed source and the rate and number of feeds are increased rapidly to meet demand. *Artemia* are counted five times per day to monitor consumption and to calculate feed requirements. Their density is determined by counting at least 5, randomly selected 10 mL samples in a petri dish.

Once larvae are feeding well on the *Artemia*, the number of feeds is increased to five per day and the feed rate is progressively increased to 1.4 *Artemia*/mL/feed at the approximate additional rate of 0.2 *Artemia*/mL/feed each day. The addition of rotifers to the tank is continued for approximately 5 days after *Artemia* feeding begins (or until water flow commences) to allow time for all of the larvae to adapt to the new feed source.

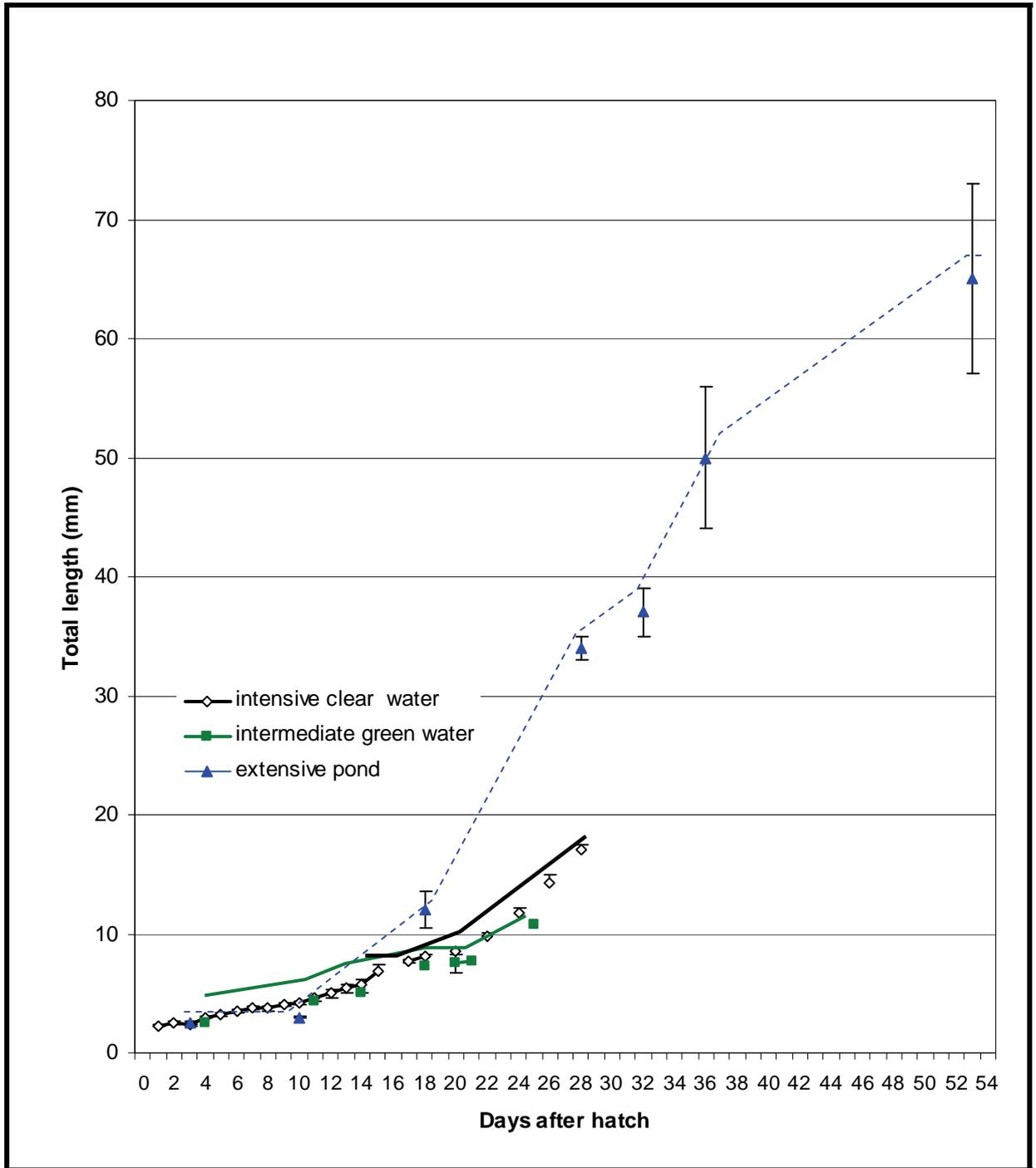
The peak *Artemia* feeding rate is achieved between days 18 and 27 depending on the larvae's growth rate and appetite. The larvae are fed the maximum ration of five *Artemia* feeds per day, at 3.2 *Artemia*/mL/feed over a period of approximately four days. From this point the number of feeds is reduced to 4 per day for two days, then 3 per day for an additional two days. On the next and final day of *Artemia* feeding, only two feeds are offered. As the number of feeds is being reduced, the ration is maintained at 1.4 *Artemia*/mL/feed. *Artemia* feeding is therefore finished between Days 39 and 43, by which time the larvae are feeding exclusively on artificial food. A typical culture of 50,000 larvae will consume between 300 and 450 million enriched *Artemia* meta-nauplii, depending on the survival of the larvae and the exact weaning schedule.

### 2.8.6 *Weaning*

Same as described for intensive Clearwater hatchery above (2.8.2).

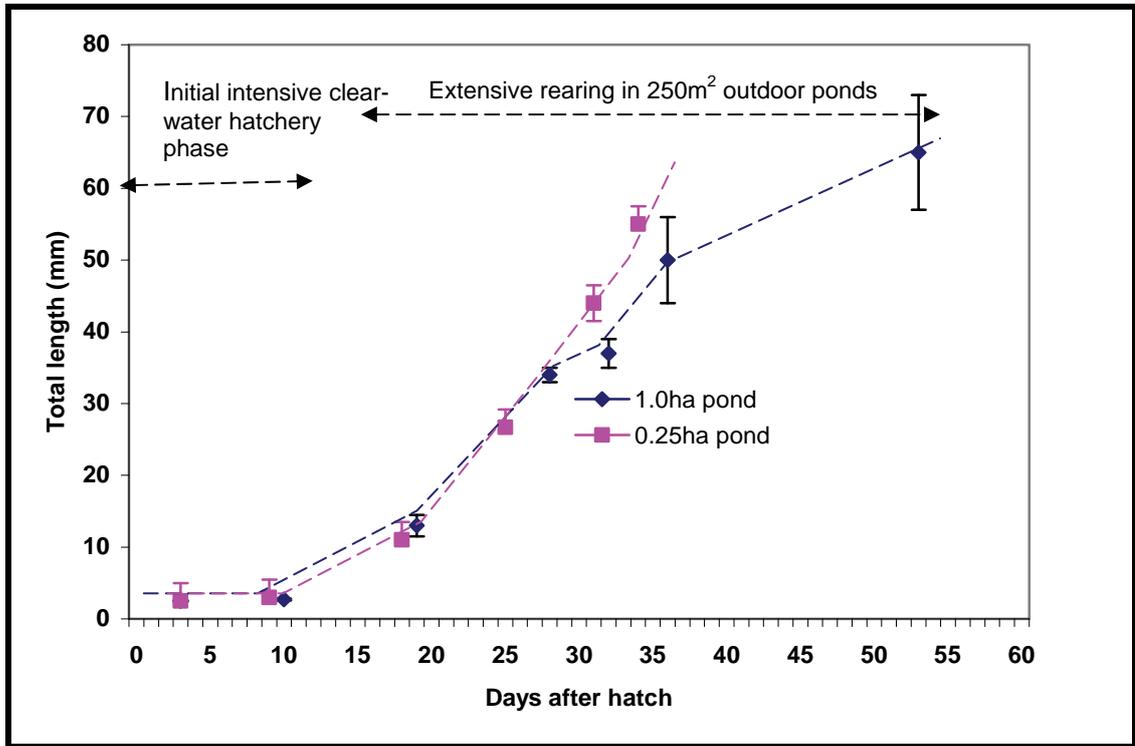
## **2.9 Extensive Outdoor Pond Culture**

Mulloway fingerlings and those of other finfish produced in extensive outdoor ponds have a number of major advantages over intensive clear water and semi-intensive green-water produced counterparts. They generally cost less, grow faster (Fig. 71) and being more vigorous are better able to survive the transition to the wild if used to enhance depleted fisheries stocks (Rutledge et al., 1990; Rutledge & Rimmer, 1991).

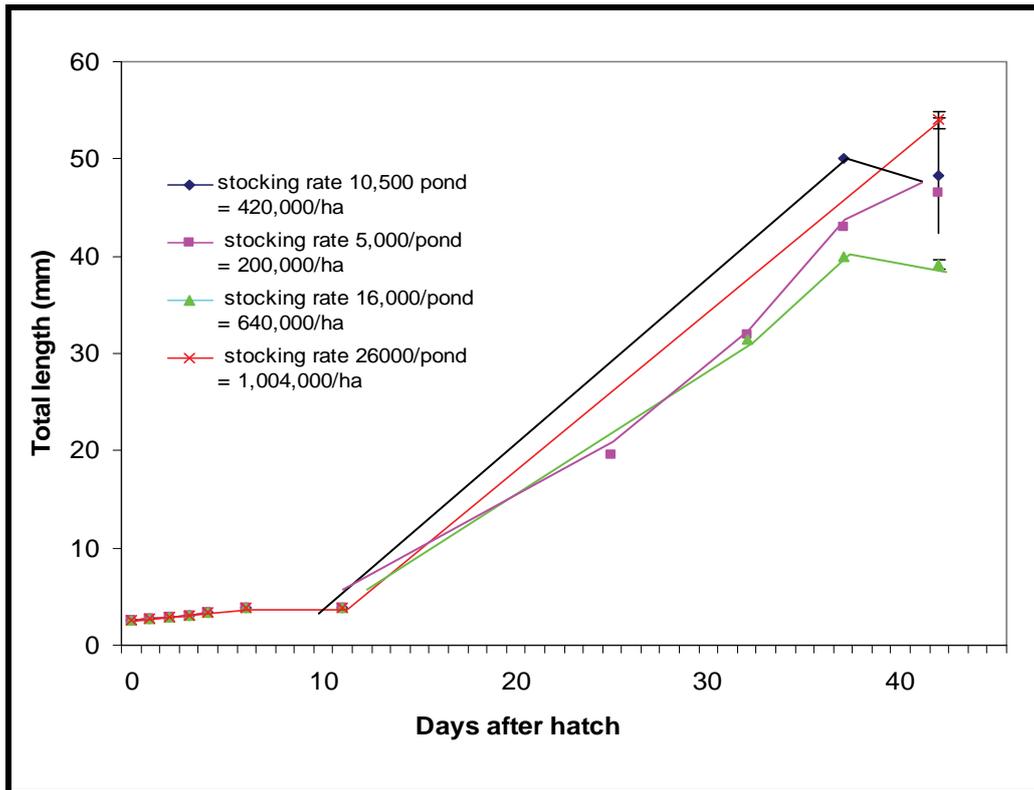


**FIGURE 71:** Comparative growth of mulloway larvae reared intensively indoors in clear water tanks, semi intensively in outdoor green-water tanks and in extensively in earthen or plastic lined ponds. (Source: redrawn from data provided in Fielder, et al., 1999).

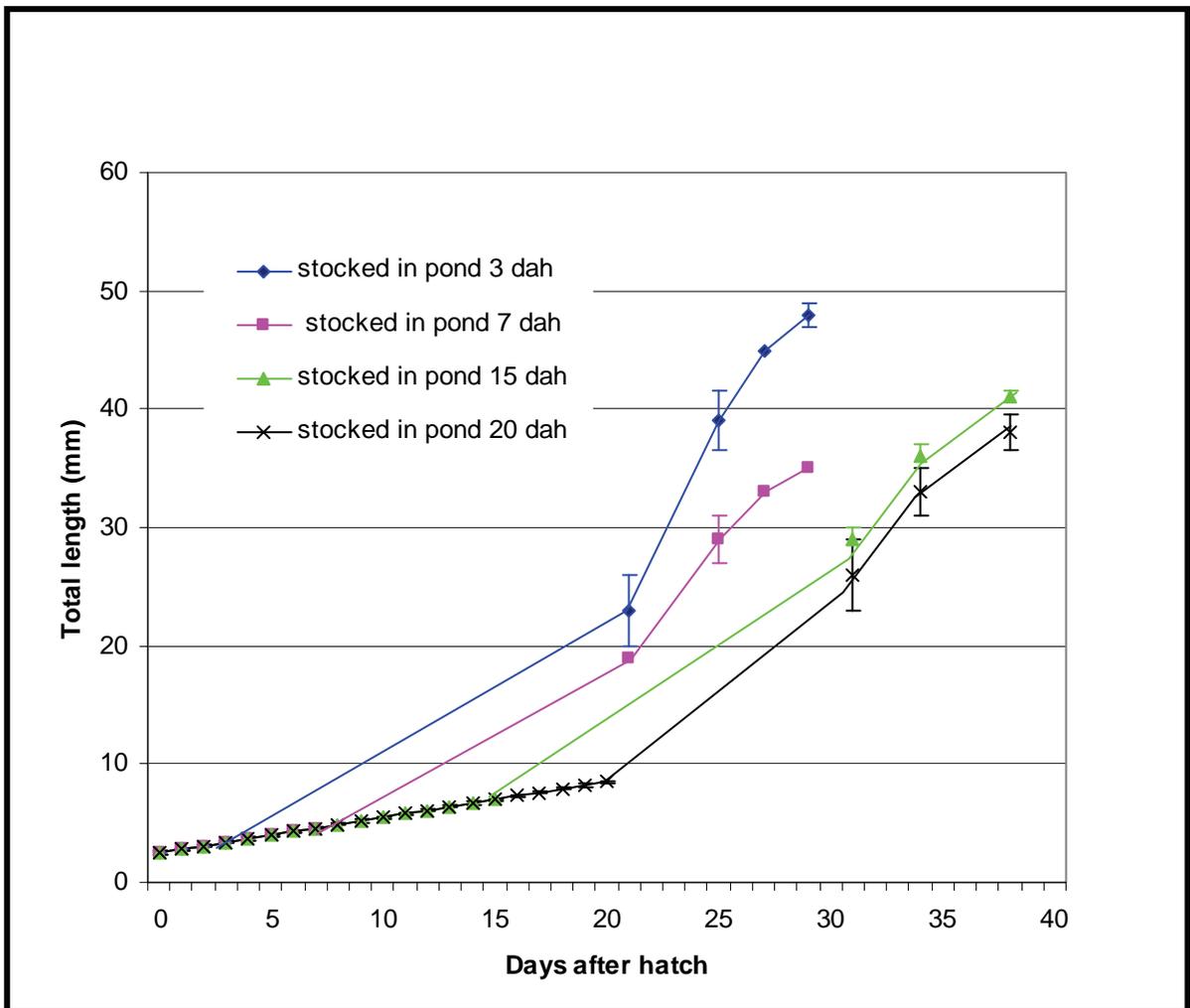
Reported growth rates of mullet reared from larvae to fully metamorphosed juveniles reared extensively in large (250–10,000 m<sup>2</sup>) outdoor ponds (Fig. 72), at stocking densities of 200,000 to 1 million larvae/ha (Fig. 73) and from starting ages of 3 to 20 days after hatch (Fig. 74), have consistently been in the range 1–1.5 mm/day. As illustrated in Fig. 74, these rates are 3 to 4 times those of 0.3–0.5 mm/day reported above for counterparts produced intensively in small indoor tanks or semi-intensively in intermediate scale green-water culture.



**FIGURE 72:** Growth of mullet larvae reared extensively in 0.25 and 1.0 ha ponds at Yamba NSW. (Source: Fielder et al., 1999)



**FIGURE 73:** Effect of stocking density on growth of mullet stocked after 11 days of initial intensive hatchery rearing into replicate 250 m<sup>2</sup> ponds at Yamba in northern NSW. (Source: Fielder et al.,1999).



**FIGURE 74:** Effect of age at stocking on growth of mulloway stocked into replicate 250 m<sup>2</sup> ponds at Yamba in northern NSW. (Source: Fielder et al., 1999).

Survival rates of > 20% to 45 days after hatch under extensive pond rearing are also superior to of 10 to 20% reported for intensive clear-water and semi-intensive green-water hatchery systems.

As discussed in relation to Australian bass (see Chapter 1), superior growth and survival attainable through extensive pond culture does not preclude use of intensive clear-water and semi-intensive green-water production of mulloway fingerlings. Indeed the latter are indispensable in the absence of suitable outdoor pond facilities or without access to large volumes of good quality marine or estuarine water. Intensive and green water techniques are also indispensable in the wake of nodavirus disease outbreaks, reoccurrence of which is difficult and often prohibitively costly to combat in extensive pond rearing systems. The other major restriction of extensive pond production is low ambient temperatures of outdoor during the colder half of the year.

### 2.9.1 *Pond Design, Preparation and Management*

The design, preparation and management of extensive ponds for production of mullock fingerlings are in most aspects the same as previously described in detail for Australian bass (see Chapter 1).

## **2.10 Summary of “best-practice” Rearing Criteria for Mullock Fingerlings**

Table 7 summarises “best-practice” larval rearing regimes for mullock at PSFI.

**TABLE 7:** Summary of “best-practice” larval rearing regimes for mulloway at PSFI.

SPECIES: MULLOWAY ( <i>ARGYROSOMUS JAPONICUS</i> )			
BREEDING & DEVELOPMENT	UNIT	COMMENT	
BROODSTOCK ORIGIN	WILD-CAUGHT AND G1	CAPTURED FROM SHALLOW (<3M DEEP) COASTAL AND ESTUARINE SITES	
BROODSTOCK TANK SIZE	22,000 – 250,000 L	1000 IU/KG NECESSARY FOR WILD-CAUGHT FISH.	
SPAWNING INDUCTION	HCG	NO LONGER USED AT PSFI	
	PHOTOTHERM MANIPULATION	POSSIBLE WITH G1 FISH ONLY – PREFERRED METHOD	
TANK SIZE FOR SPAWNING	22,000 L	1 : 1 MALE:FEMALE	
LATENCY PERIOD TO SPAWNING	34 H	AT 22 °C	
METHOD OF FERTILISATION	SPONTANEOUS SPAWNING	FERTILISATION OCCURS WITHIN SPAWNING TANK	
EGG INCUBATION TANK SIZE	500-1000 L		
TIME TO HATCH	28-30 H	AT 23 ±0.5°C	
LARVAE TANK SIZE	2000-10,000 L	INTENSIVE GREENWATER CULTURE	
	0.05 - 1 HA	EXTENSIVE, FERTILISED POND CULTURE	
LARVAL YOLK-SAC PRESENT	0-3 DAH	AT 23 ±0.5°C	
LARVAL FIRST-FEEDING	3-4 DAH	AT 23 ±0.5°C	
LARVAL SWIMBLADDER INFLATION	3-11 DAH	AFFECTED BY SURFACE SCUM, LIGHT INTENSITY, TURBULENCE, TEMPERATURE AND SALINITY	
METAMORPHOSIS	~ 10 MM TL	TIME TO METAMORPHOSIS IS DEPENDENT ON FACTORS AFFECTING GROWTH E.G. TEMPERATURE AND FEED AVAILABILITY	
CANNIBALISM	~12 MM TL	SIZE GRADING IS NEEDED TO REDUCE INCIDENCE	
WATER QUALITY PARAMETER	TARGET	DAH	ADJUSTMENT
<b>PH</b>	7.6 - 8.2	0+	
<b>DISSOLVED OXYGEN (MG/L)</b>	>6.00	0+	USE COMPRESSED OXYGEN DIFFUSER TO MAINTAIN SATURATION LEVEL
<b>TEMPERATURE (°C)</b>	22	0+	INCREASE POST SB INFLATION
<b>SALINITY (PPT)</b>	5 TO 35	0+	5-12.5 PPT OPTIMAL
<b>WATER EXCHANGE (%/DAY)</b>	100 - 200	0+	INCREASE EXCHANGE AS LARVAE DEVELOP
<b>SURFACE SKIMMER (HRS/DAY)</b>	24	4+	MONITOR SKIMMER TO ENSURE LARVAE AT WATER SURFACE ARE NOT AFFECTED
<b>PHOTO-PERIOD (L:D)</b>	(12:12) (18:06)	(0+) (6+)	INCREASE POST SB INFLATION
<b>LIGHT INTENSITY (LUX)</b>	225-400	0+	START WITH LIGHT AT LOWER INTENSITY
<b>GREEN-WATER (CELLS/ML)</b>	1.4 x 10 <sup>6</sup>	0+	PRO-AQUA* CONCENTRATE 57x10 <sup>9</sup> PER ML
LARVAL FEEDING SCHEDULE	TARGET	DAH	ADJUSTMENT
<b>ROTIFER (R/ML)</b>	20.0 - 5.0	4+	INITIAL 20/ML UNTIL FEEDING AND THEN INCREASE FREQUENCY OF REDUCED CONCENTRATION (E.G. 4x5/ML/D).
<b>ARTEMIA (A/ML)</b>	0.2 - 2.0	12+	0.2/ML UNTIL WEANED, THEN INCREASE CONCENTRATION AND FREQUENCY. START AT 5.4 MM TL
<b>WEANING DIET SIZE (µM)</b>	200 - 800	22+	COMMENCE WEANING AT 10.5 MM TL

\*Algae concentrate used Rotifer Diet-3600 (*Nannochloropsis/Tetraselmis* blend) from Reed Mariculture Instant Algae, imported via Proaqua Australia. <http://www.proaqua.net.au>