NSW GOVERNMENT | Industry & Investment

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

D. Stewart Fielder
Senior Research Scientist, Marine Finfish Breeding
Port Stephens Fisheries Institute
TAYLORS BEACH NSW 2316

Michael P. Heasman
Submariculture Pty Ltd
Aquaculture Consultancy and Project Management
35 Kanangra Avenue
CORLETTE NSW 2315

Aquafin CRC

Australian Government
Fisheries Research and Development Corporation

MARCH 2011 **WWW.INDUSTRY.NSW.GOV.AU**

Title: Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish

Authors: D. Stewart Fielder, Senior Research Scientist, Marine Finfish Breeding, Port Stephens Fisheries Institute

Michael P. Heasman, Submariculture Pty Ltd, Aquaculture Consultancy and Project Management

For updates to this publication, check http://www.dpi.nsw.gov.au

Published by Industry and Investment NSW

First Published March 2011

ISBN 978 1 74256 058 8

Disclaimer

The information contained in this publication is based on knowledge and understanding at the time of writing (March 2010). However, because of advances in knowledge, users are reminded of the need to ensure that information on which they rely is up to date and to check the currency of the information with the appropriate officer of Industry and Investment NSW or the user's independent advisor.

TRIM reference: INT10/80426

ALWAYS READ THE LABEL

Users of agricultural or veterinary chemical products *must always* read the label and any permit, before using the product, and strictly comply with the directions on the label and the conditions of any permit. Users are not absolved from compliance with the directions on the label or the conditions of the permit by reason of any statement made or not made in this publication.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Australian Government's Aquafin CRC and the Fisheries Research and Development Corporation (FRDC) for financial support in preparing this manual. In particular, we thank Dr Patrick Hone for encouraging us to compile the manual.

We thank the staff of the Port Stephens Fisheries Institute for their individual and collective contributions. We especially thank Luke Cheviot, Luke Vandenberg, Debra Ballagh, Paul Beevers, Geraldine McMahon, Ben Kearney, and Bill Bardsley for their significant technical expertise and contributions to R&D used to develop and enhance hatchery production technology.

Drs Geoff Allan and Mark Booth provided excellent editorial comments during preparation of this manual. We also gratefully acknowledge Mrs Helena Heasman for her tireless and good-humoured contributions to the preparation, design and compilation and editing of this manual. We also thank Ms Jo Pickles for her assistance with editing of the manual.

PREFACE

This manual was commissioned by the Aquafin CRC and Fisheries Research and Development Corporation (FRDC) and is the first consolidated and documented information on successful techniques for culturing juvenile Australian bass (*Macquaria novemaculeata*), mulloway (*Argyrosomus japonicus*) and yellowtail kingfish (*Seriola lalandi*) in NSW.

This manual provides specialised instruction on:

- how to collect and reproductively condition Australian bass, mulloway and yellowtail kingfish to spawn;
- how to induce them to spawn;
- how to hatchery-rear their young through the larval and early juvenile stages to an age and size suitable for on-farming or for stocking to enhance fisheries.

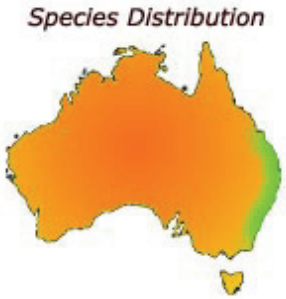
The reader will notice that the chapters are a blend of practical (hands-on) instruction and supporting scientific information. This approach is intended, to develop a manual that can be understood by the layman, while at the same time provide background and references for those who require further reading and elaboration of the concepts and methods described.

The reader will also notice that the chapters are presented with information furnished for Australian bass followed by mulloway and then yellowtail kingfish. This reflects the amount of published information and the history of culture of each species. Hatchery production of Australian bass has been developing for three decades and methods for culture are now proven reliable and sustainable. On the other hand, yellowtail kingfish is considered a 'new' species, having only been cultured for the last ten years and is currently the subject of much research effort to refine culture methods.

There are many culture methods including live feed production and equipment design and management that are common to all three species. To avoid replication in each chapter, these have been dealt with in leading chapters with reference to the applicable section provided in Chapters where information has not been expanded upon.

This manual is not intended as a stand-alone text but rather as a companion to other publications listed in the references section. In particular, we encourage the reader to acquire Partridge et al. (2003) "**Hatchery Manual for the Production of Snapper (*Pagrus auratus*) and Black Bream *(Acanthopagrus butcheri*)**, which we refer to widely in this manual, which provides an excellent account of many culture techniques common to those used for Australian bass, mulloway and yellowtail kingfish.

1. AUSTRALIAN BASS (AB)



1.1 Appearance, Distribution and Movements

Australian bass (AB), *Macquaria novemaculeata* (Steindachner), are a typical perch-like fish, having a back profile that is arched from above the eyes to the tail with only very slight tapering of the snout. The colouration of bass varies from silver, green and bronze in accordance with their surroundings. AB is a long-lived and slow growing species with females growing faster to a much larger maximum size than males. AB are catadromous, solitary (non schooling), cryptic and crepuscular spending most of their life in fresh water and migrating to estuaries to spawn to fresh and saltwater sections of eastern draining rivers from the Mary River in Queensland, southward through New South Wales and through to the Gippsland Lakes in Victoria (Harris 1983; 1987). While female AB tend to be solitary, males are more gregarious. Moreover there is a partial distributional segregation of the sexes with most males remaining in estuarine or lowland habitats where they are often found in large schools (Van der Wal, 1989), while females are generally solitary and predominate in lagoon or upland lotic (of or relating to or living in actively moving water) habitats.

Although large numbers of Australian bass do not usually enter water of salinity greater than 10 ppt, they are able to penetrate higher salinities when breeding in estuaries (i.e. 20 ppt) and move between tributaries of river systems. Furthermore, Australian bass have been caught up to 5 km off the coast after heavy floods (Williams, 1970). This highlights their potential to disperse from one estuary/river system to another and the existence of a common gene-pool over their extensive east Australian distribution (Chenoweth and Hughes, 1997)

The geographic range of AB along the south eastern coast of Australia encompasses a wide range of habitats, including headwater and main channel streams, floodplains of wetlands, and estuaries, where they experience (and tolerate) a diversity of conditions and other environmental factors (Harris, 1988). This distribution also coincides with Australia's most intensive urban and rural development with a resultant decline in distribution and abundance. This is because freshwater and estuarine environments have been substantially altered by human activities including dam, weir construction and flood mitigation coupled with inadequate provision of fish ladders, habitat degradation especially acidification, siltation and pollution and overexploitation by recreational and commercial fishing.

Highest AB population densities occur over the southern part of its range (far southern NSW and western Victoria). An intensive study of populations of AB in the Sydney Basin by Harris (1988), showed that it is sparsely distributed through sheltered parts of riverine habitat exhibiting exceptionally low productivity in terms of output per area of aquatic habitat (0.84 g /m^2 per year) and as a proportion of standing biomass (20% per year). These characteristics are in keeping with high survival resilience in environments that are unpredictable in terms of climate and food sources. Because of low natural densities, declining natural stocks and because AB is a highly-prized light-tackle sport fish, there is a large public demand to stock the fish into farm dams, lakes and impoundments (Harris, 1985; Battaglene et al.,1989). Although AB are considered excellent eating, the majority are released by anglers (87% in the case of fish in Queensland impoundments according to Wilde and Sawynok, 2005).

1.2 Breeding and Early Life History

AB are serial spawners that breed in estuarine zones of rivers (Figs. 1a and 1b) over a 1 to 4 month season within the period mid-May to December coincident with temperatures in the range 12 -18 ºC. Accordingly, the breeding season occurs significantly earlier over the northern range of this species. Fishing closures are applied at these times to protect spawning aggregations. Final gonad development, downstream migration, and hence successful annual spawning and recruitment is triggered by heavy rain, runoff and flooding. In drought years the ovaries of female AB fail to reach full ripeness and the eggs eventually regress. In such years females do not migrate downstream but rather remain within upper freshwater sections of rivers (Harris, 1983 and 1986).

AB are highly fecund with batches of 0.9 -1.0 mm eggs being spawned at a reported average number of eggs per kg body weight of 352,000 eggs by Schneirer (1982) and of 440,000 eggs (range 49,000 to 1,429,000) by Harris (1986). The eggs sink slightly under low salinity conditions associated with estuarine spawning sites at or close to the mouths of rivers (probably in the range 10 to 20 ppt based on sperm viability data provided in Figure 1c. Field studies of Harris (1986) showed that spawning, incubation and development of yolk-sac larvae in the wild occurs at salinities of 8 to14 ppt and temperatures of 11 to16 ºC.

The latter entail incubation periods of 50 to 90 hours (Van der Wal, 1985). Optimum conditions for incubation, hatching and development of yolk sac larvae in AB were identified by Van der Wal (1985) as salinities of 20 to 35 ppt and temperatures of 15 to 20ºC. It is therefore possible that the early life cycle stages of AB may occur progressively as they are swept downstream of spawning sites towards river mouths and the sea to encounter higher more favourable salinities and temperatures. AB post-larvae recruit to the shelter of macro-phyte beds such as common reeds in brackish water. Small juveniles do likewise in relation to ribbon weed in freshwater areas as they migrate upstream during spring and summer (Harris, 1986).

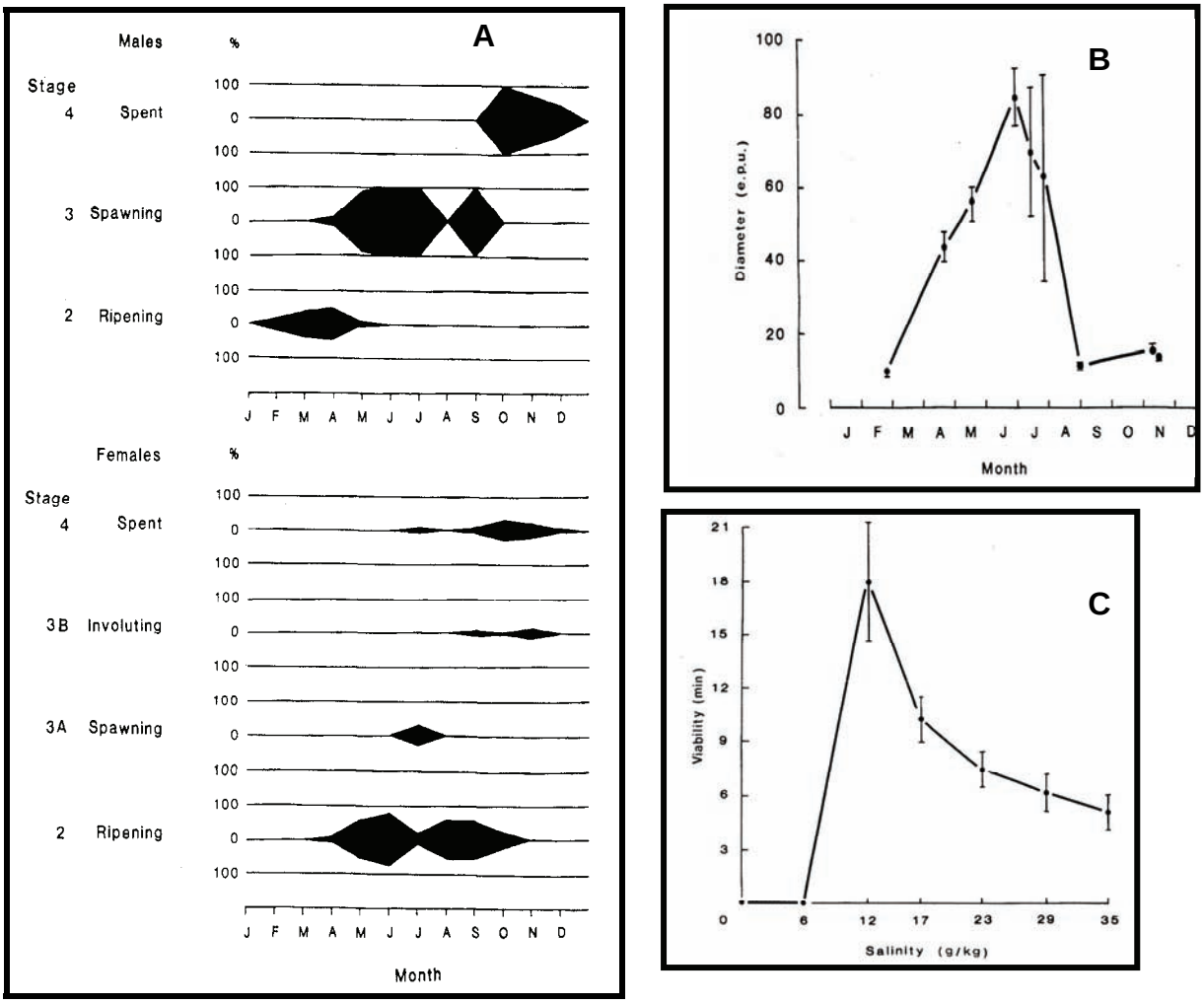


FIGURE 1: **A** Above left: Seasonal gonadal cycle of male and female AB shown by % frequency of macroscopically determined maturity stages **B** Top right: Seasonal changes in mean egg diameter **C** Bottom right: Effect of salinity on AB sperm viability (Source: Harris, 1986)

1.3 Food and Feeding

AB are broad spectrum opportunistic carnivores with seasonal and habitat variability having significant effects on specific composition of the diet and rates of feeding. The AB is a top order predator. An examination of the stomachs of 552 adults and yearlings was found by Harris (1985b) to contain almost every available prey type, including: insects (such as coleopterans, dipterans, hemipterans, odonatans and trichopterans); fish; crustaceans (such as crayfish, shrimp, prawns, crabs, copepods and cladocerans); and terrestrial vertebrates (such as skinks, frogs and birds and plant material).

Harris (1985) showed that young AB (11-47 mm TL) from the Hawkesbury River estuary in NSW feed on a far narrower range of prey species (mainly chironomids and copepods). Significant differences in the diet of AB were shown to occur between ‘summer' (November to April) and 'winter' (May to October) (Fig. 2), but especially between habitats (Fig. 3). While the diets of Australian bass are diverse and do vary significantly with habitat and season, insects are the most important food type for larger juveniles and adult AB, followed by fish and large crustaceans.

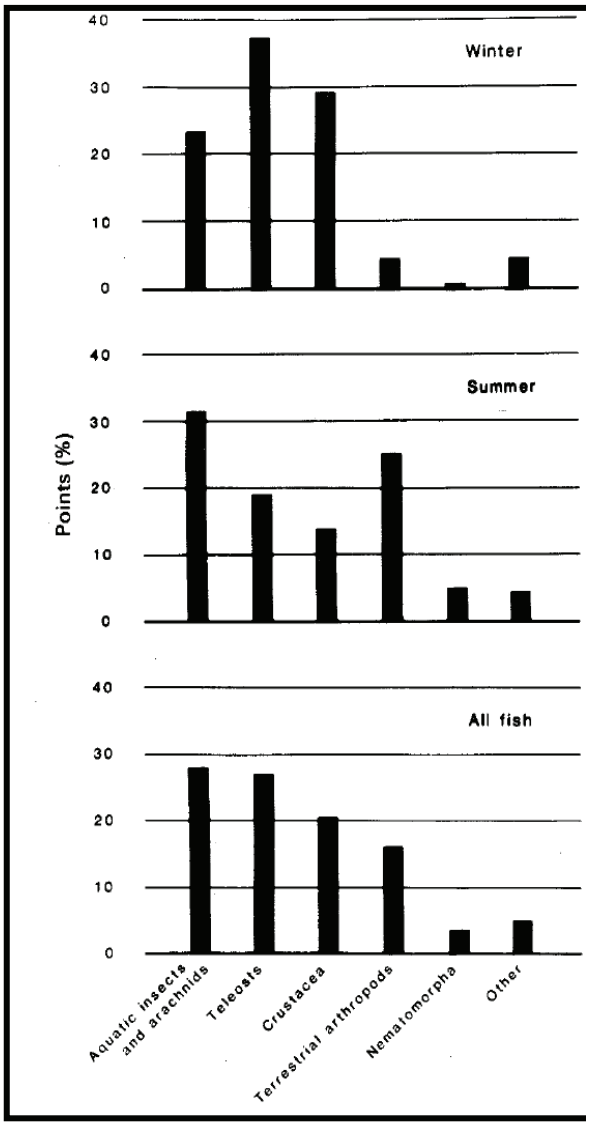


FIGURE 2: Percentage contributions of major prey groups in the diet of AB, in winter and summer (Source: Harris, 1985)

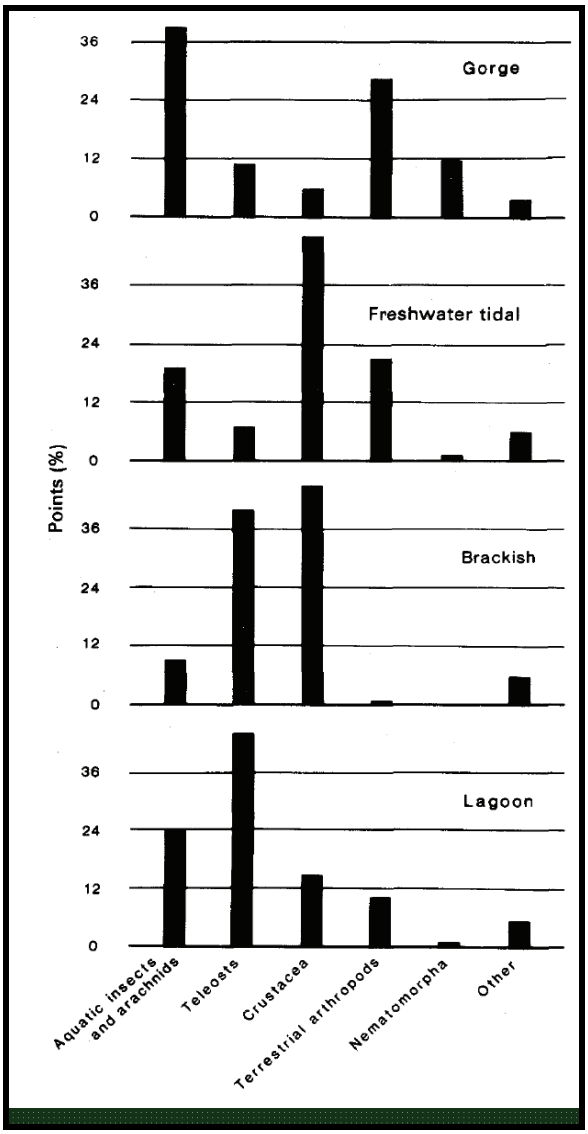


FIGURE 3: Percentage contributions of major prey groups in the diet of AB from 4 main habitat types (Source: Harris, 1985)

1.4 Growth, Longevity and Mortality

As illustrated in Figure 4, AB are long-lived (>20 years) but slow growing and exhibit sexual dimorphism, females substantially out-perform males in terms of growth rate, mean size at maturity and terminal size. Moreover, all three growth and size parameters vary substantially with habitat type and location, especially seasonal temperature regimes, food supply, population density and inter-specific competition. As discussed above, there is partial distributional segregation of the sexes in AB, most males remaining in estuarine or lowland habitats, while females predominate in lagoon or upland lotic habitats. As discussed by Harris (1987) this raises the possibility that size dimorphism is an adaptation to the different migration distances and gamete requirements of the sexes.

Growth and size parameters differences between various populations of AB also depend on whether the populations are natural within river catchments or created populations stocked as hatchery fingerlings into artificial habitats such as dams and reservoirs. Depending on such factors, the average asymptotic size (L_∞) reported for AB ranges from about 270 to 400 mm for males and from about 350 to 570 mm for females (Harris, 1987; Wilde and Sawynok, 2005). The largest recorded AB, was a 3.78 kg specimen, caught in the Clarence River in 1980. Growth data provided in Fig. 5 may be regarded as more typical for wild AB in that they relate to natural populations in the Sydney basin that lies at the centre of natural distribution of AB (Harris, 1987). Fastest recorded growth of AB is for fish stocked into 4 impoundments in south-eastern Queensland. These grew at an estimated 50-80 mm a year up to 350 mm and at 20-50 mm a year up to terminal sizes of 480-570 mm. Estimated average lifespan of these impounded fish ranged from 14.5 to 24.4 years (Wilde and Sawynok, 2005).

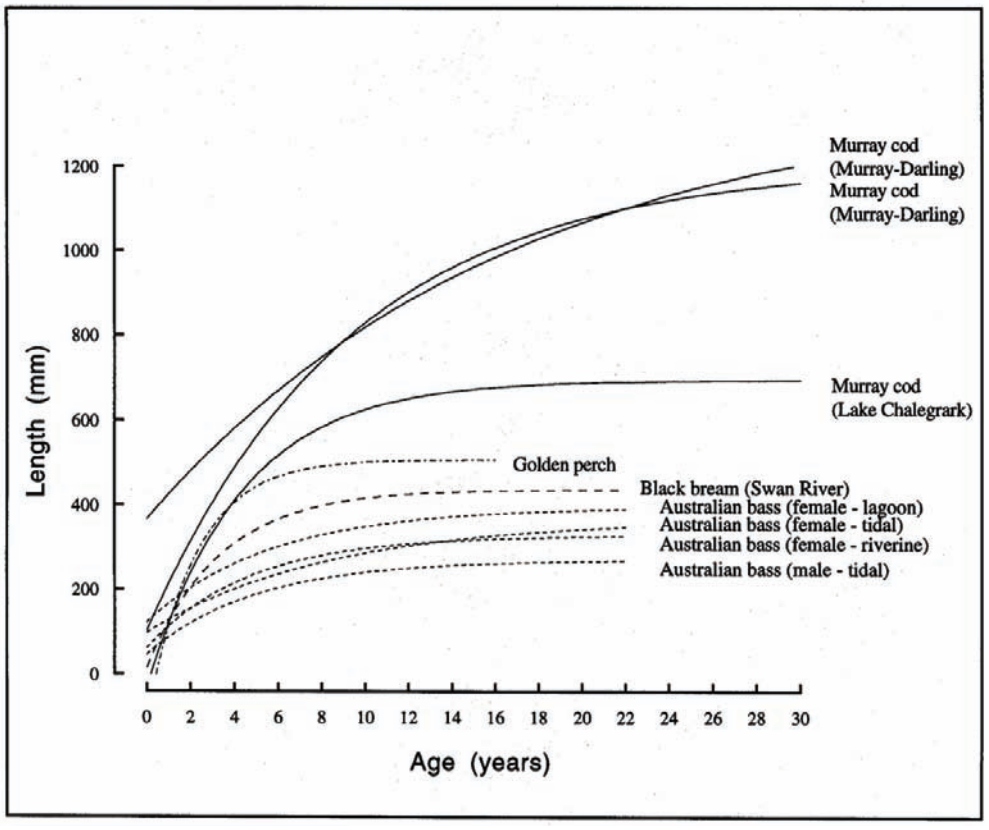


FIGURE 4: Von Bertalanffy growth curves for Murray cod, golden perch, AB and black bream. Taken by Anon (2004) from: Harris (1987); Anderson et al. (1992a,b); Gooley (1992; Rowland (1998) and Sarre and Potter (2000).

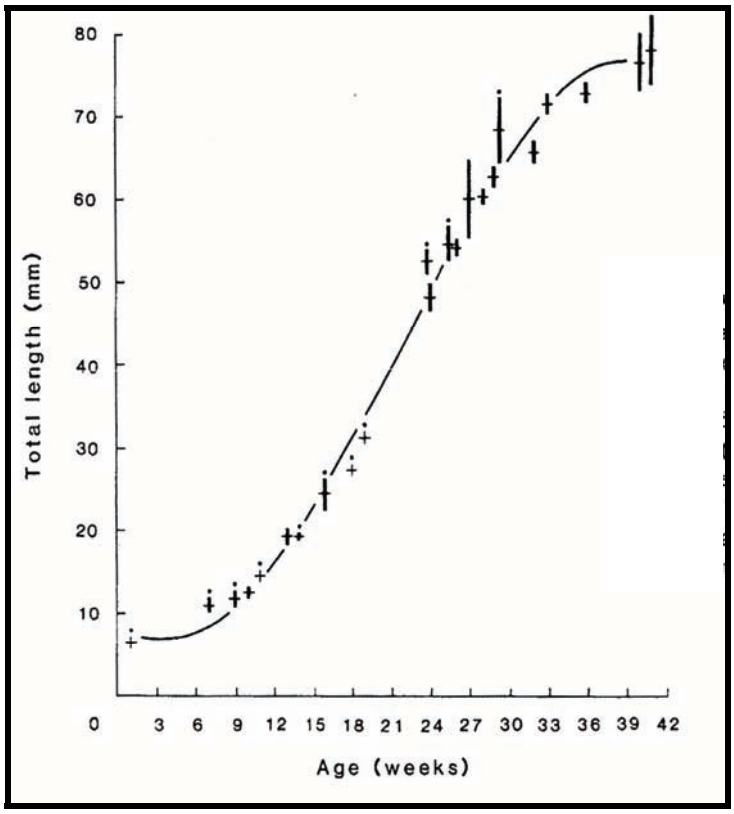


FIGURE 5: Early juvenile growth of AB in the Sydney Basin (Source: Harris, 1986)

1.5 Hatchery Production of Australian Bass.

1.5.1 Scope for Aquaculture, Enhancement of Commercial and/or Recreational Fisheries and Conservation

Though physiologically robust and of high flesh quality, the combination of slow growth rate, small definitive size and solitary life-style unsuited to high density production, precludes AB as a serious candidate for aquaculture. This is especially evident when AB are compared to an array of alternative outstanding freshwater or euryhaline candidate species endemic to Australia such as silver perch *(Bidyanus bidyanus)*, murray cod (*Maccullochella peelii peelii*), mulloway (*Argyrosomus japonicus*) and barramundi *Lates calcarifer.*

Each year Industry & Investment NSW (formerly Department of Primary Industries and Fisheries, DPI&F) stocks about 200,000 AB fingerlings into NSW freshwater impoundments. In addition angling and community groups buy a further 100,000 to 150,000 fingerlings produced by private hatcheries in NSW. The latter are for stocking under the "Dollar-for-Dollar Native Fish Stocking Program". I&I NSW's fish are produced at the department's marine finfish hatchery at the Port Stephens Fisheries Institute. Both the "Dollar-for-Dollar program" and the running costs of the hatchery at Port Stephens are supported by recreational fishing fee funds.

A project to optimise growth and survival of AB being released into freshwater impoundments was initiated by NSW DPI&F in 2008. The project is using 5 impoundments on the southern coast of NSW to quantitatively test "Predatory Impact Model "simulations in freshwater environments. Relevant data on stock dispersal, habitat and seasonal production requirements of released AB will be collected at each freshwater impoundment and simulations run to estimate optimum stocking density. AB will subsequently be stocked at optimum levels as indicated by results of the modelling and also at higher and lower (non optimum) densities to validate the accuracy of "optimum densities". Relative survival of stocked fish will also be evaluated in terms of temperature, dam levels and rainfall specific to each impoundment to determine the effects of these parameters on survival. Release fish will be marked to allow distinction between different cohorts.

1.6 Hatchery Protocols - Australian Bass

1.6.1 Broodstock husbandry

Introduction

The following account is mainly based on publications of Battaglene and Selosse (1996); Battaglene et al 1989a and 1989b; Rowland 1983 and 1986; Van Der Wal 1985 supplemented by information provided by I& NSW staff Paul Beevers, Luke Cheviot and Debra Ballagh.

The marine fish hatchery at the I&I NSW Port Stephens Fisheries Institute (PSFI) (32°745'S, 152°056'E) was the first to develop and apply mass-propagation techniques to AB. Hormone-induced breeding of AB was however first undertaken in 1973 in the basement of Fisheries House in Kent Street, Sydney. These early attempts occasionally resulted in small-scale production of eggs and larvae (Chamberlain unpublished data 1969-1975 – cited in Battaglene et al., 1989). However lack of adequate water supplies and rearing facilities precipitated the establishment of a formal breeding program and relocation to the PSFI in 1979 (Van Der Wal, 1985). Hatchery techniques subsequently developed initially followed those developed by Rowland (1983) for the closely related species golden perch (*Macquaria ambigua*). Current hatchery techniques at the PSFI as detailed below were largely established by 1994 and have been adopted by commercial hatcheries elsewhere in NSW and southern Queensland.

1.6.2 Acquisition of ripe broodstock

To augment the following information specific to AB, the reader is directed to pages 18 and 20 **chapter 4 of Partridge et al., 2003** on broodstock transportation and husbandry. Although the latter pertain to snapper and black bream, they are also pertinent to AB.

Two alternative methods of providing ripe broodstock for hatchery production of AB fingerlings have been developed and routinely practised at the PSFI over the past 30 years. These can be regarded as optional alternatives by would-be AB hatcheries.

1.6.3 Annual collection and return of broodstock from and back to the wild

One method of acquiring spawnable AB broodstock is to collect them from the wild during the breeding season and immediately induce ovulation and spawning. This method is of course subject to procurement of collection permits by state departments of fisheries in NSW, Victoria, and Queensland. Such broodstock can be captured from fresh water sections of rivers or weir reservoirs. In the case of hatchery activities at the PSFI, the perennial local collection site within 1 h drive is the weir at Seaham (Fig. 6). Broodstock for the production of fingerlings for restocking on the state's north coast are usually collected from the Clarence River ($29^{o}46$'S, $153^{o}196$'E) approximately 550 km and 7.5h driving from the PSFI ($32^{o}745$'S, $152^{o}056$'E), while broodstock for production of fingerlings for restocking on the state's south coast are usually collected from Broughton Creek ($34^{o}844$'S, 150^{o} 670'E), a tributary of the Shoalhaven River approximately 400 km and 5h driving from PSFI.

Broodstock are captured from the margins of rivers / weirs / reservoirs using manually deployed 20-60m long x 2m drop, 100mm square monofilament seine nets. Gilled fish are gently removed from the nets within minutes of being snared and immediately transferred to aerated transporter tanks prefilled with river/weir water to minimise physical trauma of capture and subsequent handling stress. Those transport tanks used at the PSFI comprise 600L fibre-glass tanks (*Fyloss P/L*, Innisfail Qld). Generally no more than 30 fish with average weight of approximately 500 g are carried in the tank at any time. Medical-grade compressed oxygen is supplied by ceramic airstone at 1-2 L/min to ensure dissolved oxygen concentration (DO) remains saturated.

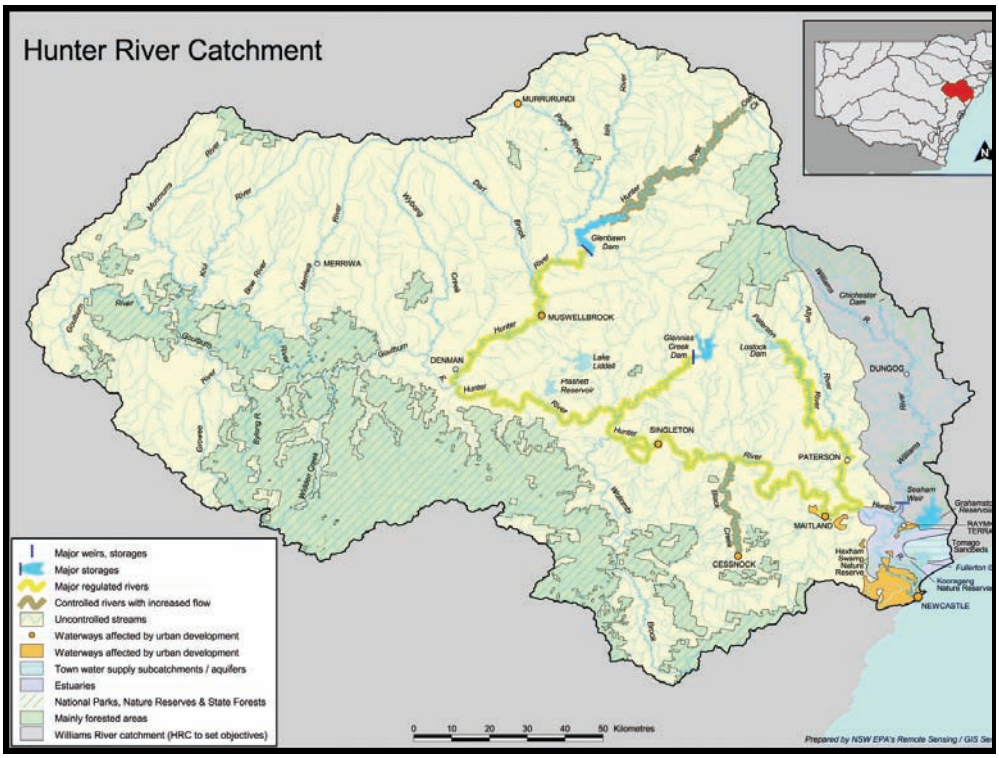


FIGURE 6: Australian Bass Broodstock collection sites local to the PSFI. Image from the NSW Department of Environment and Climate Change Website.

On arrival at the PSFI within 2-12 hours of capture, broodstock are anaesthetised before being measured, sexed and checked for suitable breeding status. A number of anaesthetics are commercially available. AQUI-S® is an anaesthetic produced in New Zealand and available within Australia. It is the only anaesthetic approved with a nil withholding period, meaning it can used with fish destined for immediate human consumption. AQUI-S® is used at concentrations of 10-20 mg/L, however, for surgical and invasive procedures such as cannulation, doses of 40-60 mg/L are recommended. AQUI-S® is also reputed to have a wide margin of safety, meaning fish can remain in the anaesthetic for relatively long periods of time. Alternative anaesthetics include 2-phenoxyethanol which is used at the rate of 0.35 mL/L or benzocaine (or MS222) at the dose of 50 mg/L. Benzocaine and MS222 are similar compounds, differing in their water solubility. Benzocaine must be dissolved in alcohol before use, whereas MS222 is soluble in water. The approximate price to achieve light anaesthesia in 100 litres of water of the various anaesthetics are; AQUI-S® $0.65, 2-phenoxyethanol $3.00, benzocaine, $1.70 and MS222 $14. Aeration, ideally in the form of pure oxygen, is required during anaesthesia[1].

The anaesthetic is either poured slowly into the stream of bubbles or dispensed under the water surface, where it is quickly dispersed. After several minutes in the bath, the fish will turn ventral surface up. They can then be gently removed from the water and placed on a flat surface (a block of high-density foam covered with a plastic sheet is suitable). When handling the fish, plastic or smooth rubber gloves should be worn to prevent slime loss.

[1] For further information regarding anaesthesia refer to the AquaInfo technical information sheet titled, "Successful Anaesthesia of Fish", available from the WA Department of Fisheries website.

Suitable males are those that yield fluid milt when the abdomen is gently compressed between thumb and forefinger anteriorly-posteriorly towards the vent. Sperm samples from suitable males need to yield viability /motility rates approaching 100% when suspended in a drop of seawater placed on a glass microscope slide beneath a coverslip and examined at 400x.

Female AB are catheterised using a sterile 2-mm diameter plastic tube inserted 2 to 5 cm into the oviduct papilla (immediately anterior of the anus within the vent) and applying slight suction (Fig. 7). Four arbitrary oocyte stages (Table 1) are used in assessing suitability for induction. Using samples of at least 100 eggs suspended in seawater, the mean diameter of the10 largest oocytes are measured to ±30 um using a stereo-microscope fitted with an eye-piece micrometer. As indicated in Table 1, fish with predominantly stage 2 eggs (mean diameters of about 0.85 mm) or stage 3 eggs (mean diameters of about 0.9 mm) are suitable for induction.

The principal advantages of annual collection of wild AB broodstock over maintenance of captive stock, are lower costs and greater genetic diversity. A major disadvantage is the relative brevity and variability of breeding season including a total absence of breeding during drought years. By contrast, access to spawnable captive stock is not subject to the vagaries of rainfall and floods and could be extended year-round subject to broodstock being held under controlled environmental conditions, especially temperature and photoperiod.

1.6.4 Use of captive broodstock

The alternative method to procuring ripe AB from the wild each breeding season, is year-round maintenance of captive fish. Following collection from the wild as described above, adult AB are quarantined in saltwater baths for approximately two weeks before being stocked into long term holding ponds in either fresh or brackish (2-15 g/kg salinity) water. In the case of the PSFI, holding facilities originally comprised six 100,000 L, outdoor concrete lined ponds, 1.2 metre deep.

FIGURE 7: Cannulation of a mature female AB for samples of oocytes prior to hormone induction.

TABLE 1: Egg stage, egg diameter and successful ovulation and spawning of hormone-induced Australian bass. Data from Battaglene and Selosse, 1996.

Predominate egg stage	Mean oocyte diameter (mm)	Mean successful rate of ovulation and spawning
I	0.50 mm	0%
II	0.85 mm	65%
III	0.90 mm	40%
IV	0.90 mm	0%

(Source: Battaglene and Selosse, 1996)

FIGURE 8: Broodstock tanks used at PSFI.

The current method of holding AB broodstock comprises an indoor 20,000 L fibreglass tank operated as a recirculating system with mechanical drum filter, suspended bead biological filter and heater/chiller unit (Fig. 8).

Photoperiod is controlled by a fluorescent light. Stocking rates of 2 -4 adult AB/m^3 comprising equal numbers of males and females commonly in the range of 500-1500 g. The stock can be adequately maintained on a mixed diet of frozen green prawns, mullet and/or commercial formulated fish pellet feeds developed for other carnivorous fish such as barramundi or Murray cod.

Feeding of captive broodstock to satiation on alternate days has proven sufficient to ensure that captive broodstock held under ambient photoperiods and temperatures in outdoor ponds and controlled conditions in indoor tanks at the PSFI, come into breeding condition. Pre-spawning condition is first attained in late May or June and is held until August or September while temperature remains in the range 9 to18ºC. However as the quality of spawned eggs suffers a continuous decline through the season (Fig. 9), earliest possible initiation and completion of hatchery operations is recommended. Criteria and methods for selecting captive AB for induced spawning are the same as those described above for annually collected wild broodstock.

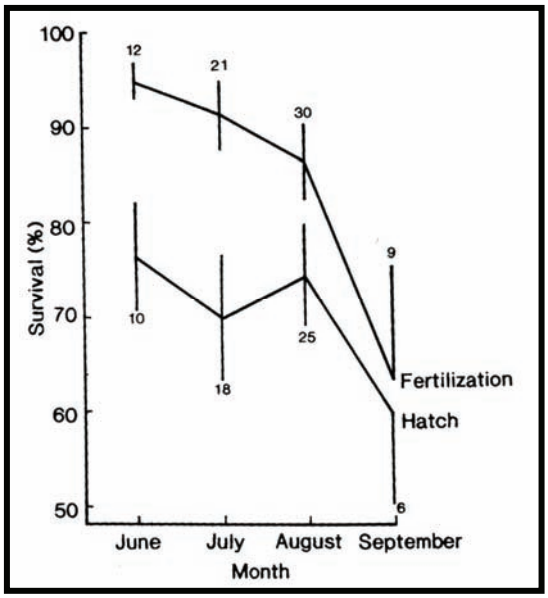


FIGURE 9: Mean monthly fertilization and hatch rates (1984-1986) for captive AB injected with hCG; bars are standard errors. (Source: Battaglene and Selosse, 1996)

1.6.5 Induction of ovulation and spawning

To augment the following information specific to AB, the reader is directed to pages 21-24 including videos 2 and 3 **chapter 4 of Partridge et al., 2003** for a clear and comprehensive instruction on induced spawning. Although the latter pertain to snapper and black bream, they also are applicable with minor modification to AB.

As previously discussed, adult female AB mainly reside in deeper lotic areas in of upper regions of rivers. They require complex natural cues generated by heavy rainfall, runoff and flooding in Autumn and winter to initiate downstream migration to brackish water regions to undergo final gonad maturation and unite with males to spawn. Accordingly, reproductively ripe AB, whether newly collected from the wild or captive, will not ovulate and spawn without the administration of exogenous hormones. The latter entails intra-peritoneal injection of human Chorionic Gonadotropin (hCG) such as *Chorulon®*, (Intervet International B.V., Boxmeer-Holland). This is

administered with a 1ml sterile syringe at the posterior base of the pelvic fin as shown in Fig. 10. Use of, hCG is recommended as it is widely available, relatively cheap and has excellent storage characteristics and is available in standardized dosage forms. Wild broodstock are best collected early in a working week and injected at 1600 and 1900 h on the day of collection. This ensures that all collection, spawning or stripping operations can be completed during working week days (Monday to Friday) and that intensive hatchery operations following spawning or stripping can be completed within standard working hours (0600 to 1800 h).

Following hormonal injection, groups of one-three male and one female AB are transferred without the need of salinity acclimation into 500 to 1000 L cylindrical fibreglass or plastic spawning tanks (Fig. 11) filled with 10 µm filtered and disinfected seawater (30 -35 ppt) to await ovulation and spawning, or in the case of failure of unassisted spawning, stripping of eggs and sperm followed by in vitro fertilization. These tanks are run static (without water exchange) but are aerated and maintained at an optimum temperature of 18 ±1ºC.

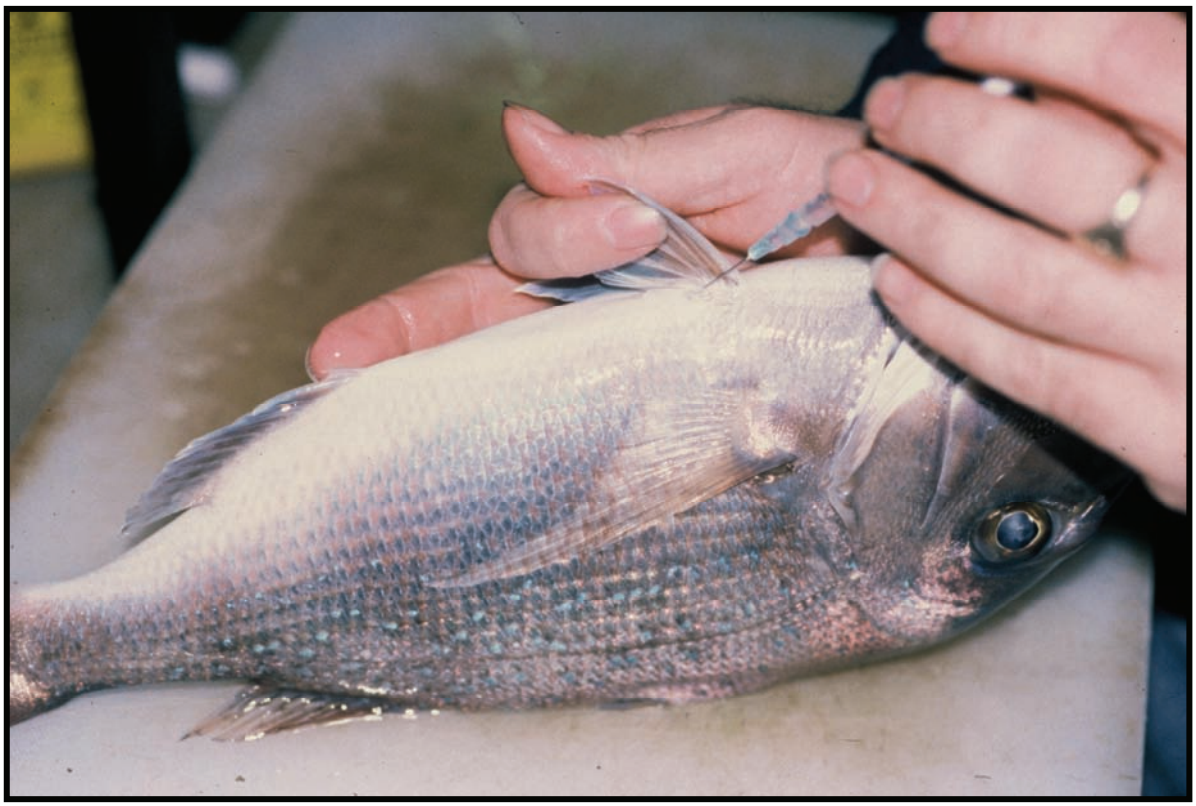


FIGURE 10: Injecting hCG at the base of the pelvic fin of a mature female snapper (*Pagrus auratus*).

FIGURE 11: 500 L cylindro-conical spawning tank.

It should be noted, NSW hatcheries that produce AB for stocking to public waterways must do so following a Hatchery Quality Assurance Scheme (HQAS). A stipulation of the HQAS is that an effective population size of 50 (Ne50) must be used for stocking over a 5 year period. That is, 25 paired matings must contribute to all AB progeny. AB are mass spawners and most female AB will spontaneously release eggs after hormone induction with high fertilization success when they are held in a communal tank with male and female bass. In tanks where there is only one female present, spontaneous spawning has proven unpredictable with approximately only 25% of the females releasing eggs unaided after ovulation.

The latency period between hCG injection and spawning is the same for captive and wild broodstock (Fig. 12), mean duration at 18 ±1ºC being 34.2 + 0.4h. The time at which stripping intervention is recommended for female AB that fail to spawn is 36-h after hCG injection which is 2 h after the average spawning response time of 34 h. This recommended stripping time represents a compromise between sufficient time for completion of ovulation and the deterioration of egg quality that follows ovulation. A delay of even a few hours in the stripping of ovulated eggs can greatly reduce subsequent fertilization rates and/or post fertilisation viability. On average, 70% of female AB stripped at 38 hours after hCG injection at 18 ±1ºC can be expected to yield good quality viable eggs and larvae (Fig. 13). Significant reduction in hatching success can be expected in AB beyond 41 h of hCG injection regardless of whether eggs are spawned unaided or require stripping (Fig. 13).

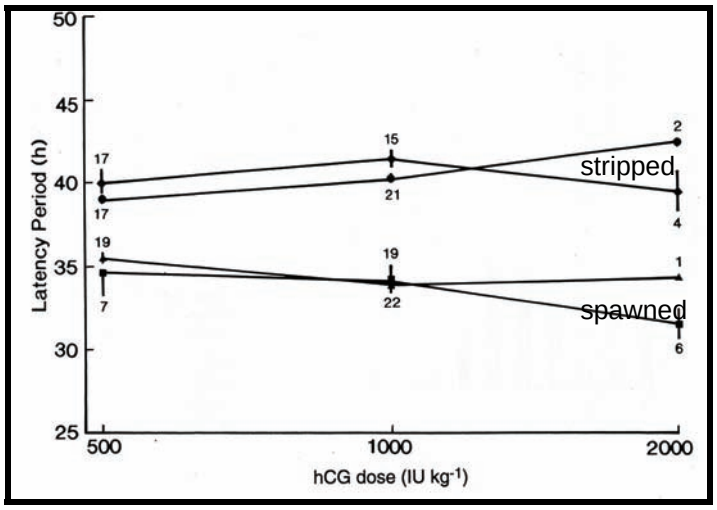


FIGURE 12: Mean latency period at 18 ±1º C for captive and wild AB injected with 500, 1000 or 2000 IU hCG./kg. Bars are standard errors, n. number of fish; (♦) stripped captive fish; (■) captive fish which spawned; (●) stripped wild fish; (▲) wild fish which spawned. (Source: Battaglene and Selosse, 1996).

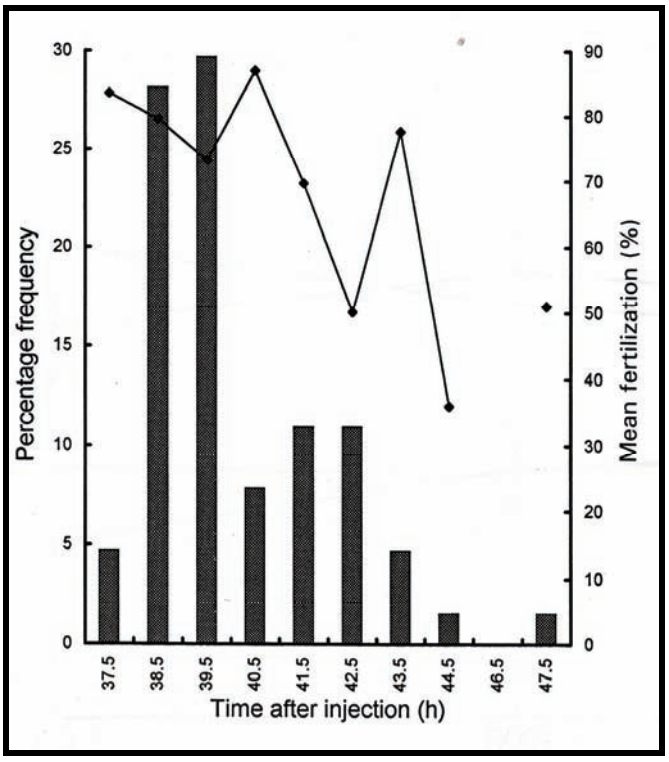


FIGURE 13: Frequency histogram of the period between injection and successful stripping at 18 ±1º C showing the mean percentage fertilization for each time interval. Data are for wild and captive fish injected with 500 or 1000 IU kg-' hCG (n = 64). (Source: Battaglene and Selosse, 1996**)**

Fecundity of female AB, though subject to a high degree of variability between equal size individuals, is linearly related to live-weight (Fig. 14). Although the relationship of live-weight to fecundity is similar for captive and wild collected broodstock (Fig. 15) captive fish yield marginally more eggs on average (500,000 eggs/kg) than their wild collected counterparts (450,000 eggs/kg).

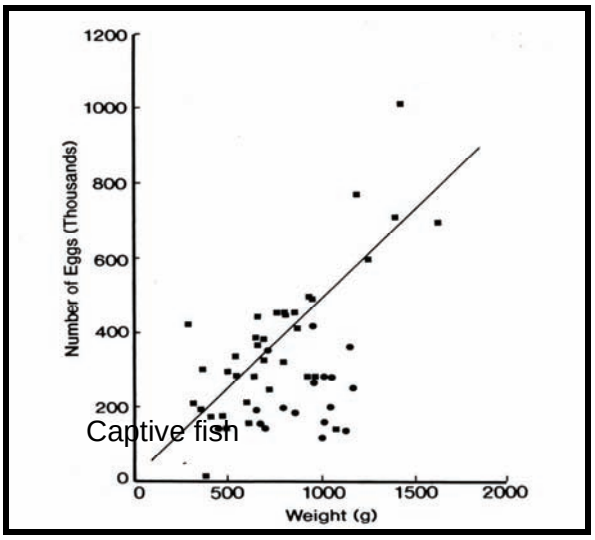


FIGURE 14: Relationship between weight and number of eggs spawned (■) or stripped (●) from wild AB injected with 500 or 1000 IU kg hCG following capture in May,June or July (Source: Battaglene and Selosse, 1996).

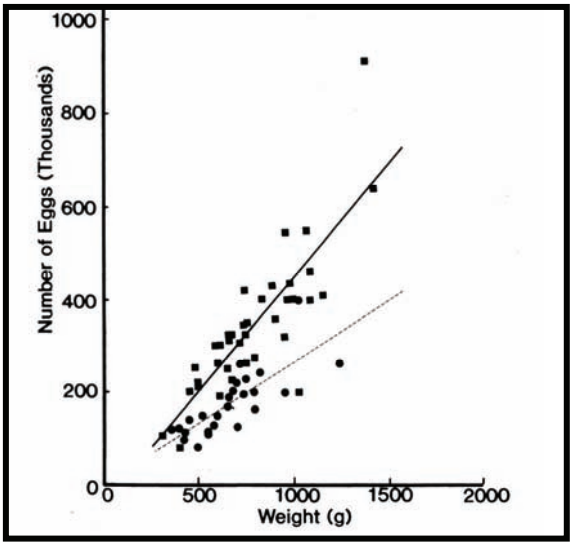


FIGURE 15: Relationship between the weight and number of eggs from spawned (■) and stripped (●) captive AB injected with 500 or 1000 IU kg hCG. (Source: Battaglene and Selosse, 1996).

The reader is directed to pages 27 and 28 including videos 4 and 5 **chapter 4 of Partridge et al., 2003** for additional comprehensive instruction on stripping and artificial fertilisation Although this information pertains to snapper and black bream, it is also applicable with minor variation to AB.

It is important to be aware that not all ripe female AB (those carrying stage 2 and 3 oocytes) ovulate and spawn even when induced with the optimal dose of 500 IU (International Units) hCG/kg. Accordingly, females that ovulate but do not spawn, need to be stripped and generally produce fewer, inferior quality eggs. This may be because initial stripping is taking place prior to the completion of ovulation.

Female AB that fail to spawn within 38 h of hCG injection should be removed from induction tanks using a soft mesh landing net, then anaesthetised and catheterised as described above to establish the status of ovulation (final ripening and hydration of oocytes). Only females that have a distended abdomen (Fig. 16), inflamed genital papillae and/or have started to ovulate and freely shed fully hydrated eggs under mild compression of the abdomen, should be stripped and these must first be rinsed clean of anaesthetic with fresh water. If firm compression of the abdomen is required to extrude eggs, it should be assumed that ovulation is not complete and the female returned to the holding tank.

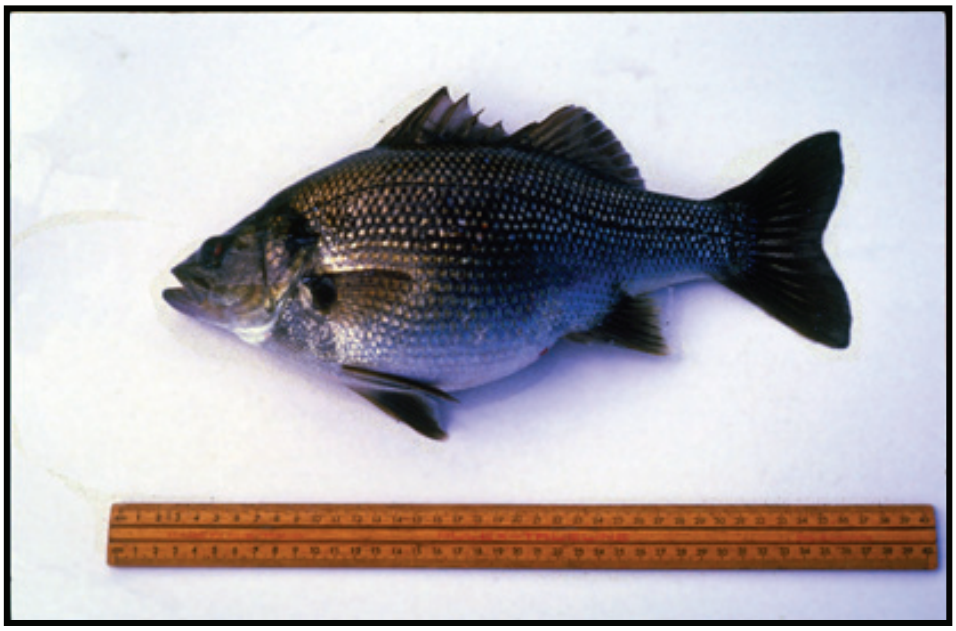


FIGURE 16: Female bass with distended abdomen after ovulation and egg hydration.

Whilst the procedure is relatively simple, correct timing is critical to obtain good-quality eggs, as once the fish ovulate the eggs only remain capable of being fertilised for a short period. This period, known as the window of viability, is generally only a few hours for AB.

As AB appear to spawn naturally from late afternoon to early evening a rule-of-thumb is to inject the hormones in the late afternoon and inspect the spawning tanks 36 h later at 18^{o}C. If spontaneous spawning has not occurred then stripping should be commenced. Females that have ovulated will present with a heavily swollen abdomen. If ovulated eggs are not released spontaneously, the fish may become egg-bound. As this condition can kill the fish, it is necessary to strip all female fish that have not spawned. Induced fish must be kept under close observation and may require repeated handling, therefore they should be returned to their small spawning tank where they can be quickly and easily recaptured and stripped once ovulation has occurred. If the fish are being held in a communal tank and natural spawning after hormone induction is expected

to occur, they should be returned to the main broodstock tank and an egg collector fitted to the overflow (see Chapter 1.7.5 Egg harvesting, counting and incubation).

The fish should be anaesthetised prior to each stripping occasion. After anaesthesia, the fish is removed from the water, its abdominal region rinsed with fresh water and gently towelled dry. The eggs are then manually exuded from the vent into a clean container by exerting pressure along the abdominal region, starting from the front of the fish, behind the pectoral fins, and working towards the vent. Stripping into a graduated cylinder allows an accurate determination of the volume of eggs collected. The eggs are very fragile at this stage and, should be treated accordingly.

Ovulating females and spermiating males are best laid on a table or bench and wrapped in wet soft cloth (wet cotton sheet, flannelette or towelling). Eggs and milt are best stripped from fish still wrapped in wet cloth. The fish are held and gently restrained using surgically gloved hands to prevent infection. Batches of eggs are stripped directly into sterile 1-5 L plastic graduated measuring jugs (Fig. 17). The stripped eggs should be then gently mixed using a plunger homogeniser while milt (0.1 ml milt /100 ml eggs) of predetermined high sperm motility from a companion male is stripped directly into the jug. The eggs should then be transferred into to a clean sterile bucket or tub, topped up a known volume with filtered seawater at 18-20 ºC and 25-35 g/kg salinity and the egg suspension gently homogenised for several minutes. A minimum of 5 x 1 ml subsamples should be taken with a 1 ml pipette (with 2 mm aperture) during the mixing operations and each sub-sample dispensed onto a petri dish for counting under a dissecting microscope (Fig. 18). The mean count of the sub-sample is then used to accurately assess density of fertilised eggs of normal healthy appearance and thence to estimate total number of eggs/embryos in the bucket/tub.

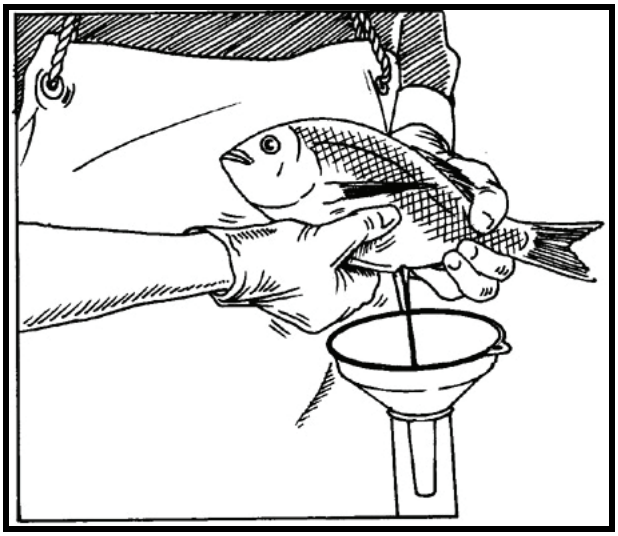


FIGURE 17: Stripping ovulated oocytes from a fish. (Source: Partridge et al., 2003).

Fresh sperm needs to be on hand to enable the stripped eggs to be fertilised. After anaesthesia, the abdomen of the male fish is rinsed with fresh water and dried and the sperm exuded with pressure as described for the female. The sperm should be drawn directly into a 5 ml syringe that is then capped and chilled (either in the refrigerator at 4°C or on cloth-covered ice). AB sperm has been used successfully after several days of storage. It should be noted, however, that sperm viability will decrease with increasing storage time. During the collection process it is vital that the sperm does not contact water, as this will activate the sperm and limit its viability to a few minutes.

It is best to have sperm available when the females are stripped, so any eggs obtained can be fertilised immediately. Fertilisation is achieved by gently mixing the eggs and sperm in approximately 300 mL of filtered seawater for several minutes. A general rule of thumb is 1 mL of sperm to 1 L of eggs. The suspension is then left to stand for a further 10 minutes before being gently but thoroughly rinsed with filtered seawater on a 200 µm screen.

1.6.6 Egg harvesting, counting and incubation

The following advice on egg harvesting counting and incubation, is largely derived from **chapter 5 of Partridge et al., 2003.** Although the latter was complied in relation to hatchery production of snapper and black bream, equipment and operating procedures extend with only minor variation to AB.

Where pairs of AB broodstock successfully complete spawning and unassisted fertilisation, parent stock should be removed from spawning tanks as soon as practicable. Aeration should be stopped for about 20 minutes to allow the slightly buoyant high quality eggs to rise and concentrate into a narrow but dense layer at to the surface to be harvested while non viable non buoyant eggs can be settled out and drained to waste. Before harvesting, eggs should first be examined to establish overall fertlisation rate and an appearance characteristic of a normal healthy development up to first or second cleavage (cell division) (Fig. 18). Batches of water hardened eggs of acceptable fertilisation rate and appearance can either be harvested by hand using fine mesh (500 µm, polyester) dip nets or automatically by overflowing into collection tubs fitted with drainage panels of the similar mesh materials as dip nets, located in harvesting tanks of the type illustrated in Fig. 19 that receive egg bearing surface skimmed water either via a central standpipe or outlet placed high on the wall of the spawning tank.

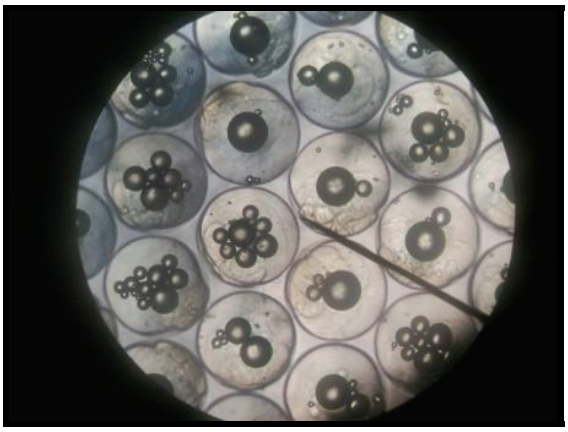


FIGURE 18: Microscopic observation of newly-fertilised AB oocytes. Note the normal, variable size and number of oil globules in oocytes.

FIGURE 19: Automatic 500 µm screen egg collector mounted in a 500L sump on the outer wall of spawning tank.

The harvested eggs are placed into a 20 L bucket filled with disinfected seawater and aerated to ensure uniform distribution. A minimum of 10, 1 ml samples are then taken from the tank with a pipette and counted under a dissecting microscope. The counts are then averaged to obtain the average number of eggs per millilitre. To obtain the total number of eggs, this value is then multiplied by 1,000 (to convert to eggs per L), then multiplied by the volume of water in the collecting tank.

At the PSFI, fertilised AB eggs are harvested from spawning tanks. If of marginal quality (<70% fertilization) they are incubated in a dedicated incubation tank. High quality fertilised eggs are however stocked directly into cylindro-conical larval rearing tanks where egg shells and any unhatched eggs can be easily siphoned from the tank. The advantage of this method is that the eggs only need to be handled once. Prior to stocking eggs into the larval rearing tanks, a period of acclimatisation must be implemented before eggs can be stocked due to the difference in water sources between the broodstock tank and the larval rearing tank. Eggs are acclimated in 60-L perspex tanks (acclimators) floated in the larval rearing tanks. Enough aeration is supplied in the acclimators to mix the eggs. The duration of acclimatisation is dependent on the degree of difference between the two water bodies. Generally, a change of 2°C/hr and 1 pH unit/hr is acceptable. Each acclimator is approximately half filled with water from the broodstock tank, and up to 200,000 eggs are added. Water from the larval rearing tank is then added gradually, depending on the difference in water quality parameters between tanks - slowly for considerable differences, and more rapidly if parameters are similar. Eggs are released into the rearing tank once water quality in both the acclimator and rearing tank are matched, generally once temperatures are within 0.5°C. Optimum conditions for incubation and hatching of AB eggs include a salinity range of 25-35 g/kg (Fig. 20) and mean ± range of temperature of 18-20 $\pm 1^0$C (Fig. 21).

Considerable effort is invested in setting up larval rearing tanks. A separate incubation phase is therefore considered when there is a chance of a poor hatch. In such cases eggs can be incubated in simple, low-cost 500-1000 L conical or flat-bottomed tanks, with no provision for recirculation or water flow- through (i.e. static operation). Such tanks (incubators) are placed on an elevated stand in the broodstock room. Incubators are filled with 10 µm filtered, disinfected seawater and water quality in the incubator is maintained by draining down approximately 50% of the water and topping up daily with filtered, disinfected seawater. The tank water is siphoned out through a 53 µm screen to retain the eggs and newly hatched yolk-sac larvae within the incubator.

Hatching at the recommended mean ± ranges of incubation temperature of 18 to 20 ± 1^0C will commence after 40 to 50 hours (Fig. 22) and once started, will be completed over a further duration of 8 to 9 hours with expected hatch rates exceeding 90% (Fig. 20). Use of lower incubation temperatures down to 12ºC progressively protracts on-set of hatching up to 90 hours and significantly reduces yields of hatchlings to as low as 70%. Higher incubation temperatures up to 24ºC marginally shorten the time to onset and duration of hatching, but do so at the risk of imposing severe larval deformities, including curvature of the spine, mouth deformations and *cyclopic* larvae (single eye in centre of the head).

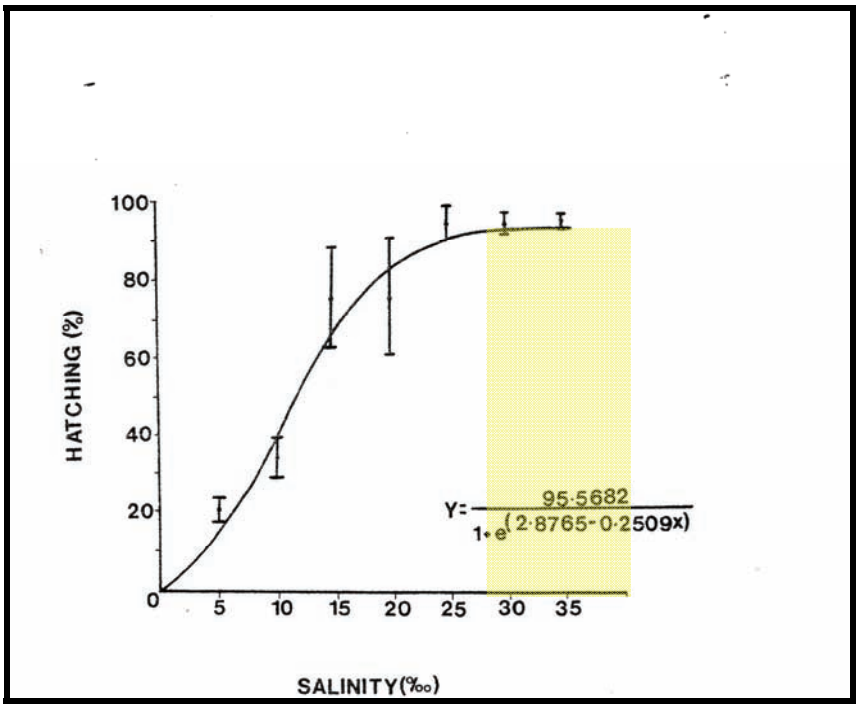


FIGURE 20: Effect of salinity on hatch rate of AB. Shaded area is recommended salinity band. (Source: Van der Wal 1985).

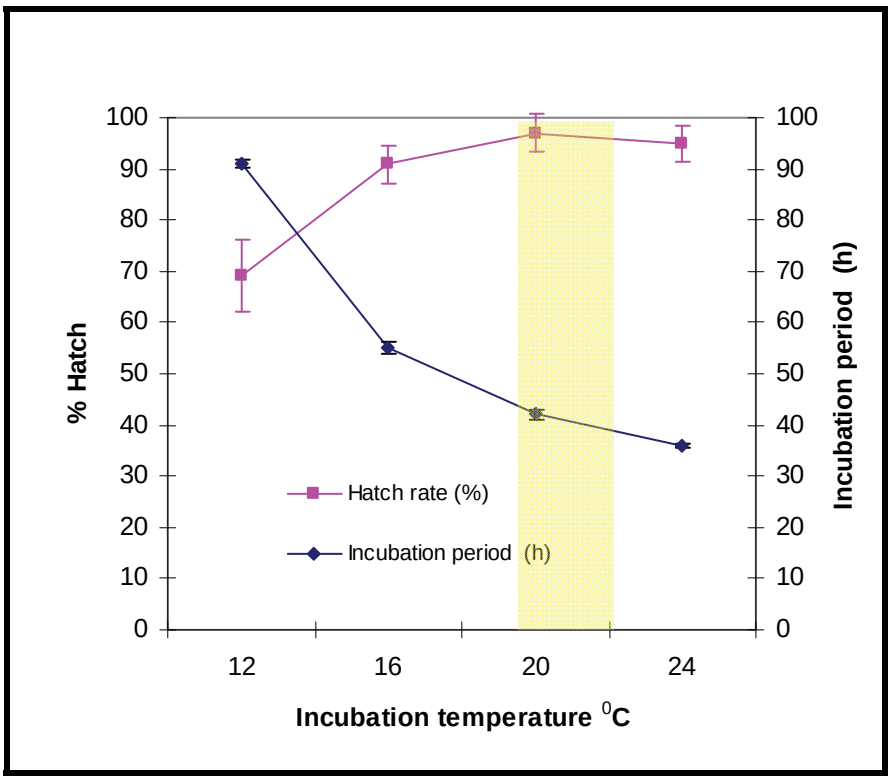


FIGURE 21: Effect of temperature on hatch rate and incubation time of AB eggs. Shaded area is recommended temperature band. (Based on data from Tables 1 and 2 of Van der Wal 1985).

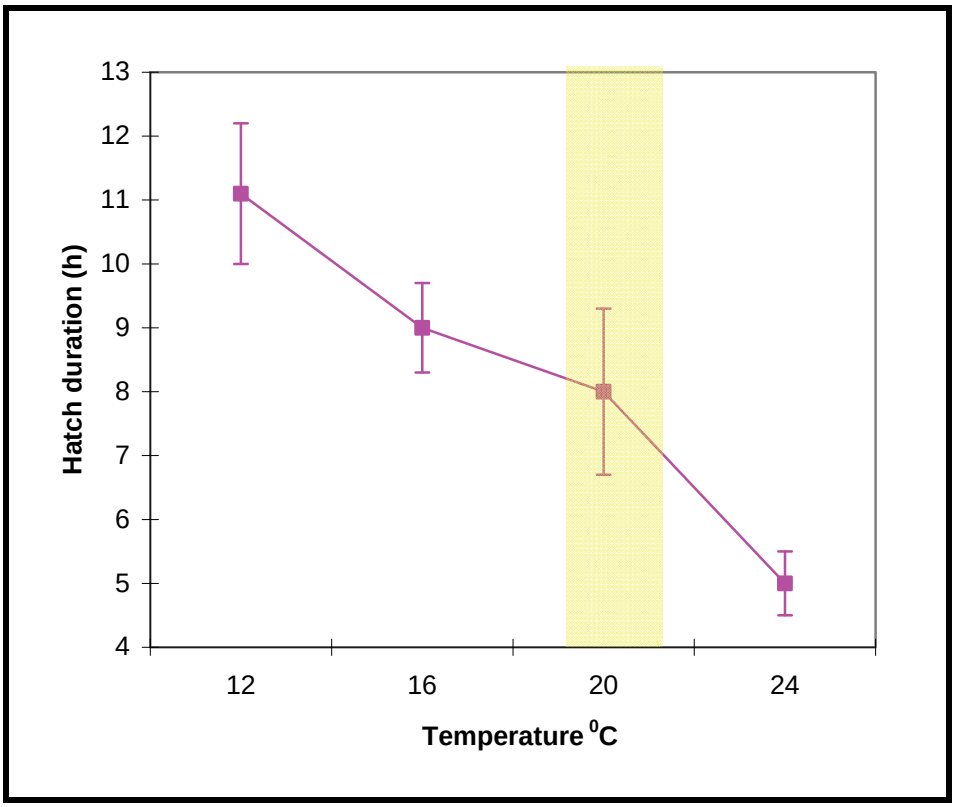


FIGURE 22: Effect of temperature on hatch duration of AB larvae (based on data from Tables 1 and 2 of Van der Wal, 1985). Shaded area is recommended temperature band.

1.6.7 Interim harvesting, counting and stocking of newly hatched larvae

AB larvae hatched in incubators (rather than directly stocked as fertilised eggs into larval rearing tanks) need to be counted and harvested before transferring to larval rearing tanks. Numbers of larvae to be transferred are estimated while still in the incubator. Estimating numbers of larvae while still in the incubator is preferred as it eliminates the need for double handling after harvesting and is generally accurate enough for production purposes. Accurate estimation of larvae numbers relies on larvae being evenly distributed through the water column, and sufficient size (volume) and number of samples. Larvae may be well mixed by brief, vigorous aeration. Counting of larvae within five aliquots of 250 ml to 1000 ml should provide an adequate estimate of mean larval density. Once numbers of larvae are calculated, decisions can be made regarding stocking density and the number of tanks to be stocked.

Newly hatched larvae being very fragile are intolerant of netting, strong water flow, prolonged turbulence, sudden increase in light intensity and changes in water quality. Consequently, harvesting from the incubator is a delicate process. Larvae should be wet drain-harvested from the incubating tank into a 53 µm harvesting net (Fig. 23). Harvesters are positioned as close to the height of the incubator outlet as practical to minimise the height of fall from incubator to harvester. Recommended draining rate to harvesters is 3 to 4 l/min. Low aeration should be provided to the harvester. Alternatively, the incubator water volume can be lowered by siphoning through an immersed 500 µm screen and then taking 10 L buckets of larvae directly from the incubator.

FIGURE 23: Harvester for collecting newly hatched larvae from incubation tanks. (Source Partridge et al., 2003)

Once larvae are concentrated into harvesters, they should immediately be moved into acclimators. An acclimator is a tank of suitable size to hold larvae and that can be floated in the larval rearing tank. At PSFI, 60-L Perspex tubs are used as acclimators. Larvae are best transferred by beaker from the harvester to the acclimator. It is preferable to scoop under the larvae, rather than pushing the rim of the beaker under the surface and allowing larvae to be drawn into the beaker. Take care not to remove the beaker too quickly, as this will rapidly drop the water level in the harvester and run the risk of stranding larvae on the harvester mesh. Acclimators need to be filled with sufficient water from the incubator to avoid pouring larvae from the beaker. The beaker should be lowered below the surface of the acclimator and removed from beneath the larvae to minimise turbulence.

Acclimators can then be left floating in larval rearing tanks and provided with gentle aeration. Principles for acclimating larvae are the same as outlined for stocking eggs. Once water quality parameters of the acclimator and rearing tank are similar, the acclimator can be submerged and the larvae released gently into the rearing tank.

1.7 Larviculture

1.7.1 Introduction and background

Although routine intensive hatchery production of three-week-old (21days after hatch) AB larvae around 6 mm TL (see Fig. 24 D) was established by the mid 1980's (Battaglene et al., 1989), intensive on-rearing was commonly marred by mass mortalities of larvae around 8-12 mm TL, 30 days after hatch (dah) (Fig. 24E). Typical symptoms of ailing larvae included erratic swimming, fainting, constipation, failure to digest food, pale colour and copious mucous production. Histopathology comprised abnormal liver development, and cystic calculi in the bladder. These observations were consistent with a primary non-infectious metabolic disorder most probably a dietary deficiency. The chief suspects were essential fatty acids (EFA's) and /or B group vitamins (Langdon and Battaglene, unpublished data,1989).

Over the past two decades, nutritional deficiencies have frequently been encountered and overcome in relation to hatchery production of many catadromous, estuarine and marine finfish around the world. Within Australia notable examples are barramundi, snapper and mulloway.

A common solution to dietary deficiencies has been enrichment of live-feeds, namely rotifers (*Brachionus* spp) and brine shrimp (*Artemia* spp). Enrichment is achieved by boosting rotifers and brine shrimp with either cultured micro-algae (or concentrates thereof) high in EFA's, other essential nutrients such as B group vitamins or with specially formulated supplements comprising micro-particulate powders or emulsions. An increasingly popular alternative or addition to enriched live-feeds is an ever expanding array of "complete live-feed replacement diets" of continuously improving quality, that are mainly emanating from Japan and Western Europe. (The reader is directed to **Appendix 13** and to **chapter 6 of Partridge, 2003**).

Solving the problem of reliable cost effective hatchery production of AB fingerlings has however proven more complex than just "fixing live-feed nutritional deficiencies". AB eggs have relatively high levels of EFA's in comparison to other Australian native fish (Anderson et al., 1990) and while enrichment of rotifers and brine shrimp with supplements has greatly improved survival of many intensive hatchery reared fish including barramundi (Rimmer and Reed, 1989), only marginal (<5%) improvement in survival was achieved by this means for intensively reared AB, Battaglene and Talbot (unpublished data, 1990).

Lack of swim bladder inflation was also recognised as a major and confounding factor contributing to poor yields and vigour of AB fingerlings produced using intensive clear water techniques. Indeed prior to 1990, intensively reared AB larvae frequently lacked functional swim bladders with up to 100% of larvae being afflicted. Battaglene and Talbot, 1990, showed that the first 10 to 12 days after hatch were critical to normal swim bladder development (Fig. 25) and hence ultimate viability of AB larvae. They also demonstrated that ≥70% of intensively reared AB larvae will undergo normal swim bladder inflation provided that they are kept in total darkness for the first 11 days after hatch (Fig. 26) and provided with other key physio-chemical conditions of low aeration, salinity in the high range of 25-35 g/kg and mean ± range in temperature of 18 to 20±1°C (Fig. 27). As illustrated in (Fig. 28), ranking of critical physiochemical parameters in terms of their potential to inhibit normal swim- bladder inflation in AB larvae are light > aeration related surface turbulence > low salinity.

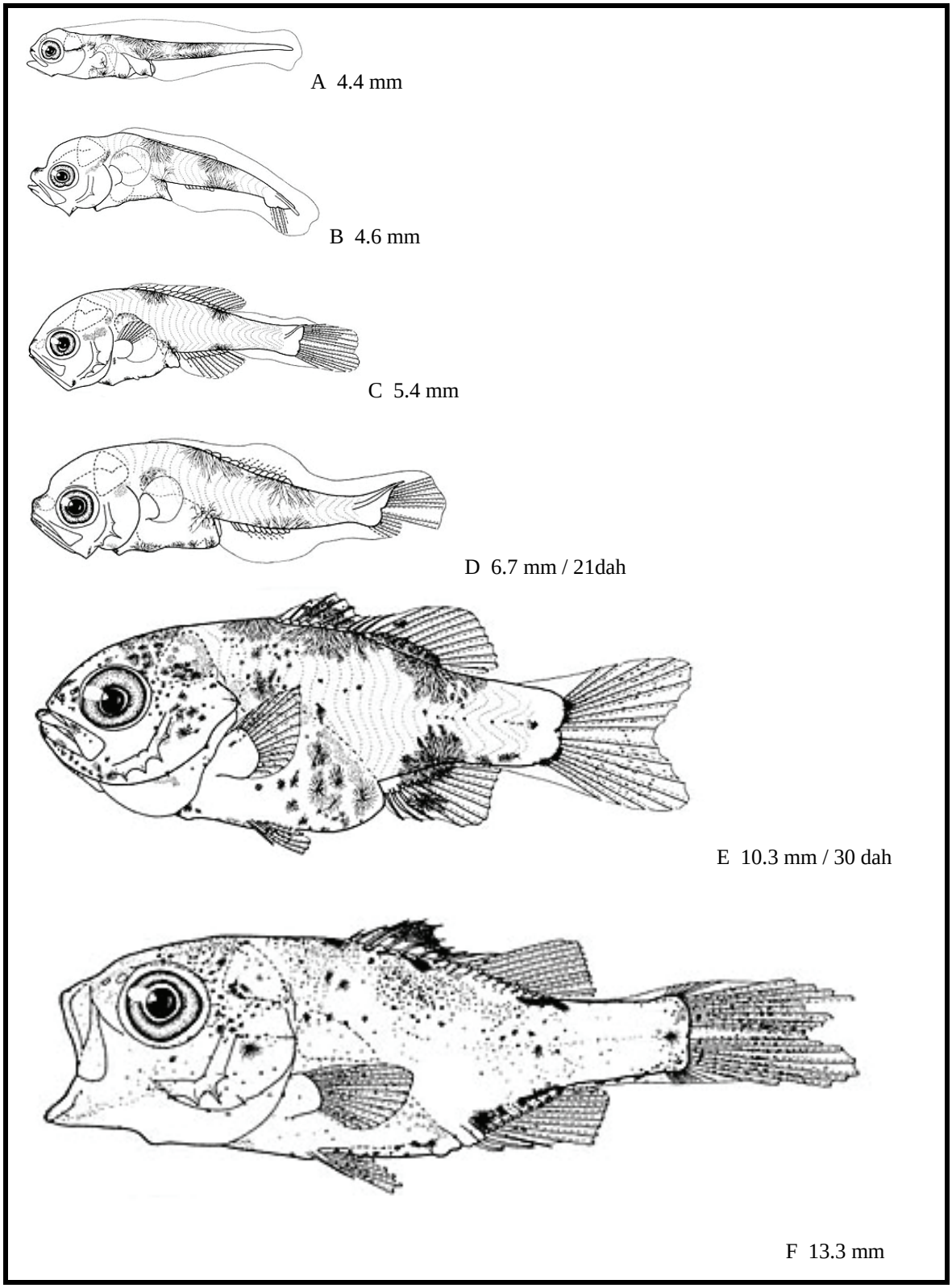


FIGURE 24: Life stages of AB. (Source: Trinski, T, Hay, A.C. & Fielder, D.S., 2005). Images down loaded from the Australian Museum Larval Fishes Website: http://amonline.net.au/larval_fishes/descriptions/macquaria-novemaculeata.html

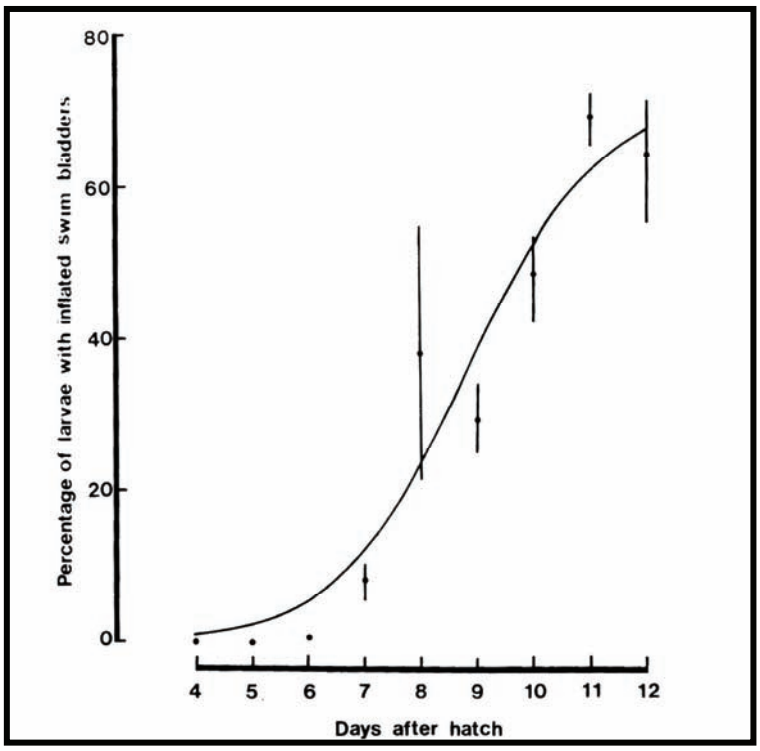


FIGURE 25: Percentages of AB larvae with inflated swim bladders from day 4 to day 12 after hatch. (means± se; n = 3) (Source: Battaglene and Talbot, 1990).

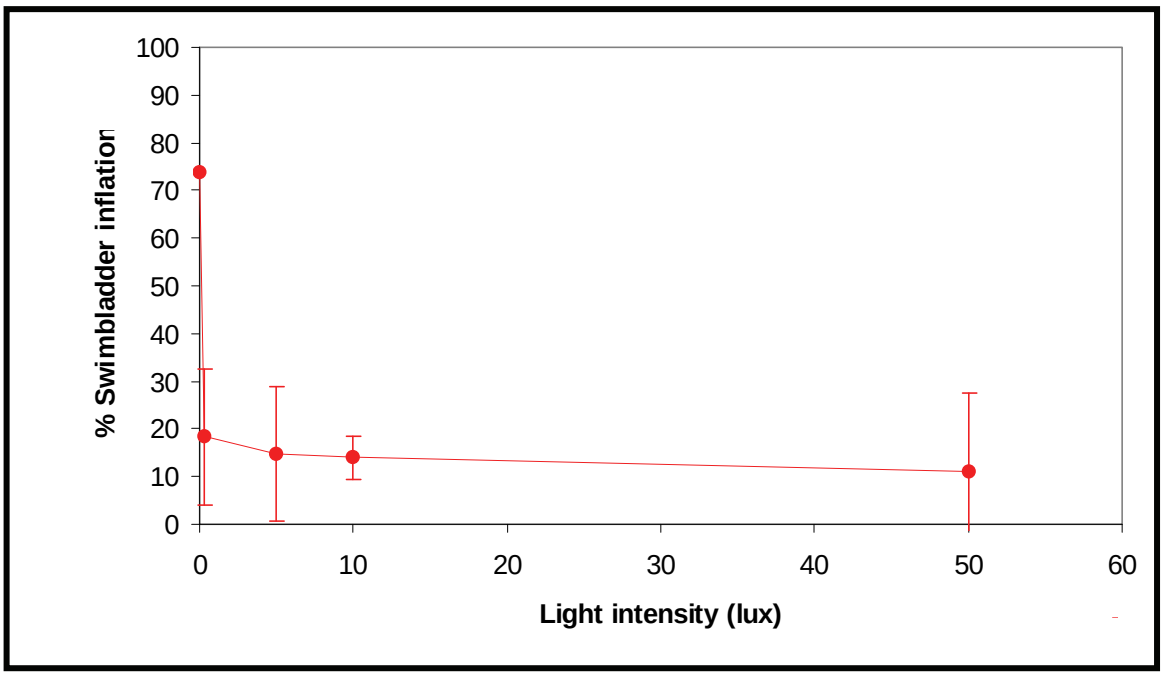


FIGURE 26: Effect of light intensity on initial swim bladder inflation (means ± sd) in larvae over the first 11 days after hatching when reared at optimum salinity (25g/kg), temperature (19 ±1 ºC), nil aeration and, with the exception of the continuous darkness (0 lux) treatment, a 12: 12-h light: dark regime. (Source: based on data from Table 2: Battaglene and Talbot, 1990).

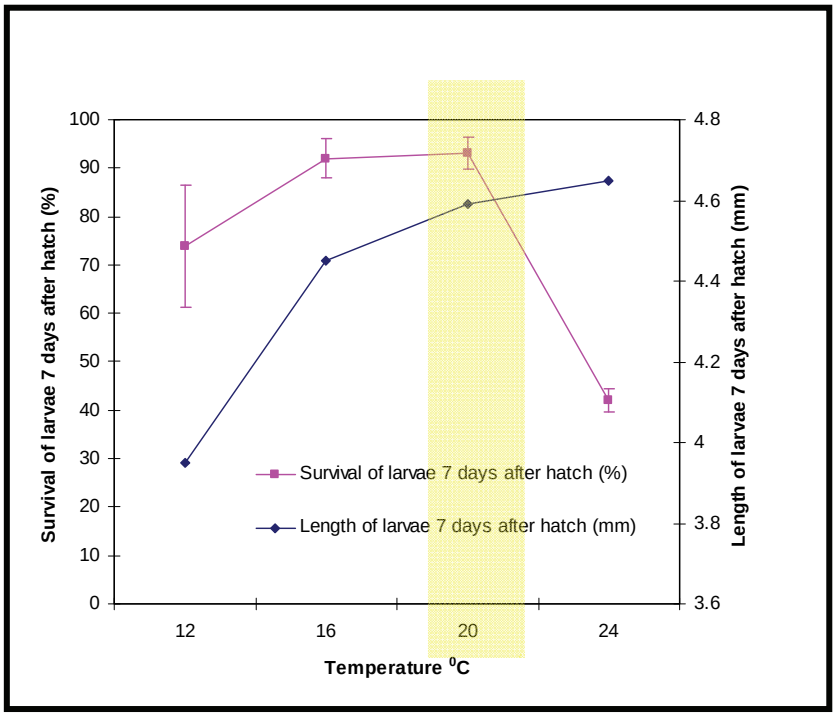


FIGURE 27: Effect of temperature on the growth and survival (mean ± s.d.) of AB larvae 7 days after hatch Shaded area is recommended temperature band. (Source: based on data from Table 1: Van der Wal, 1985).

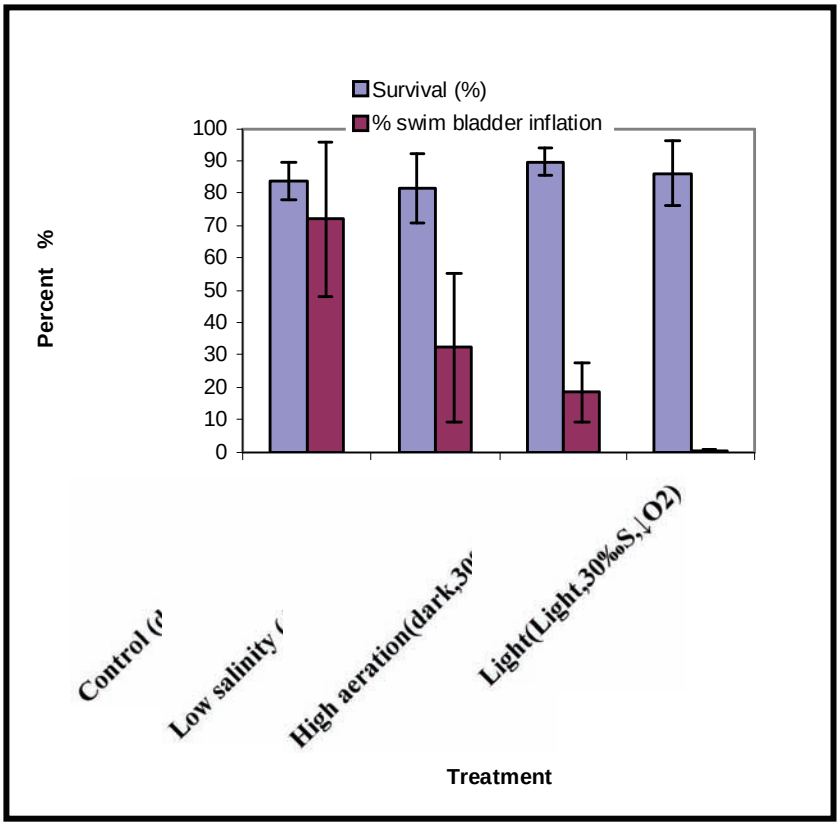


FIGURE 28: Separate effects of light, low salinity and high aeration on survival and swim bladder inflation in 11 day old AB larvae reared at 19±1 ºC. (Source: based on data provided in Table 1, Battaglene and Talbot, 1990).

Burke 1994 investigated the age at which AB larvae can fully osmoregulate in fresh water when acclimated at alternative salinities of 15 and 28‰. Results (Fig. 29) showed that larvae which survived acclimation continued to develop of full osmoregulation which occurred at between 21 and 28 dah.

However results also showed that larvae can survive direct transfer to salinities at least as low as 2 ‰ by 7 dah. This finding paved the way for extensive production in brackish water ponds by some hatcheries that circumvent intensive rearing and hence the need and high costs of culturing microalgae and enriched live feeds such as rotifers and *Artemia.*

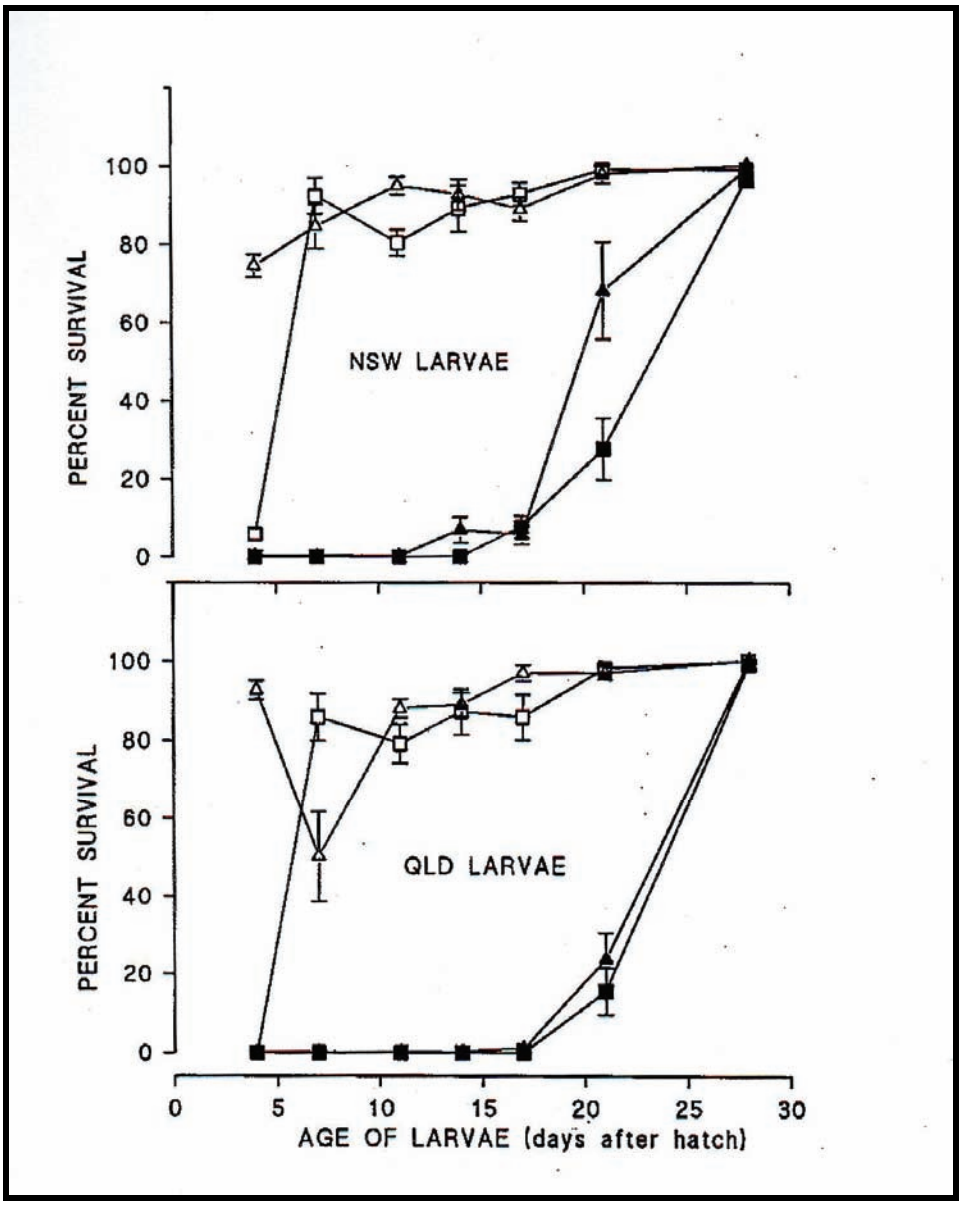


FIGURE 29: Percent survival at different ages of AB larvae from the Noosa River (Qld Larvae) and the Williams River, New South Wales (NSW larvae), in salinities of 0‰ and 2‰ following acclimation at salinities of 28‰ and 15‰ *Symbols*: ■ acclimated at 28‰ tested at 0‰ ; □ acclimated at 28‰ ,tested at 2‰; ▲ acclimated at l5‰ , tested at 0‰ ; Δ acclimated at 15‰, tested at 2‰. (Source: Burke, 1994).

Although multi factorial experiments have not been used to investigate combined effects of temperature and salinity on early growth and survival of AB larvae, there is evidence to suggest that effects are compound rather than additive. For example, a mean ± sd survival rate of 81.5 ± 10.9% (Fig. 28) was reported by Battaglene and Talbot (1990) for 11 dah when AB larvae were reared at a salinity of only 10 ‰ but at an optimum mean ± sd temperature of 19 ± 1 ºC, in the dark and under low aeration. This result contrasts markedly with a yield of 4±3% (encircled point in Fig. 30) reported by Van der Wal (1985) for 7 dah AB larvae reared at a treatment salinity of 10‰ but in combination with a sub-optimal temperature of 15ºC within lightly aerated rearing vessels and (presumably) ambient light conditions. The poor survival of larvae in this treatment also contrasted dramatically with rates above 80% for counterparts in the same experiment but reared within the optimal salinity range of 25 to 35‰.

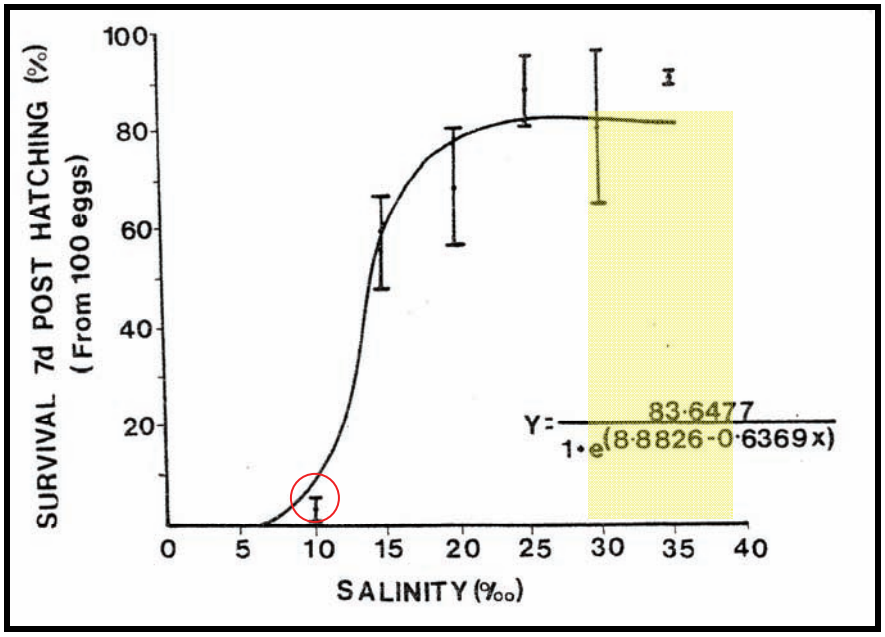


FIGURE 30: Effect of salinity on the survival rate (means ± sd) of 7dah AB larvae at 25-35‰ and 15°C. Shaded area is recommended salinity band. Encircled data point referred to in text. (Source: Van der Wal 1985).

On the basis of the above findings an intensive rearing phase of 21days including an initial dark period (5-11 days depending on water temperature and rates of swimbladder inflation), has been widely adopted as standard hatchery practice to ensure high rates of survival and normal swim bladder inflation. Optimum mean ± ranges of physio-chemical rearing parameters during this initial phase are, of 18-20 ±1ºC, 25-35±2 S‰ and low aeration (less than 70 ml/min per 100 L of rearing volume). As discussed above, slow and gentle handling and acclimation of AB larvae to changes in the holding conditions and physiochemical properties of rearing water are also critical to optimising growth and survival.

1.7.2 Larviculture protocols

Two alternative protocols for on-rearing AB larvae beyond the initial intensive clear-water phase through to 20-25 mm fully metamorphosed fingerlings for replenishing depleted wild populations or for stocking public impoundments and privately owned farm dams, have been developed by I&I NSW. These comprise:

- Continued intensive green-water culture in indoor tanks under optimised temperature, salinity and light regimes using enriched live feeds.
- Extensive green-water on-rearing of 7 to 21 dah, 6.5 mm larvae in large outdoor ponds under management regimes that promote favourable phyto-plankton and zooplankton communities, especially rotifers and copepods and favourable ranges of temperature and salinity.

Intensive clear-water rearing under optimum controlled physiochemical conditions and feeding regimens

Standard intensive methods involve rearing larvae at high densities in flowing, clear seawater. Sophisticated filtration, temperature and lighting equipment are often used to maintain precise control over the rearing process. Intensive methods are comparatively expensive in terms of labour and capital. Consequently, it is necessary to rear larvae at high densities to be economically viable. High larval densities, coupled with the potentially inferior nutritional quality of intensively cultivated live feed (due to rapid loss of enrichment if not immediately consumed), can result in reduced growth rates of larvae when compared with extensively cultured fish. If strict hygiene protocols are not enforced, such high larval densities may result in rapid deterioration of water quality and the swift transfer of disease.

A succession of significant trials has been conducted on intensive rearing of AB, snapper, mulloway and yellowtail kingfish at the PSFI using largely common equipment and operating protocols. These have previously been described by Bardsley and Fielder (pages 49 to 53 of Partridge et al., 2003) in reference to larviculture of snapper. What follows largely comprises a revision of the latter suitably amended and augmented in specific reference to intensive green-water production of AB. Survival of AB larvae from hatch to fully weaned, metamorphosed juveniles is reliable and typically generates yolksac larvae to 20-25 mm juvenile yields of 10-20% survival.

The PSFI hatchery is located at the mouth of an estuary flowing into Port Stephens, approximately 10 km from the ocean. Locally accessible estuarine water can vary in quality (salinity and pH) and pumping protocols generally target high tides to optimize water quality.

Culture set up

All intensive larviculture tanks should be housed in insulated and light-proof controlled temperature rooms isolated from other sections of hatcheries. Fibreglass tanks in the range 500 to 2000 L, (1.0 - 2 m diameter) with a 45° sloping conical bottom of the type illustrated in (Fig 31), are recommended. Sides of the tanks should be dark coloured (black or deep green or blue), to better enable larvae to see their prey against a dark background. Tank bases should be white to allow easy viewing of larvae and to more readily show detritus as it accumulates on the bottom. Individual tanks can be enclosed in a black plastic film shroud supported by a lightweight plastic pipe or timber frame. The latter is particularly useful if separate lighting regimes or asynchronous batch rearing in individual tanks are required.

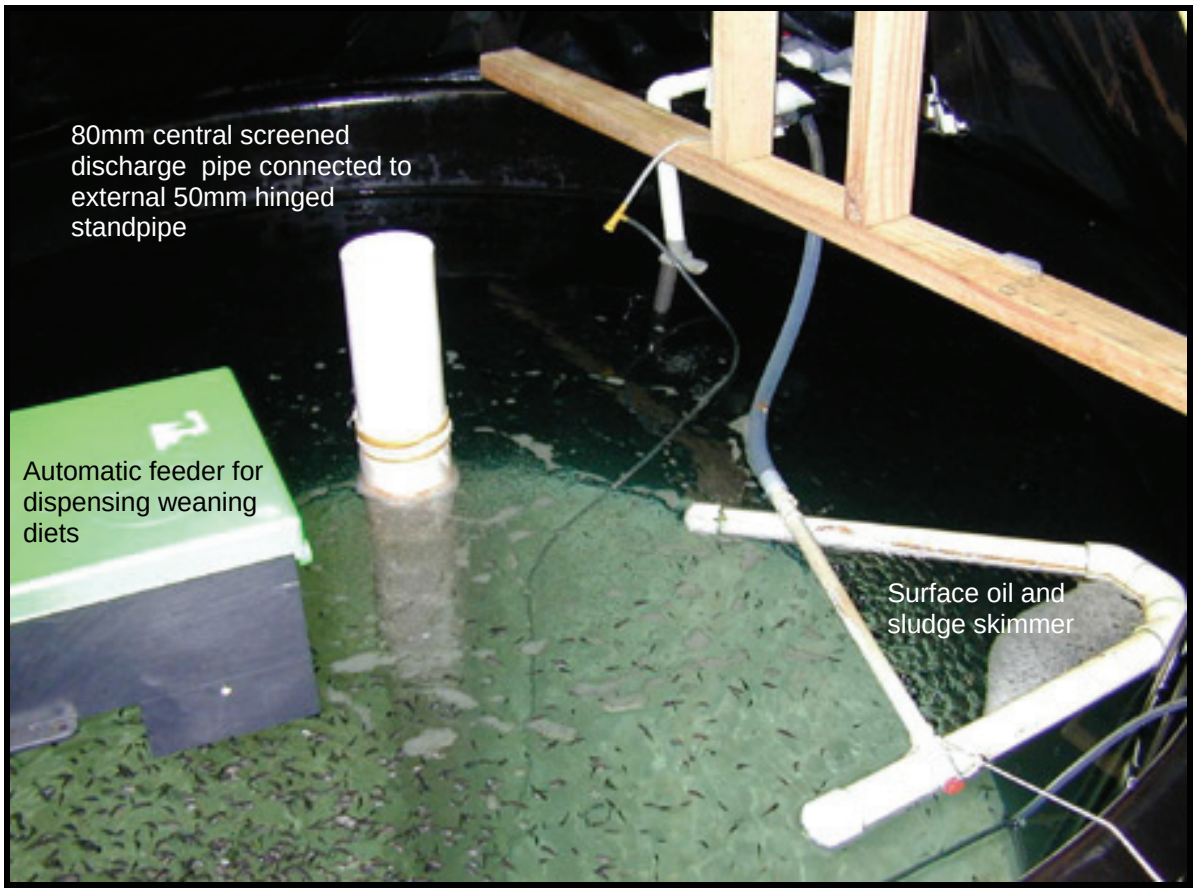


FIGURE 31: 2000 L intensive clear-water finfish hatchery rearing tank at PSFI. Note black sidewall and white conical bottom.

Maintenance of the optimum rearing temperatures of 18-20±1ºC for AB larvae can be achieved cost effectively with a dual (reverse) cycle air-conditioner or a standard (cooling only) air-conditioner in combination with immersion heaters (1.0 kW/m^3 of tank volume). Beyond the initial 5-11 day of total darkness needed to maximise swim bladder inflation, provision of light at an intensity at the water surface of 100 -500 lux will help ensure high visual feeding efficiency by larvae. Such lighting can be created using standard 40W fluorescent globes. Depending on the size of the rearing vessels, 1 or 2 such lights should be suspended about 0.5m above the water surface. Beyond the initial dark phase, a 18: 6h light: dark regime controlled with a timer switch should be imposed. Adequate aeration can be supplied with a single 10 cm air-stone suspended at or slightly above the nadir of the cone shaped floor of the tank. As discussed above, aeration during the initial swim bladder dark phase should be maintained below 70 ml/min/100l of tank volume. The hatchery tank is furnished with a surface air skimmer throughout the larval rearing period as a means of reducing the risks to normal swim bladder inflation and/or occlusion of gills.

A simple way to retain larvae whilst exchanging water and/or removing suspended waste particles and nutrient depleted live food is to provide a central screened 80 mm diameter standpipe (Fig. 31) fitted via a reducer into a 50 mm discharge drain. An initial screen mesh size of 200 µm should be increased to 500 µm then 1200 µm as the size of larvae and their food increase. Tank depth is controlled by an external hinged standpipe, which directs discharged water to a 50-200l bio-filter of the type illustrated in Fig. 30. The bio-filter is stocked with *bio-balls* (Fig. 32) packed into plastic mesh "onion bags" for ease of handling, and is strongly aerated by a bubble-ring at the base of the bio-filter. Multiple air-lifts (4 in the case of the bio-filter illustrated in Fig. 32 provide water circulation, and several air-stones provide added oxygenation. Bio-balls within onion bags are preconditioned prior to incorporation into water reuse systems. Preconditioning to establish a viable flora of nitrifying bacteria is conducted in a separate 500-1000 L seawater filled

tank over periods of 4-6 weeks, and can be promoted by the daily addition of 1-2 g/day of ammonium chloride.

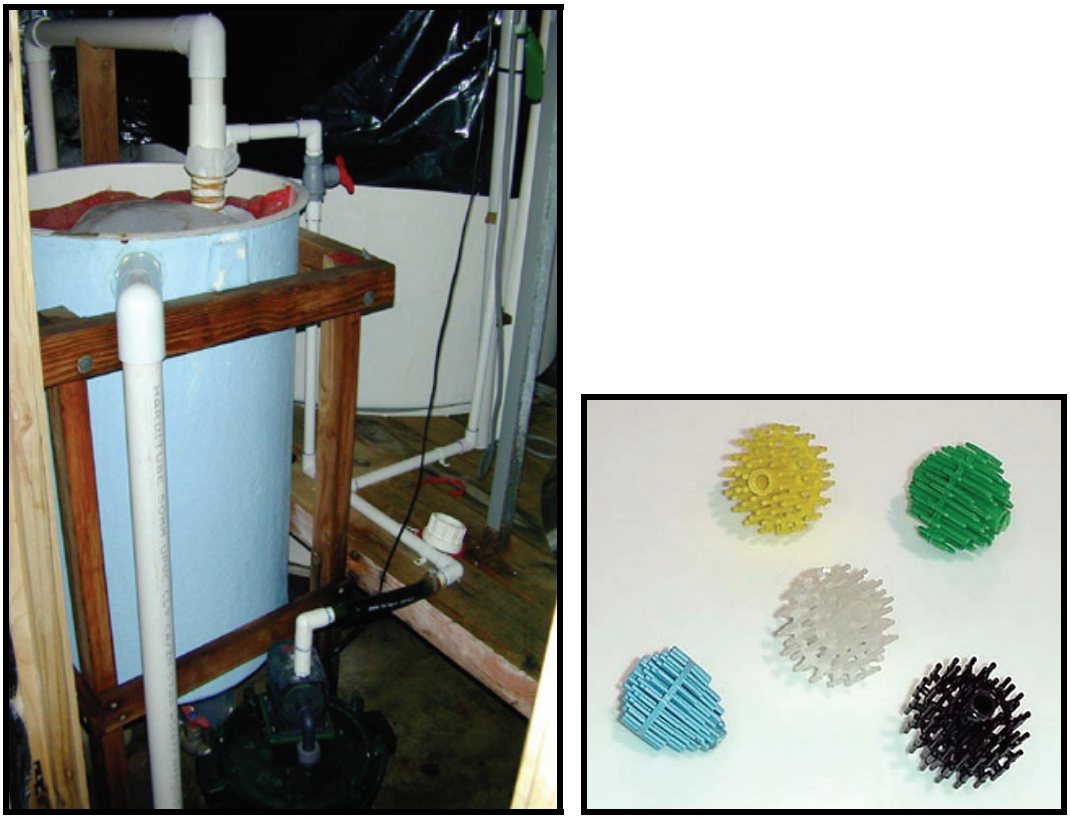


FIGURE 32: External bio-filter packed with onion bags filled with bio-balls.

Recycled 25-35 ‰ seawater is pre-filtered through a 50 µm felted polyester bag secured to the tank overflow en route to the bio-filter. This removes uneaten live food and waste particles thereby reducing the risk of clogging and overloading of the bio-filter. Recycled water can also be passed though a tub filled with glass filter-wool downstream of the bio-filter to further reduce residual particulate waste.

Water should be recycled through larval rearing tanks initially at 3-15 L/min (~100% exchange every 2-3 h). Water re-entering the tank is best passed through a baffle such as an immersed, perforated tube covered with fine mesh (200 µm). This is to reduce strong directional currents and air bubbles from entering and generating turbulence that in turn inhibits larvae from reaching the water surface to inflate their swim-bladders as well as impairing feeding efficiency and reducing the risk of gas bubble disease. Provision of a by-pass on the recirculation pump allows additional water to be looped back into the bio-filter to augment nitrification.

Some water movement is necessary to ensure that larvae and live feeds are evenly distributed through the water column. Inadequate water movement may lead to high density aggregations of live food and/or larvae that in turn may induce localised depletion of dissolved oxygen. Mild water turbulence also mixes food evenly through the water column and prevents formation of pockets of stagnant water "dead spots”.

It should be noted that recirculating aquaculture systems can be difficult to disinfect following a pathological event in the hatchery tank. Biofilms coating the tank components, especially biofilter media, can harbor pathogens including bacteria and viruses, and are hard to remove from the surfaces even with strong oxidizing agents such as chlorine and caustic soda. To reduce risk of disease, PSFI no longer uses recirculating systems for larval rearing of any marine fish species. Instead, a static system is used where water is exchanged each morning by drain-down and top-up of at least 50% volume and the tank is then left static until the following day.

1.7.3 Other water quality management requirements and practices

DO

Adherence to equipment and operating protocols as described above should enable dissolved oxygen to be maintained close to the optimum level of 100% saturation (8 to 8.5mg /L at an optimum rearing temperature/salinity combinations of 18 to 20ºC and 25 to 35 ‰, respectively) and always above a health risk threshold of 5 mg/L. It is however important to maintain necessary dissolved oxygen levels without generating excess turbulence in the larval rearing tank. Adding aeration or compressed pure oxygen directly into the biofilter achieves increased dissolved oxygen levels in the tank, whilst also improving the nitrifying efficiency of the biofilter.

pH

The pH in recirculating systems should be kept as stable as possible at around 8.2. Recirculation systems have a tendency to reduce pH, necessitating buffering with sodium carbonate (Na_2CO_3). To estimate the quantity of buffer to add to the larval rearing tank, it is advisable to conduct a trial with a small amount of water from the rearing tank. The buffer is then added slowly to prevent rapid increase in pH.

Ammonia

Ammonia produced as a waste product of fish metabolism is controlled by the biofilter (converting NH_4+ to much less toxic nitrate, NO_3^-). The total ammonia concentration range usually experienced in PSFI larval rearing tanks (pH 8.0 - 8.2) is 0 – 0.4 mg/L. As already discussed, efficient removal of suspended detritus from the larval rearing tank also helps to prevent total ammonia accumulation. Particles settling out of the water column must be vacuumed from rearing tanks or whenever detritus begins to accumulate, however, vacuuming is a time consuming process. If detritus is fine and evenly spread, it is often useful to gently scrape it down to the central standpipe, where it can accumulate into larger piles and then be siphoned out of the tank. This process must be conducted slowly as it is important not to re-suspend detritus into the water column. The diameter of the siphon hose is dictated by the swimming capabilities of the larvae: stronger, larger larvae will resist the suction of larger diameter hoses. Vacuum hoses of 4 mm and 8 mm are used at PSFI to clean larval rearing tanks. The start of tank vacuuming may vary with larval batch, largely due to differences in stocking densities; however, vacuuming of tanks rarely begins until artificial diet is introduced (Day 30 onwards). Vacuuming is then generally done each day. An 8 mm hose is suitable for use in our tanks when larvae are approximately 10 mm TL. Additional routine maintenance in intensive cultures utilising recirculating systems includes the cleaning of mechanical filters (53 µm bags and glass filter wool), which are generally emptied and cleaned at least twice daily.

Stocking density

Larval rearing tanks can either be stocked directly with newly fertilised eggs or with newly hatched larvae. Egg and larvae stocking densities used at the PSFI have varied over the range 10 - 100 larvae/litre depending on end use and available numbers. It should be noted that the low end of this range, used experimentally, may not be economically viable for commercial hatcheries. If high quality eggs are stocked directly into the larval rearing tanks , hatch and survival rates of larvae to first-feeding at 5 to 6 dah at 18 to 20±1ºC are high (typically ≥80%). However numbers of pre-feeding larvae can be simply and accurately estimated at day 3 while larvae, still being held in the dark, are evenly distributed through the water column. Numbers of surviving larvae can be accurately estimated from mean counts of 10 or more 1 L samples when collected at randomised locations and depths throughout the tank.

1.7.4 Live food and feeding protocols

Van der Wal and Nell, 1986, showed that at the optimum rearing temperature of 20±1ºC, AB larvae complete yolk absorption (endogenous nutrition) 5 to 6 days after hatch and that larvae if unfed, starve to death within 7 days of hatch. Although the "point of no return" for delayed feeding of AB larvae after yolk reserves have been exhausted has not been determined, common sense suggests that no delay is the best strategy for maximising survival and hence ensuring reliable hatchery production. Australian bass larvae can be transferred to fresh water 4 weeks after the completion of yolk absorption, when metamorphosis has taken place (van der Wal, unpublished data, 1984). Accordingly the need for saltwater food organisms exists only during the first 4 weeks of feeding. Van der Wal and Nell, 1986 also showed that exogenous feeding requirements of AB larvae can be fully satisfied with diets of rotifers during the first 2 weeks and with A*rtemia* nauplii during the subsequent 2 weeks respectively, with overall post larval yields of up to 65%.

1.7.5 Rotifer feeding phase

Readers are directed to pages 77 to 84, **Chapter 8, Partridge et al., 2003** for comprehensive instruction on rotifer and rotifer enrichment equipment and operating protocols. Rotifers are offered to AB larvae from first feeding (completion of yolk absorption) 5 - 6 dah for 14 days through to 19-20 dah. An optimum rotifer feeding rate of 9 rotifers /ml (Fig. 33) determined by Van der Wal and Nell (1986) is used to feed AB larvae over the first 2 weeks of exogenous feeding.

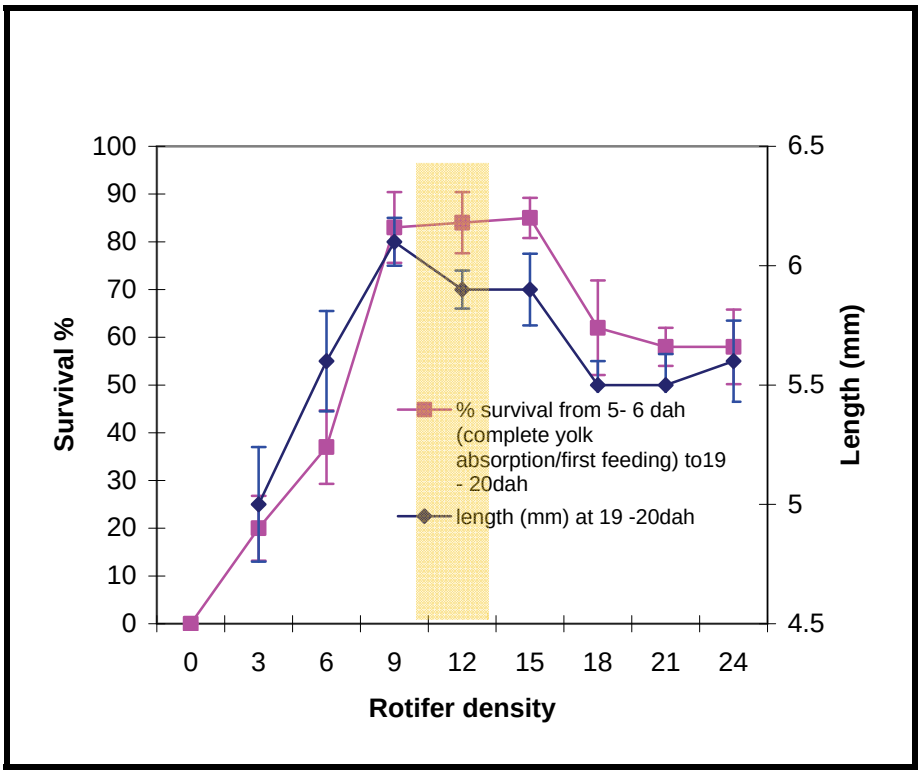


FIGURE 33: Effect of large strain rotifer (*Brachionis plicatilis*) concentration on growth and survival of AB larvae from first feeding/complete yolk absorption on 5-6 dah to day19-20 dah when reared at optimum salinity (28 -34g/kg) and temperature (20 ±1 ºC). Shaded area is recommended feeding concentration of 9 rotifers/ml. (Source: derived from data presented in Table 1, Van der Wal and Nell, 1986)

A large species of rotifer, *Brachionus plicatilis* (L-type) and a small species, *B. rotundiformis* (S-type) have both been routinely propagated at PSFI to feed early stage marine finfish larvae. However on the basis of the results of Van der Wal and Nell (1986) (Fig. 33) that were achieved using L – type rotifers and findings of Bardsley et al. (1998), that L-type rotifers at 10/mL support significantly better growth and survival of snapper larvae than do equivalent densities of S-type rotifers or blends of the two types of rotifer, AB larvae are usually only fed L-type rotifers. In the event that only S-type rotifers are available, feeding densities will be increased to 20/mL, to achieve satisfactory survival and growth of larvae AB.

The number of L –type rotifers required to feed a tank of larvae each day is calculated from the tank volume and the target feeding density of rotifers. Rotifers are nutritionally enriched overnight by feeding the microalgae Tahitian *Isochrysis galbana* and *Pavlova lutheri* as well as Algamac 3050 (Bio-Marine Inc, California, USA) before being stocked into intensive hatchery tanks at a density of 9-10 rotifers/ml. Attempts are made to maintain this density of rotifers throughout the day and for the entire 14 day period that rotifers are fed. As larval feeding and water filtration progressively reduce the concentration of rotifers during the day, it is necessary to feed rotifers 2-4 times a day to maintain prey density at 9-10 rotifers/mL. Typically, rotifer density in larval rearing tanks is assessed twice daily by counting residual rotifers in two, 1 mL samples taken from random points throughout the tank.

To calculate the number of "top up" rotifers required, the target density of 10 rotifers/mL is multiplied by the tank volume. For example, the total number of rotifers required to provide an initial morning feed of 10 rotifers/mL, and an afternoon top up of 5 rotifers/ml for a 2000 L tank is:

- 15 (rotifer/mL) x 2,000,000 (mL) = 30,000,000 rotifers

Rotifers lose their nutritional value following enrichment (within 6 h at 30°C; Fielder et al., 2000). Consequently, it is beneficial to minimise the numbers of residual nutritionally depleted rotifers prior to the addition of newly enriched rotifers at the start of each day. Recirculation rates can be increased overnight (coincident with cessation of feeding) thereby entrapping and removing nutritionally depleted rotifers in physical filters.

Rotifer availability can limit hatchery production as it is not always possible to match fluctuating feeding demands with supply. Decisions on how to allocate restricted amounts of rotifers are largely dependent on the development stage of the finfish larvae. As young AB larvae need high prey densities to effectively feed, the most sensible allocation is to supply a large ration of rotifers in the morning followed by a small supplementary ration later in the day. By contrast for more advanced larvae with well developed vision and predatory skills, limited rotifers are best fed as two equal rations.

Where adequate rotifer densities cannot be maintained, it is better to sacrifice a tank/s to enable retained larvae to be fed at rates that ensure good continuous growth and health. Alternatively, if tanks have been conservatively stocked, a practical option may be to combine several tanks of larvae. This increases the density of larvae, but reduces overall water volume and therefore the total numbers of rotifers needed to maintain acceptable feed density. Lowering the volume of water in an individual tank has the same effect. However it is cautioned that such options should only be considered if larval rearing systems are capable of maintaining water quality at increased stocking densities or reduced volumes. Improved use of limited rotifers can also be achieved by recapturing rotifers from tank discharge water. Such recycled rotifers must however be concentrated, rinsed and re-enriched before being restocked into the larval tanks.

1.7.6 Artemia feeding phase

Van der Wal and Nell, (1986) determined that the optimum *Artemia* nauplii feeding density of 6 *Artemia* nauplii /mL for AB larvae over the 3rd and 4th weeks of exogenous feeding. This regime supported mean ± sd survival rates of 78±2.6% and growth to a mean ± sd length of 10.8±0.17 mm at 33 dah (Fig. 34).

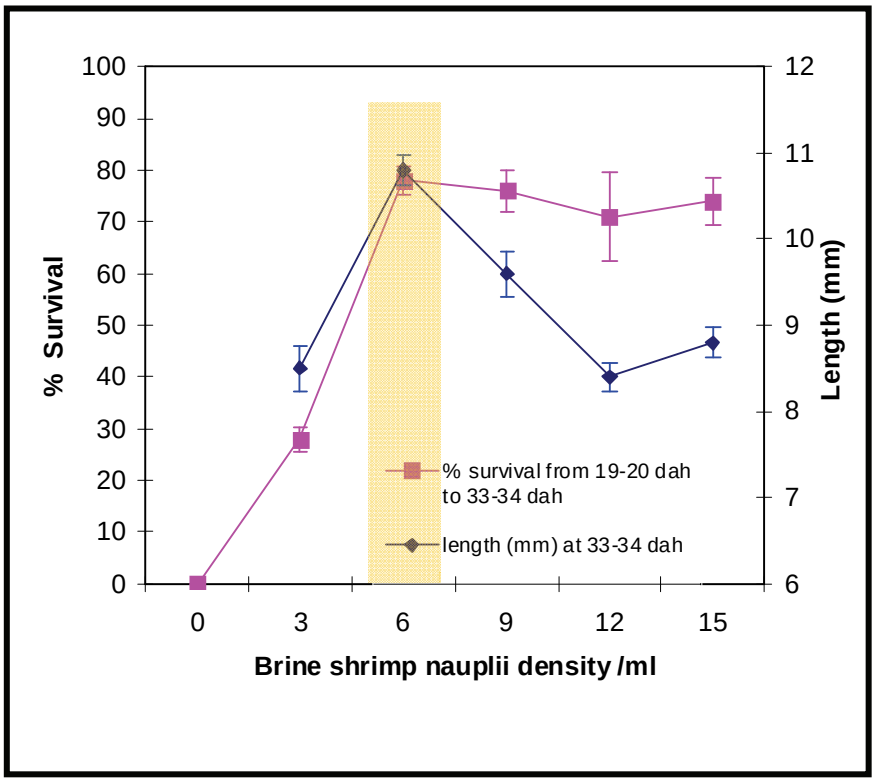


FIGURE 34: Effect of *Artemia* nauplii concentration on growth and survival of AB larvae over a 14 day period from 19 dah to day 33 dah when reared at optimum salinity (28 -34g/kg) and temperature (20 ±1 ºC). Shaded area is recommended feeding concentration of 6 *Artemia* nauplii /ml. (Source: derived from data presented in Table 3, Van der Wal and Nell, 1986).

Feeding newly hatched *Artremia* nauplii has nevertheless resulted in significant mortality of pre-metamorphoric marine finfish larvae in Australia and abroad and may partially account for low and inconsistent yields of AB fingerlings experienced in the past especially prior to 1990. It is also possible that early feeding stage AB larvae have difficulty digesting and assimilating lipids in newly hatched brine shrimp nauplii, but more probable that the major problem is EFA deficiencies associated with *Artremia* nauplii. Such nutrient deficiency problems, can be simply averted by either:

- Replacing newly hatched *Artemia* nauplii with enriched meta-nauplii
- Entirely replacing *Artemia* by extended feeding with enriched rotifers in combination with either a mixed zooplankton diet produced in fertilised outdoor ponds

Currently the standard *Artemia* feeding regime employed at PSFI for intensive hatchery production of AB larvae involves production and use of enriched *Artemia* meta-nauplii. *Artemia* meta-nauplii are enriched for 12 h with microalgae and Algamac 3050® (see detailed procedures Chapter 8 of Partridge et al., 2003) before feeding to larvae, in conjunction with rotifer feeds, at a starting density of approximately 0.1 *Artemia*/ml (fed 4 times each day). *Artemia* density increases as the larvae grow, with a peak feeding rate of 0.5 *Artemia* /ml (fed 4 times each day). This feeding phase commences from the end of the rotifer feeding phase at a length of 6.0 - 6.5mm (21 dah) and continues until fish are ready to be released into waterways and

impoundments as 20-25 mm fully metamorphosed fingerlings. The optimal rearing parameters and feeding schedule for mulloway larval rearing used at PSFI are shown in Table 2.

TABLE 2: The optimal rearing parameters and feeding schedule for Australian bass larval rearing used at PSFI.

Species: Australian Bass (*Macquaria novemaculeata*)			
Parameter	**Target**	**Dah**	**Adjustment**
pH	7.6 - 8.2	0+	
Dissolved Oxygen (mg/l)	>6.00	0+	Use compressed oxygen diffuser to maintain saturation level
Temperature (°C)	18 - 20	0+	Increase post SB inflation
Salinity (ppt)	15 - 35	0+	
Water Exchange (%/day)	100 - 200	0+	Increase exchange as larvae develop
Surface Skimmer (hrs/day)	24	4+	Monitor skimmer to ensure larvae at water surface are not affected
Photoperiod (L:D)	(12:12) (18:06)	(0+) (6+)	Increase post SB inflation
Light intensity (Lux)	85-225	0+	Larvae are very sensitive to high light at first feeding and SB inflation
Green-water (cells/ml)	1.4 x 10^6	0+	Pro-Aqua* concentrate 57x10^9 per ml
Rotifer (R/ml)	20.0- 5.0	4+	Initial 20 until feeding and then increase frequency of reduced concentration (e.g. 4x5/ml/d).
***Artemia* (A/ml)**	0.2 - 2.0	12+	0.2 until weaned, then increase concentration and frequency
Weaning Diet size (µm)			No weaning onto formulated pellet diet if fish are to be used for stock enhancement

*Algae concentrate used Rotifer Diet-3600 (*Nannocholoropsis/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 ≈100,000 lux ≈ 10,000 foot candle ≈ 500 µmol/m2/sec (microeinsteins/square metre/second)

1.8 Extensive Outdoor Pond Culture

Some hatcheries favour production of AB fingerlings in extensive outdoor ponds. This is because fingerlings, generally cost less, grow faster, and are more vigorous and better able to survive the transition to dams and impoundments than those reared intensively (Rutledge et al., 1990; Rutledge & Rimmer, 1991). Outdoor ponds that commonly range from 250 to 2500 square metre surface area are best constructed on self sealing clay or clay loam soils. If not, they need to be sealed with either heavy duty plastic or rubber liners. Between 2 and 4 weeks prior to stocking, ponds are filled with marine or estuarine water in the range of 25 – 35‰, pre-filtered through 200 micron mesh screens or bags to exclude juvenile and adult stages of potential predators and competitors. After 3 – 4 weeks salinity is progressively reduced to 3 - 5‰.

Ponds are fertilised to encourage successive blooms of microalgae and zooplankton (Fig. 35) on which the larval bass feed and may also be inoculated with water from other ponds carrying diverse and rich zooplankton to better insure successful preparation. They are fertilized to promote algal and zooplankton growth. Some hatcheries choose to stock ponds with first feeding (5 - 6 dah) AB larvae at a rate of approx 1000/m^2 but at the cost of yields in the highly variable range of 1 – 20%. Offsetting these variable yields, is that surviving stock are better able to survive the trauma of transport and release into private dams and impoundments. *(*AQUABLUE website: http://www.aquablueseafoods.com.au)

Research and Development at the PSFI has improved methods for large-scale extensive pond production of AB as well as of mulloway (see Chapter 2) and snapper. However pond temperatures as low as 9 ºC and poor zooplankton food levels in ponds at the PSFI during the winter breeding season of AB prompted the relocation in 1988 of extensive pond hatchery trials to a private prawn and native fish farm at Palmers Island, Yamba , 400 km further north. In an initial trial, newly hatched AB yolk-sac larvae seeded into two 250 m^2 prawn nursery ponds all died. By contrast, seeding of 21 dah AB larvae into two other ponds yielded advanced 13 to 16 mm larvae at survival rates estimated at 3 to 5% and 12 to 15%, respectively. This was at salinities of 8-12 % and in spite of high pH fluctuations of 8.5-10.2. (Battaglene and Allan, 1990).

Spurred on by these encouraging results, an additional 20 *ad hoc* pond trials were undertaken at the same farm during the subsequent 1989 and 1990 winters. Results summarised in Table 3 showed that simple addition of *Artemia cysts* at the rate of 4 kg/ha every 2 to 3 days to supplement pond zooplankton food, raised mean yield of 20 mm fully metamorphosed fingerlings from < 1% to more than 12%. Moreover, when A*rtemia* supplementation was combined with green-housing of ponds using simple plastic film covers, mean yield of fingerlings was further quadrupled to more than 50% and turn-off time halved from 88-118 down to 48-55 days.

TABLE 3: Summary of results of 20 ad hoc extensive pond hatchery rearing trials with AB conducted in 250 m^2 earthen ponds at a prawn farm on Palmers Island, Northern NSW. (Source: Battaglene and Allan, 1990).

Treatment	**Total number of ponds stocked**	**± Plastic thermal covers i.e green-housed or ambient**	**Temperature range**	**Artemia supplements** (100g cysts/pond = 4kg/ha, every 2-3 days)	**Mean ± sd yield of 20mm, fully metamorphosed fingerlings**	**Growth rate** (mm/day)	**Time to full metamorphosis**
1	4	Ambient	12 - 23 ºC	-	<1%	NR	NR
2	8	Ambient	12 - 23 ºC	+	12.3 ± 8.8%	0.24 - 0.33	88 - 118 days
3	8	Green-housed	18 - 23 ºC	+	50.8 ± 23.5%	0.37 - 0.48	48 - 55 days

Common factors across trials					
Pond dimensions length x width x depth	AB larval stocking rate	Age of AB larvae at seeding	Salinity range	DO range	pH range
16x16x1m (250m^2)	100/m^2 (= 1 million/ha)	10 - 20 dah	11 - 19‰	5 - 14 mg/L	6.7 - 9.4

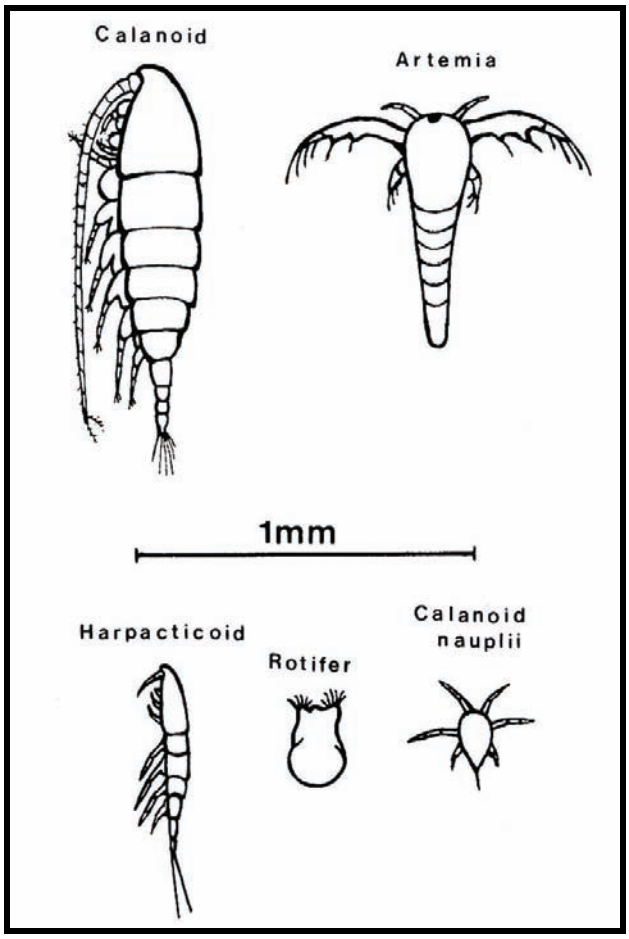


FIGURE 35: Left: Relative sizes and dominant categories of food items eaten by AB larvae in earthen prawn nursery ponds. (Source: Battaglene Talbot and Allan, 1992). Right: Photo of green- housed ponds at PSFI.

These combined green-housing and *Artemia* supplementation techniques were progressively refined back at the PSFI between 1996 and 2006 with the commissioning of a new experimental outdoor pond system that including four perched, fully drainable, aerated green-housed ponds. Standard extensive pond production protocols for producing advanced 20-25 mm AB fingerlings progressively developed between 1996 and 2006 are as follows.

1.8.1 Pond design, preparation and management

Extensive rearing was undertaken in plastic lined 350,000 L ponds (38 x 10.5 m, with filled depth of 1.4 m at the deeper end). Ponds are battered at a gradient of around 3:1 and a fall of 0.3 m over their length allowed ponds to drain into a concrete sump with a volume of 2.15 x 0.92 x 0.3 m. Effluent water is drained from a harvest sump via a 150 mm diameter pipe. Pond depth and water exchange rates are controlled using an external standpipe, A 1.67 x 0.9 x 0.95 m frame fitted with sandfly netting (1mm mesh) located in the harvest sump prevents the escape of larvae during flushing and draining operations. Influent estuarine water is screened through a 0.5 mm mesh bag to remove potential predators.

The ponds are covered by twin-domed polyhouse structures. To allow some thermal regulation, the greenhouses are elevated 1.45 m above the pond batters and the side walls enclosed with polyhouse curtains capable of being winched up or down to allow air ventilation. This capacity is used to help stabilise water temperatures, sides being lowered on cooler days to trap sun heated air, and elevated on warmer days to expel solar heated air. Comparative water temperature data for green-housed and non green-housed ponds through a typical winter production cycle in Fig. 36 illustrate the great benefits of green-housing in relation to AB larvae and fingerlings that have optimal mean ± range rearing temperatures of 18-20±2 ºC.

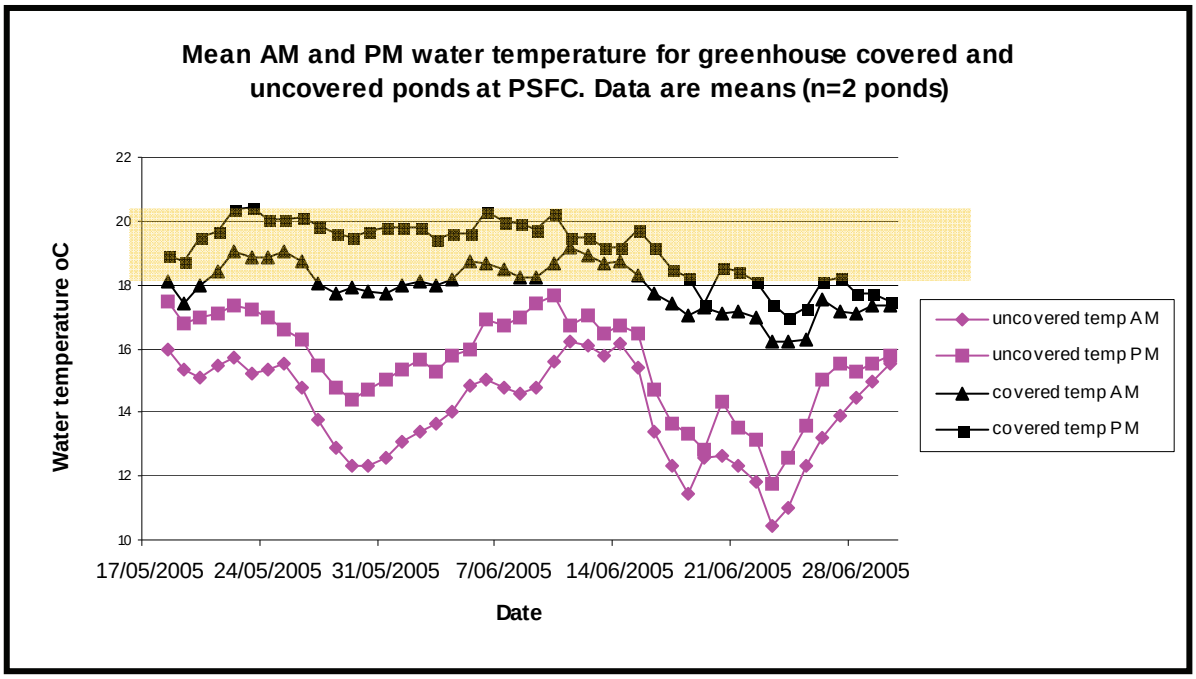


FIGURE 36: Typical effects of green-housing on 350 m^2 PSFI outdoor extensive fingerling production ponds. Shaded area is optimum rearing temperature for AB larvae. (Source: Fielder and Allan, 2008b)

An additional three 500,000-L plastic lined ponds are used for supplementary live food production. To do this the ponds are filled with 0.5 mm filtered estuarine seawater and fertilised with organic and inorganic fertilisers to promote phyto- and zooplankton blooms. Both fingerling and supplementary live food ponds are fertilised using both organic (lucerne chaff and dynamic lifter) and inorganic *(Liquifert P, Liquifert N)* fertilisers. The initial dose of organic fertiliser comprises pesticide free lucerne chaff at 300 L/ha. Thereafter, organic fertiliser is dosed three times weekly, alternating between *Dynamic Lifter* (a poultry manure, blood meal and bone meal based product) at 60 kg/ha and lucerne chaff at 300 L/ha. The initial dose of inorganic fertiliser consists of a mix of *Liquifert N*, calculated to provide 1 ppm of nitrogen, and *Liquifert P*, to provide 0.25 ppm of phosphorous. Thereafter, inorganic fertilisation is administered (typically weekly) as quarter doses of the above, as dictated by phytoplankton dynamics.

On the day of stocking, one third of a companion 500,000-L food production pond at the PSFI is pumped into each of the 350,000-L larval rearing ponds to be stocked. Temperature, dissolved oxygen, pH, and salinity are monitored twice daily. Zooplankton densities are monitored by siphoning 150 L of pond water through a 53 µm box screen. Concentrated plankton is harvested into a 1-L beaker and 1 mL aliquot counts used to infer total plankton densities.

Development of the phytoplankton, zooplankton succession to a status ready to support feeding of AB larvae of optimal age and size for seeding into the ponds (21 dah; TL 6.5±0.6 mm capable of feeding on zooplankton up to 1 mm) is around 21 days after filling and fertilising. AB Larvae are concentrated in the hatchery tank by partial draining and transferred by bucket into a 150 L tank which is filled to a volume of 50-L. Larvae are then homogenised in the water column by hand-stirring, and the mean count within three 1 L aliquots used to estimate the total number of larvae. The tank is next covered with black plastic to exclude light and moved to an area close to the ponds. Larvae are next moved to a 250 L tank filled with water from the hatchery tank. A submersible pump is then used to add pond water for 1.5 hours. Finally the larvae are wet drain harvested into a 53 µm mesh lined box screen for stocking into the out door green-housed ponds.

Rotifers and copepods comprise the predominant prey items of marine finfish larvae (Fielder and Allan 2008a). The latency period of 21days before stocking of the AB larvae allows pre warming of green housed ponds to optimum temperature of 18-20ºC and initial proliferation of rotifers (Fig. 37) and copepods to densities of 5-10/mL and ≥ 0.2/mL, respectively appropriate to support satiation feeding of the larvae. Continued exponential increase in rotifer and copepod dominated zooplankton over subsequent weeks (Fig. 38) is in keeping with feeding demands of AB and other marine finfish larvae such as snapper (Fig. 39) that also exhibit continued exponential growth over this period that is further enhanced under more favourable temperature regime in green-housed ponds.

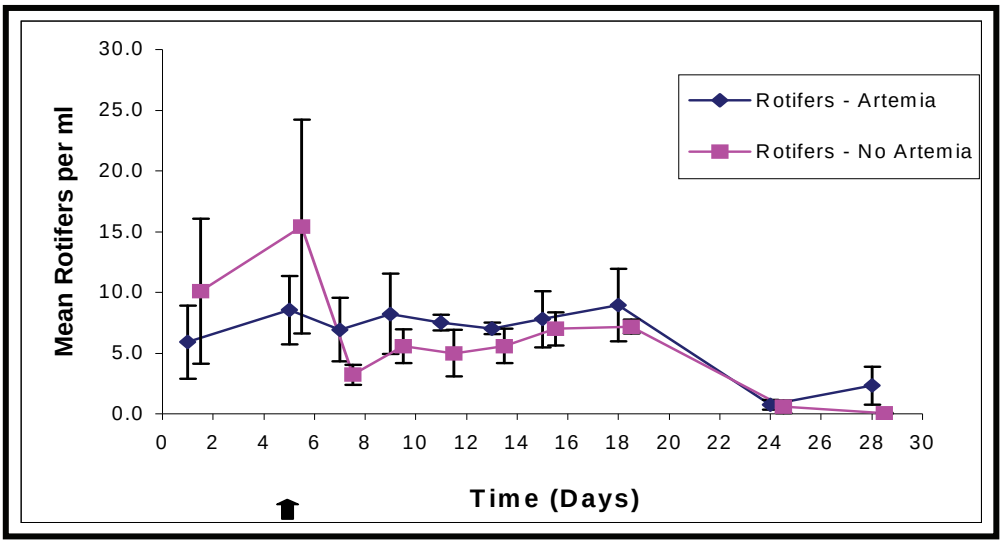


FIGURE 37: Mean number of rotifers in green housed ponds at the PSFI. Time is duration after filling. Data are means ± S.D (*n*=3 ponds). (Source: Fielder and Allan, 2008b).

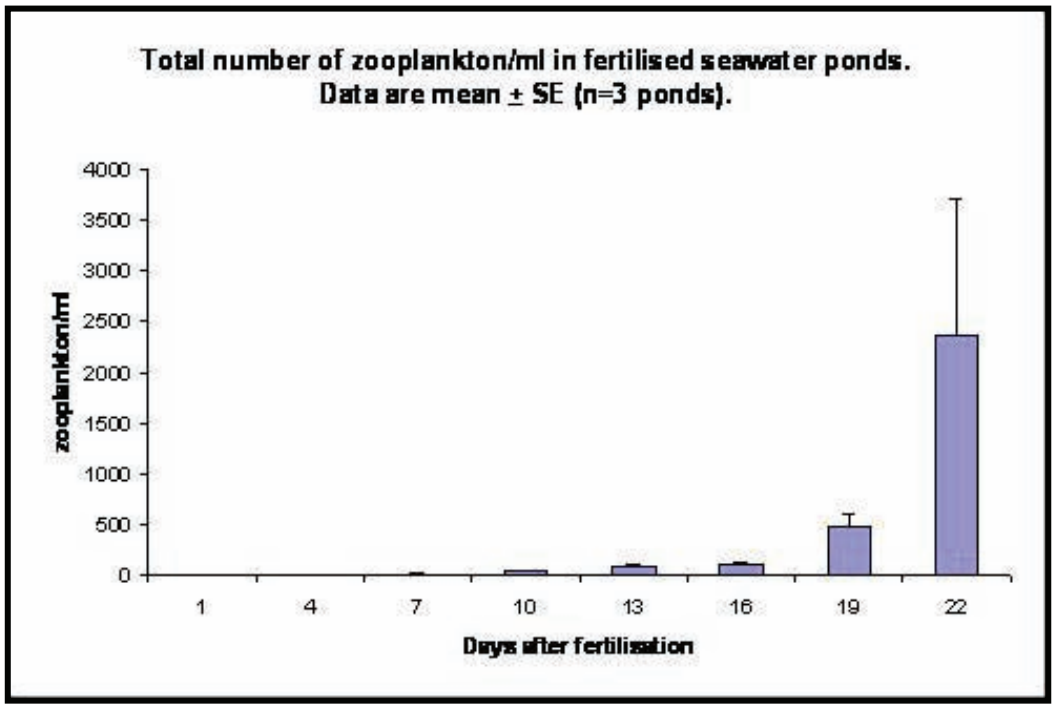


FIGURE 38: Total density of zooplankton density in plastic-lined ponds filled with seawater and fertilized. (Source: Fielder and Allan, 2008b).

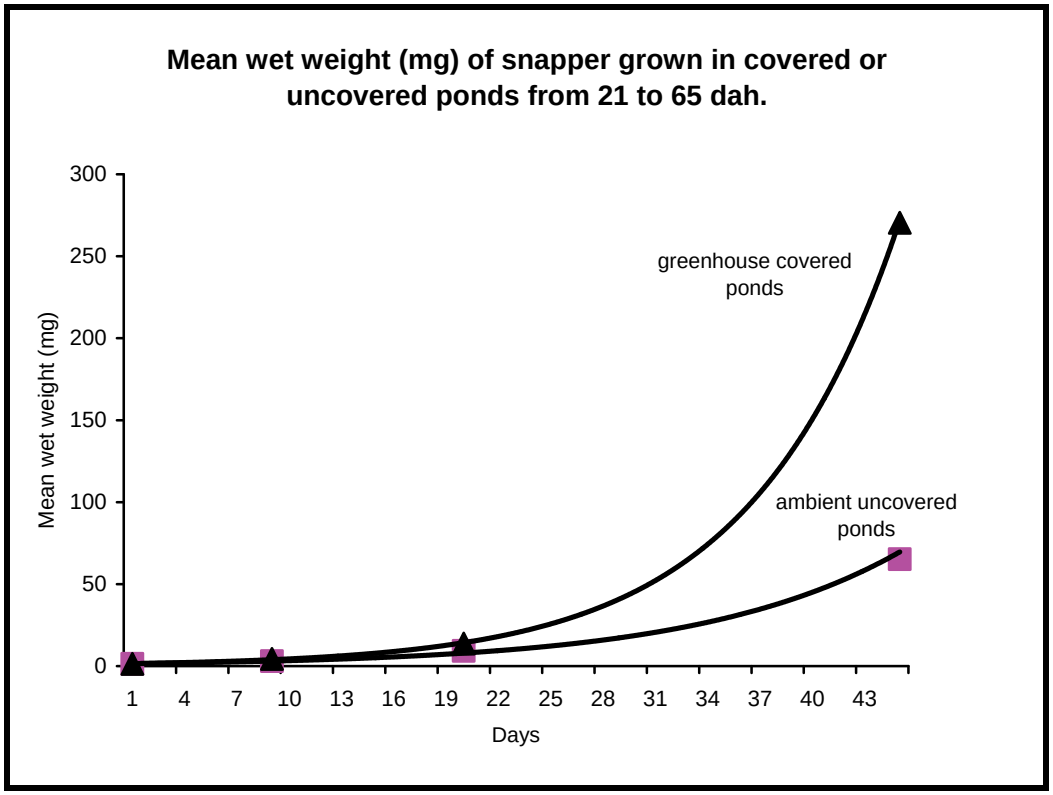


FIGURE 39: Mean wet weight (mg) of snapper grown in covered or uncovered ponds at the PSFI from 21 to 65 dah. (Source: Fielder and Allan, 2008b).

1.9 Summary of “best-practice” Rearing Regimes for Australian Bass.

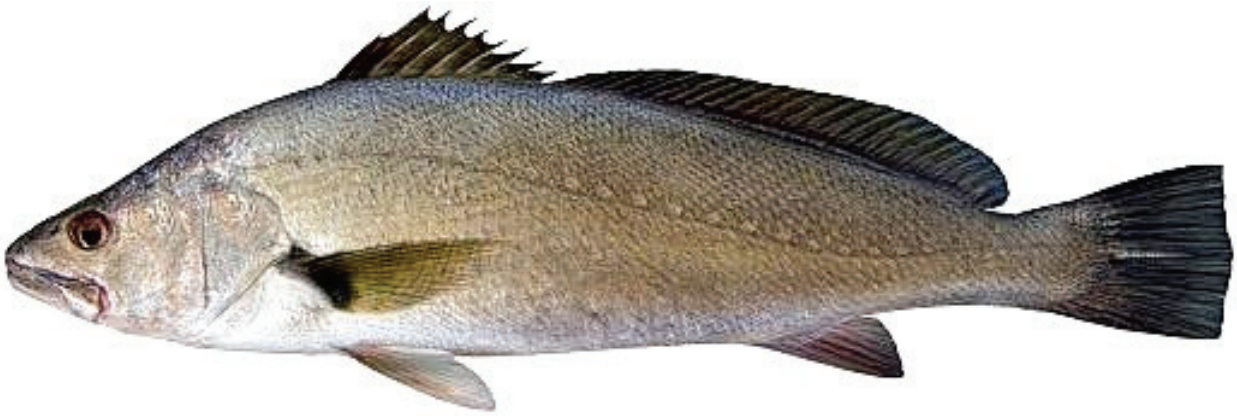
Best-practice rearing protocols for AB larvae are summarized in Table 4.

TABLE 4: The "best-practice" regime for Australian bass larval rearing used at PSFI.

SPECIES: AUSTRALIAN BASS (*MACQUARIA NOVEMACULEATA*)			
BREEDING & DEVELOPMENT	**UNIT**		**COMMENT**
BROODSTOCK ORIGIN	WILD-CAUGHT		EASTERN DRAINAGE ESTUARIES/FRESHWATER RIVERS
BROODSTOCK TANK SIZE	4000-22,000 L		
SPAWNING INDUCTION	HCG		500 IU/KG
TANK SIZE FOR SPAWNING	500-1000 L		1 FEMALE WITH UP TO 3 MALES
LATENCY PERIOD TO SPAWNING	34 H		POST-HORMONE INJECTION AT 18^{O}C
METHOD OF FERTILISATION	SPONTANEOUS SPAWNING OR HAND-STRIPPING		HAND-STRIPPING OF MILT AND OOCYTES NECESSARY IF FISH FAIL TO SPAWN SPONTANEOUSLY
EGG INCUBATION TANK SIZE	500-1000 L		
TIME TO HATCH	40-50 H		AT 18 ^{O}C
LARVAE TANK SIZE	2000-10,000 L		INTENSIVE GREENWATER CULTURE
	0.05 - 1 HA		EXTENSIVE, FERTILISED POND CULTURE
LARVAL YOLK-SAC PRESENT	0 – 5/6 DAH		AT 20±1 ^{O}C
LARVAL FIRST-FEEDING	5-6 DAH		AT 20±1 ^{O}C
LARVAL SWIMBLADDER INFLATION	4-12 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			NOT OBSERVED TO OCCUR
WATER QUALITY PARAMETER	**TARGET**	**DAH**	**ADJUSTMENT**
PH	7.6 - 8.2	0+	
DISSOLVED OXYGEN (MG/L)	>6.00	0+	USE COMPRESSED OXYGEN DIFFUSER TO MAINTAIN SATURATION LEVEL
TEMPERATURE (°C)	18 - 20	0+	INCREASE POST SB INFLATION
SALINITY (PPT)	15 - 35	0+	CAN TOLERATE FRESHWATER AT 7 DAH
WATER EXCHANGE (%/DAY)	100 - 200	0+	INCREASE EXCHANGE AS LARVAE DEVELOP
SURFACE SKIMMER (HRS/DAY)	24	4+	MONITOR SKIMMER TO ENSURE LARVAE AT WATER SURFACE ARE NOT AFFECTED
PHOTOPERIOD (L:D)	(12:12) (18:06)	(0+) (6+)	INCREASE POST SB INFLATION
LIGHT INTENSITY (LUX)	85-225	0+	LARVAE ARE VERY SENSITIVE TO HIGH LIGHT AT FIRST FEEDING AND SB INFLATION
GREEN-WATER (CELLS/ML)	1.4 x 10^{6}	0+	PRO-AQUA* CONCENTRATE 57X10^{9} PER ML
LARVAL FEEDING SCHEDULE	**TARGET**	**DAH**	**ADJUSTMENT**
ROTIFER (R/ML)	20.0- 5.0	4+	INITIAL 20 UNTIL FEEDING AND THEN INCREASE FREQUENCY OF REDUCED CONCENTRATION (E.G. 4X5/ML/D).
***ARTEMIA* (A/ML)**	0.2 - 2.0	12+	0.2 UNTIL WEANED FROM ROTIFERS, THEN INCREASE CONCENTRATION AND FREQUENCY
WEANING DIET SIZE (µM)			NO WEANING ONTO FORMULATED PELLET DIET IF FISH ARE TO BE USED FOR STOCK ENHANCEMENT

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

2. MULLOWAY



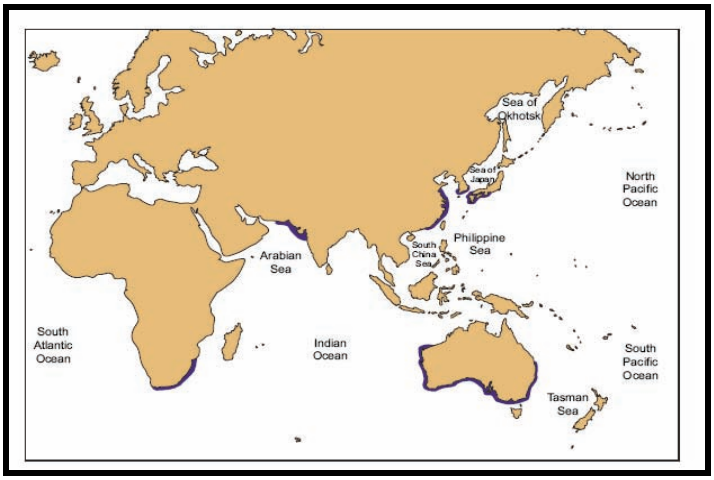


FIGURE 40: Global distribution of mulloway (Source: Silberschneider and Gray, 2005)

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 seaboards 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.

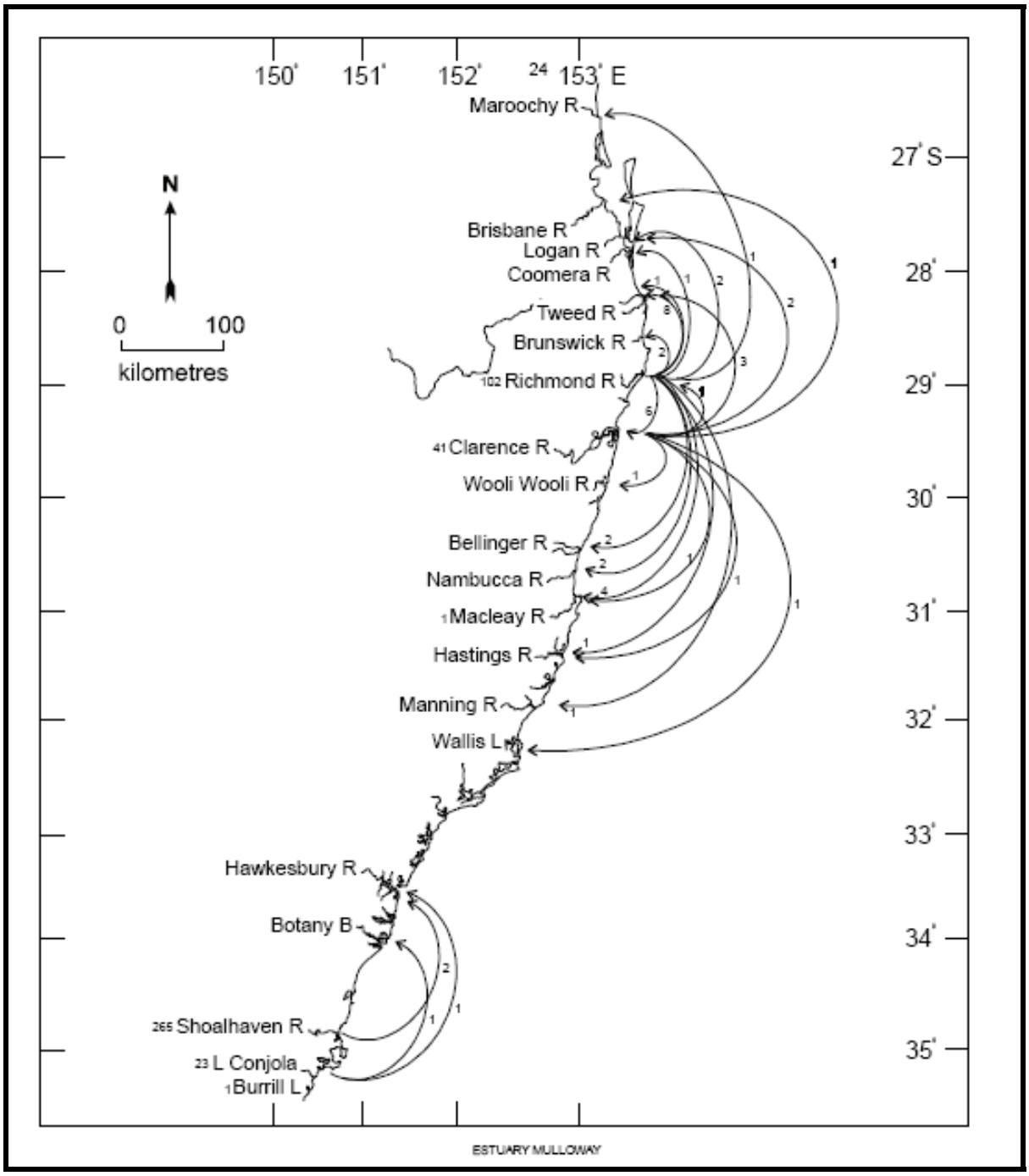


FIGURE 41: Map showing locations of release and recapture of tagged mulloway in New South Wales, (Source: Silberschneider & Gray, 2005)

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.

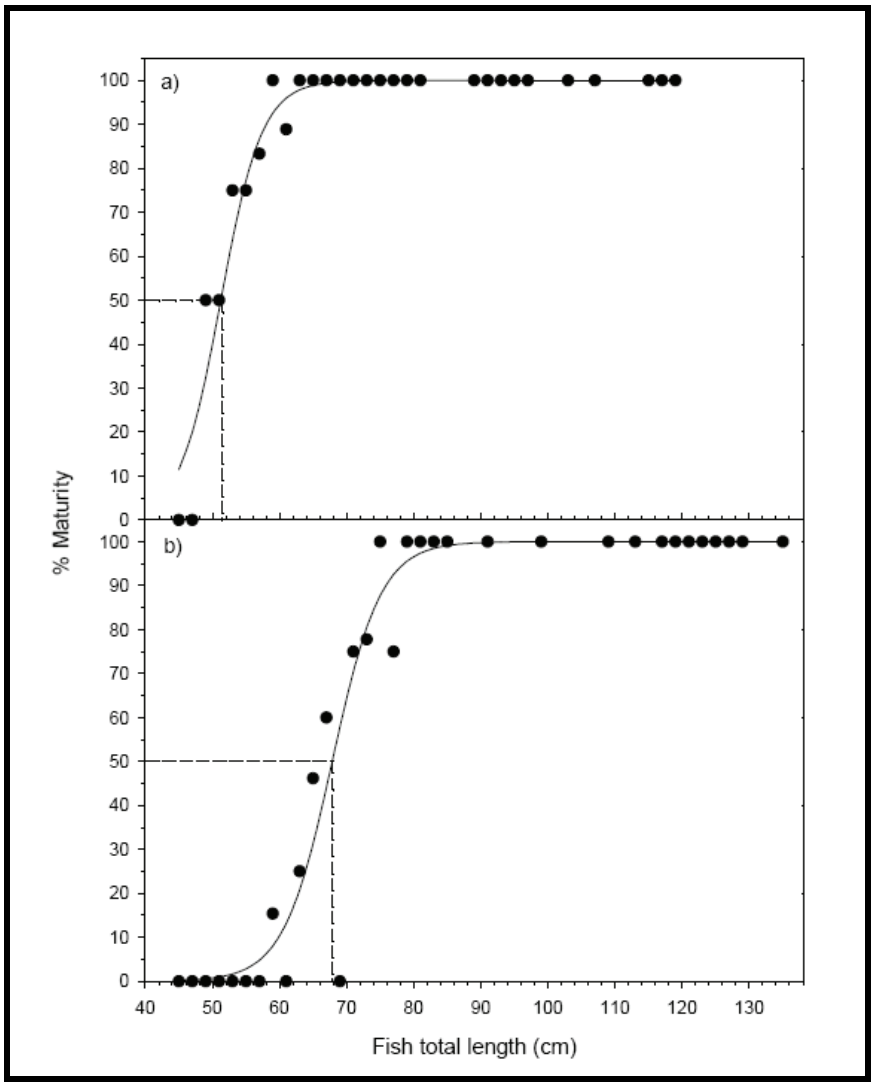


FIGURE 42: Size and sexual maturity of mulloway from New South Wales (Source: Silberschneider and Gray, 2005).

As with the size and age at maturity, the spawning season of mulloway 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, mulloway 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 mulloway 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 mulloway 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 mulloway are pelagic, approximately 0.938 ± 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, mulloway 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 mulloway 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. Mulloway 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 mulloway 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 mulloway (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, mulloway 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 mulloway 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 mulloway 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

Mulloway 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 mulloway. Crustaceans accounted for between 14 and 81% of the reported diet of juveniles. The importance of crustaceans in the diet of mulloway appears to decrease with increasing fish size, resulting in fish and squid being the prey of greater relative importance in larger mulloway.

2.4 Growth, Longevity and Mortality

Growth of mulloway varies greatly between different geographic regions. In South Africa, Mulloway 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 mulloway in Australia show that juvenile Mulloway 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.

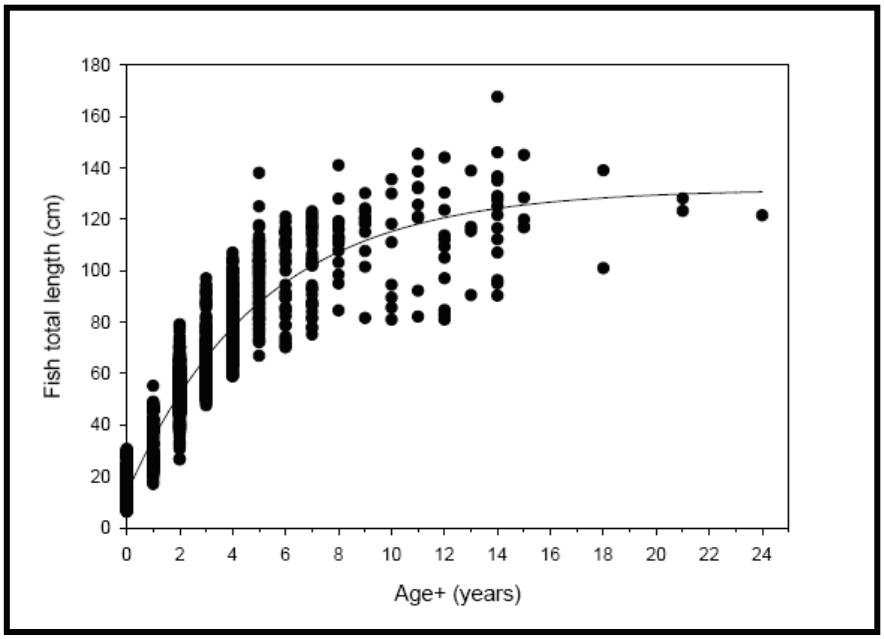


FIGURE 43: Length data with fitted growth curve for mulloway in New South Wales Lengths are presented as total length (TL). (Source: Silberschneider and Gray, 2005).

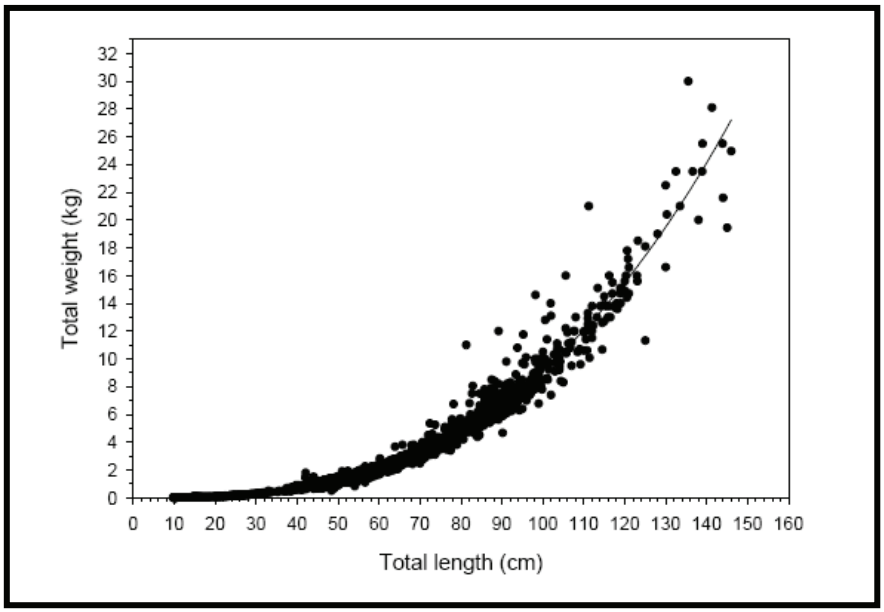


FIGURE 44: Length-weight relationship of mulloway sampled in NSW (n = 2865). (Source: Silberschneider and Gray, 2005).

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.

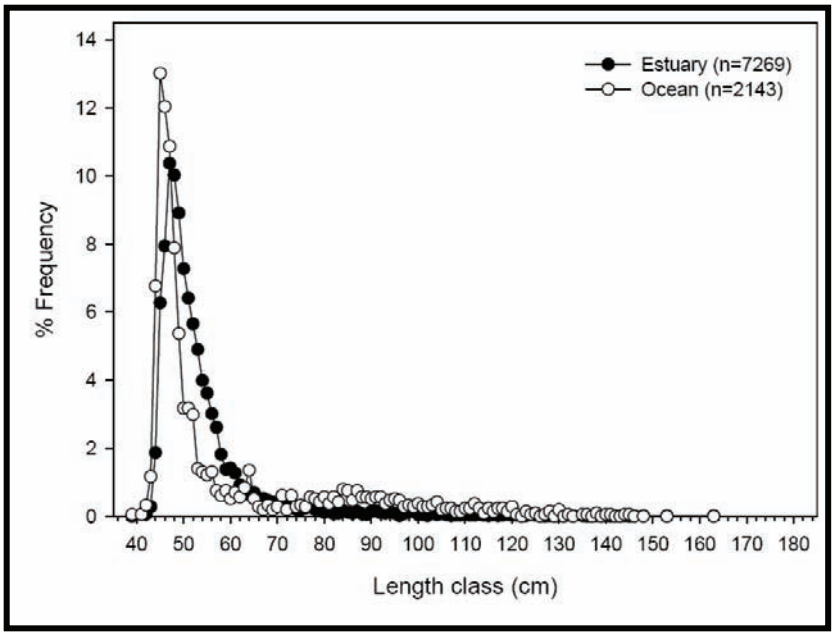


FIGURE 45: Length composition of sampled estuarine and ocean commercial catches of mulloway (pooled across regions). (Source: Silberschneider & Gray, 2005)

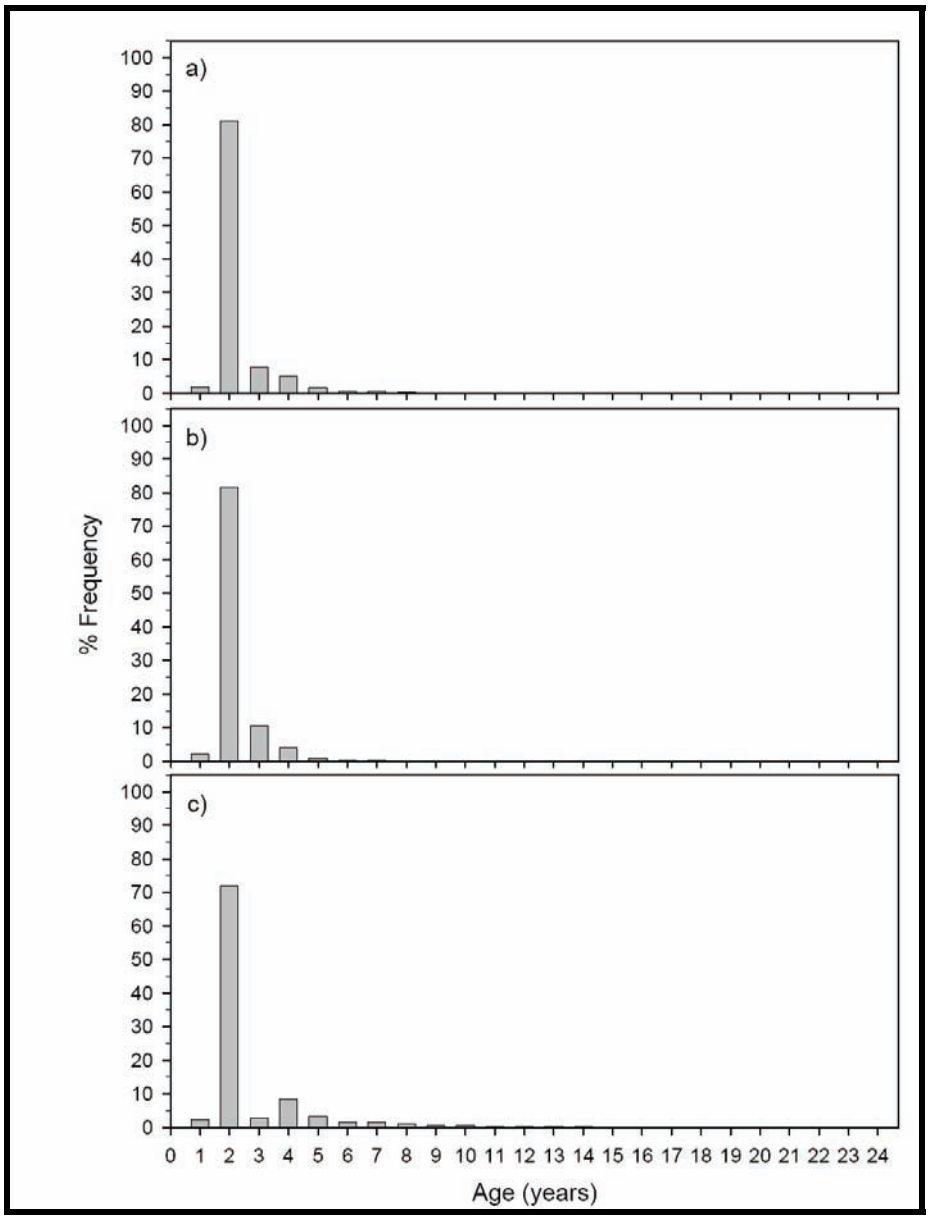


FIGURE 46: Estimated age compositions of a) the total commercial catch (n = 2605), b) the estuarine catch (n = 1681), and c) ocean retained commercial catches (n = 381) of mulloway in NSW 2003 to 2005. (Source: Silberschneider & Gray, 2005)

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 (Battaglene 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.

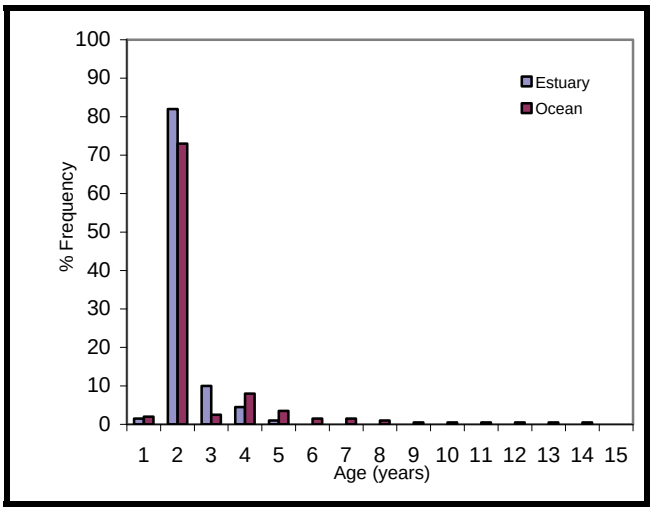


FIGURE 47: Size and age frequency composition of commercial catches of mulloway in NSW (pooled across regions). (Source :Silberschneider & Gray, 2005)

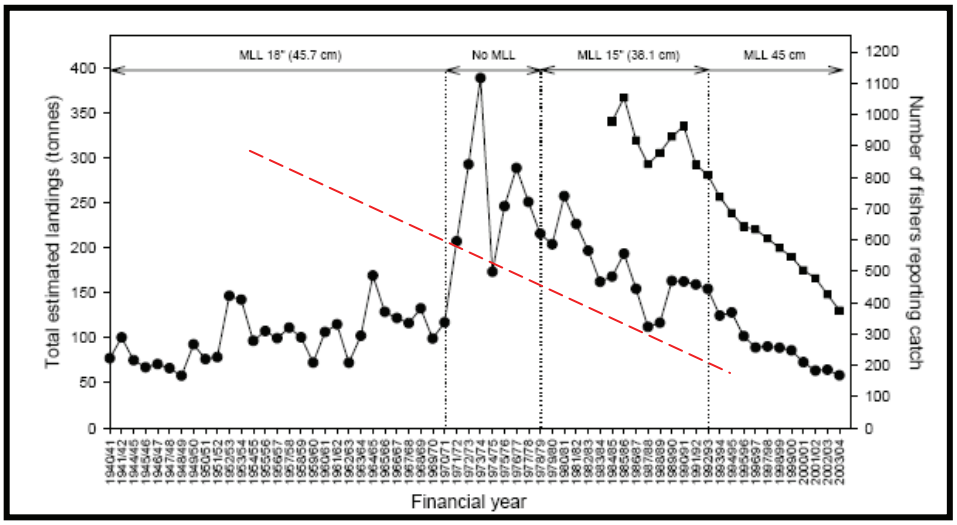


FIGURE 48: Commercial catch data for mulloway in NSW 1940/41 to 2003/04

2.6.2 Acquisition of ripe broodstock

Fertilised mulloway 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[2] (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)

[2] 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 mulloway is largely based on information sourced from Battaglene and Talbot, 1994 and Fielder, Bardsley and Allan, 1999. Supplementary information relating to the propagation of snapper and black bream but also relevant to mulloway 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 mulloway 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 mulloway 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 oxygengenation 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 mulloway 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 oxytetra-cycline 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

Mulloway broodstock in the range 2 to 15 kg should be held in tanks at least 20 m^3 in volume without sharp corners i.e. that are either round or preferably cylindro-conical and in the range 20 to 50 m^3 (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 mulloway release eggs that are positively buoyant. As already discussed in relation to Australian bass, (see Chapter 1), water level in mulloway 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 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 mulloway, 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^3 should be adopted to reduce captive stress on wild collected mulloway broodstock. Thus 20, 50, and 100 m^3 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 to10 kg respectively.

Physiochemical conditions

As mature mulloway are lower estuarine and inshore coastal fish, salinity should be maintained at 30±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 mulloway 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.

Mulloway 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, E2) 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® (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® and Luteinising Hormone Releasing Hormone analogue (LHRHa). Ovaprim® 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.

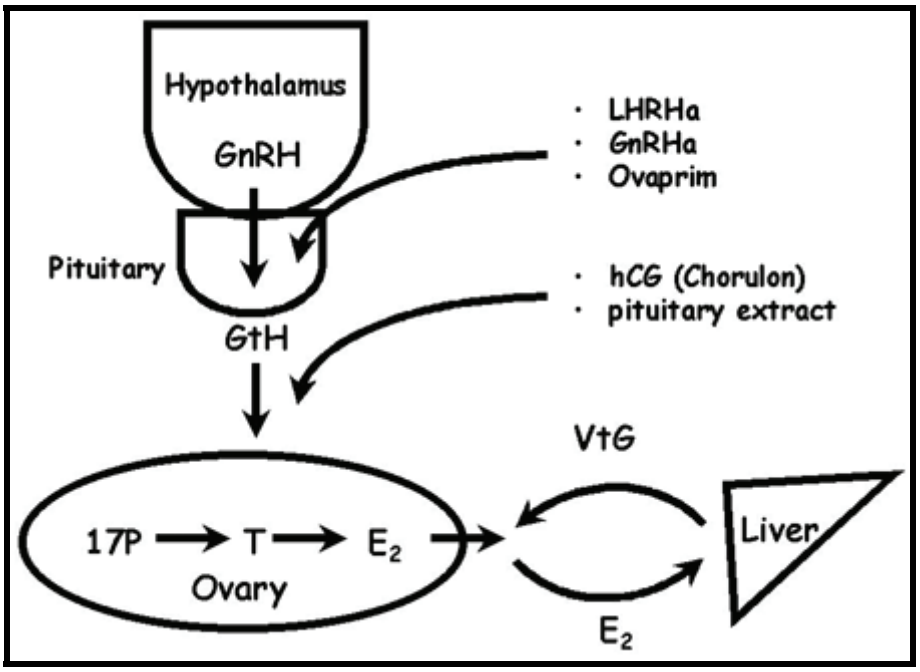


FIGURE 53: Schematic diagram of the reproductive-endocrine pathway in teleost fish indicating the action of various hormone treatments. (Source: Partridge et al., 2003).

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 mulloway 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 mulloway must be greater than 500 µm in diameter (Fig. 54).

HCG induction can also be used to increase milt volume and motility in males. For mulloway, 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 (Battaglene, 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 mulloway with oocytes as small as 400 µm in diameter to ovulate successfully. Induction of females with oocytes above 500 µm 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 µg/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.

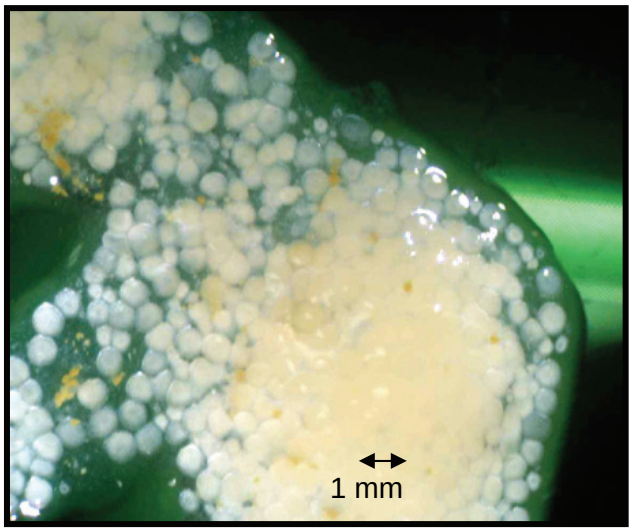


FIGURE 54: Microscopic view of an ovarian sample. (Source: Partridge et al., 2003).

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 mulloway. 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 mulloway 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 mulloway 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^{o}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 mulloway and YTK as well as snapper for which it was originally designed.

Date	Day-length	Light ON	Light OFF	Temperature °C	Day
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

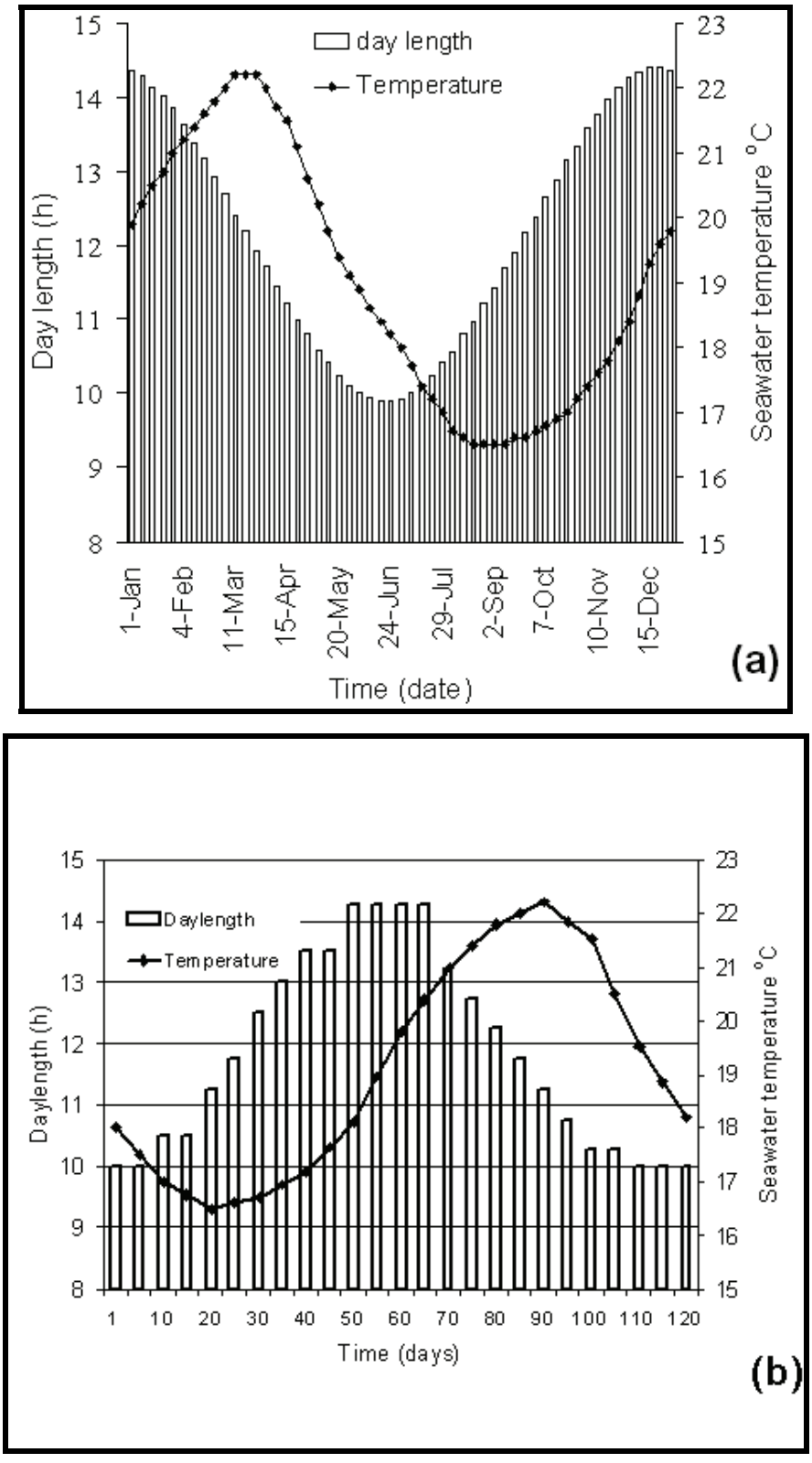


FIGURE 55: Natural (a) and compressed (b) temperature and photoperiod regime suitable for inducing breeding in broodstock mulloway and YTK as well as in snapper for which it was originally designed.

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^{o}C.

2.7.3 Egg harvesting counting and incubation

As for Australian Bass previously described in Chapter 1.

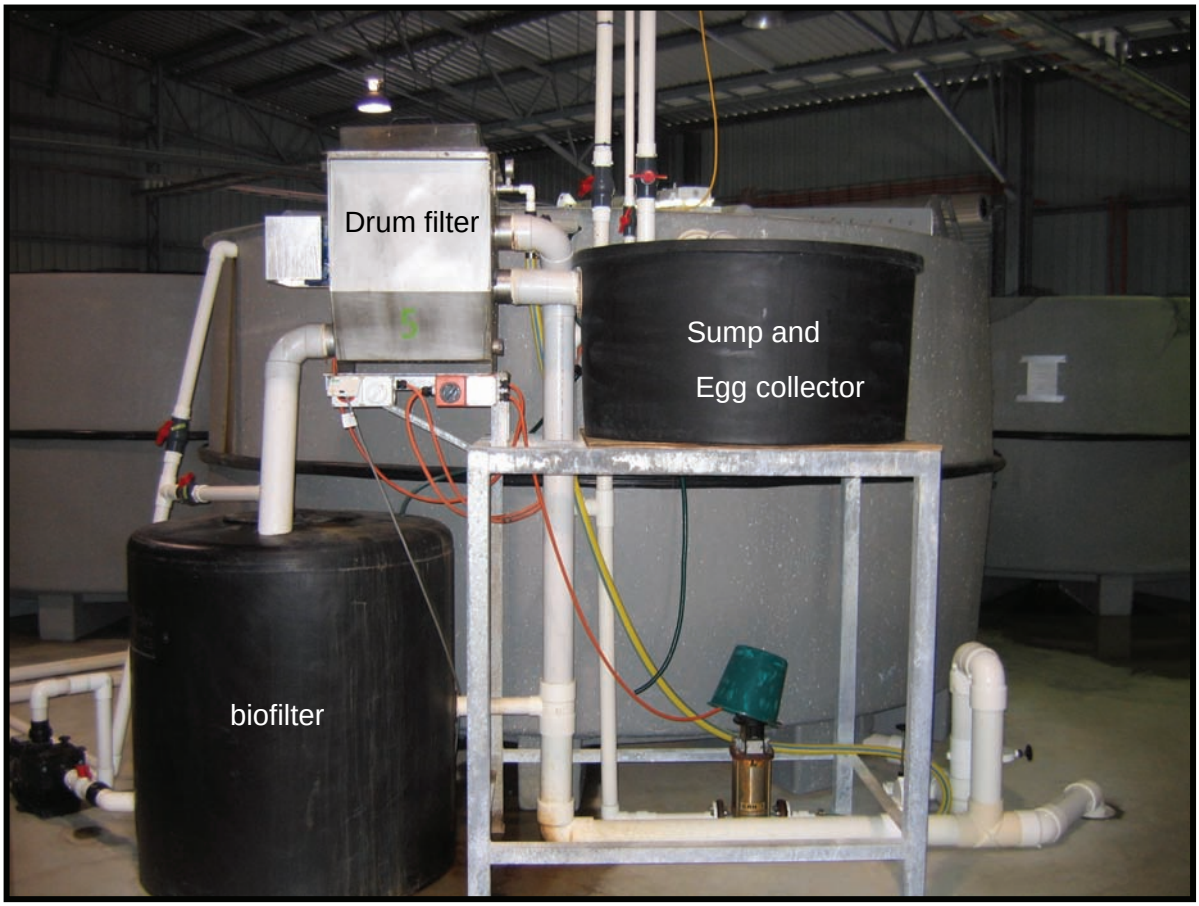


FIGURE 56: Controlled environment recirculation tank for broodstock maintenance at PSFI. Note the plumbing from the pump goes to an outdoor heater/chiller unit and then returns to the tank.

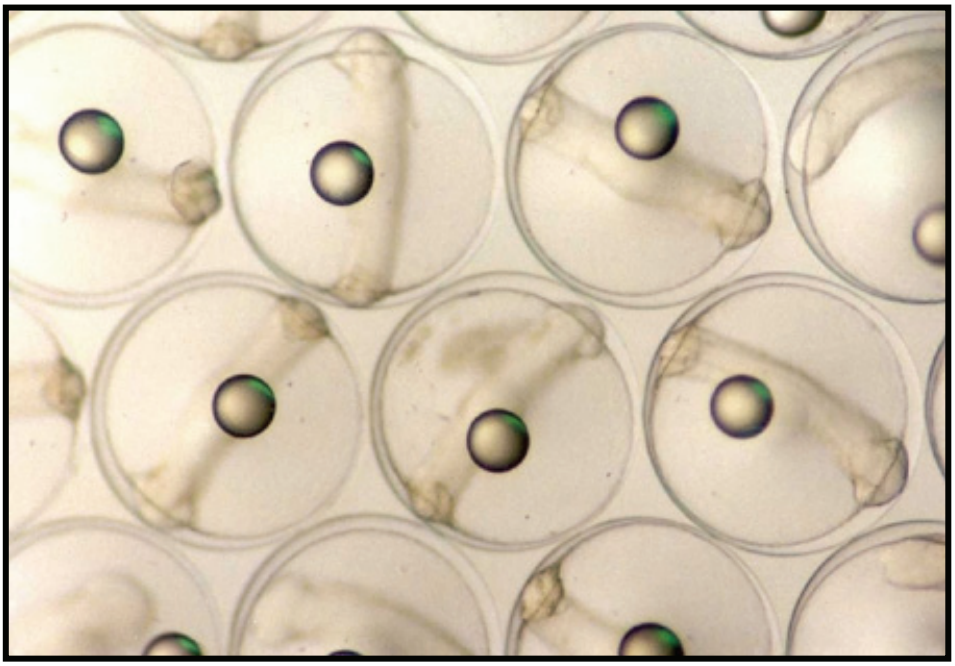


FIGURE 57: Mulloway eggs have a mean diameter of approximately 938 ± 24 µm with a single oil globule with mean diameter of 270 ± 30 µm.

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 ± s.d total length (hereafter referred to as length) of 2.25 ± 0.09 mm with a yolk sac of 0.88 ± 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 24ºC, 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.

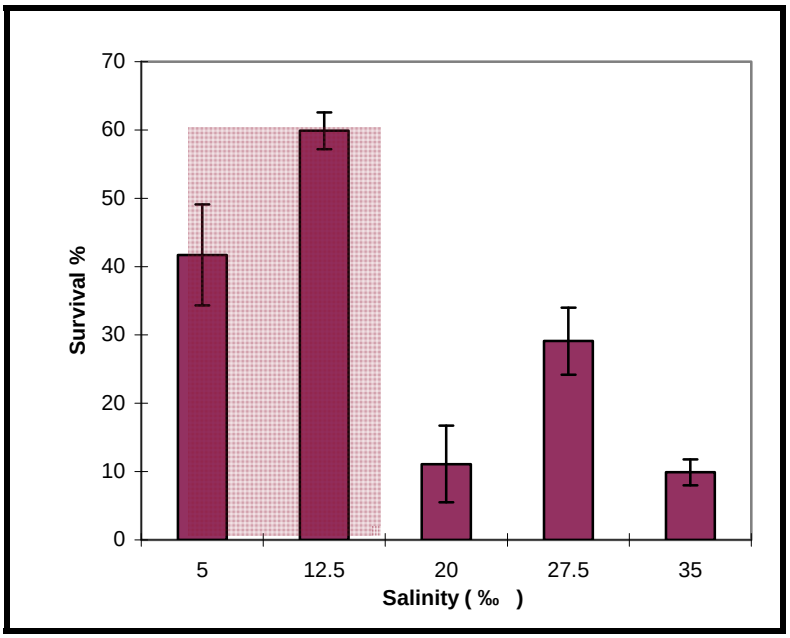


FIGURE 58: Effect of salinity on the survival rate (means ± se) of 20 dah mulloway larvae at 23 ±1°C. Shaded area is recommended salinity band. (Source: Fielder, Bardsley and Allan, 1999).

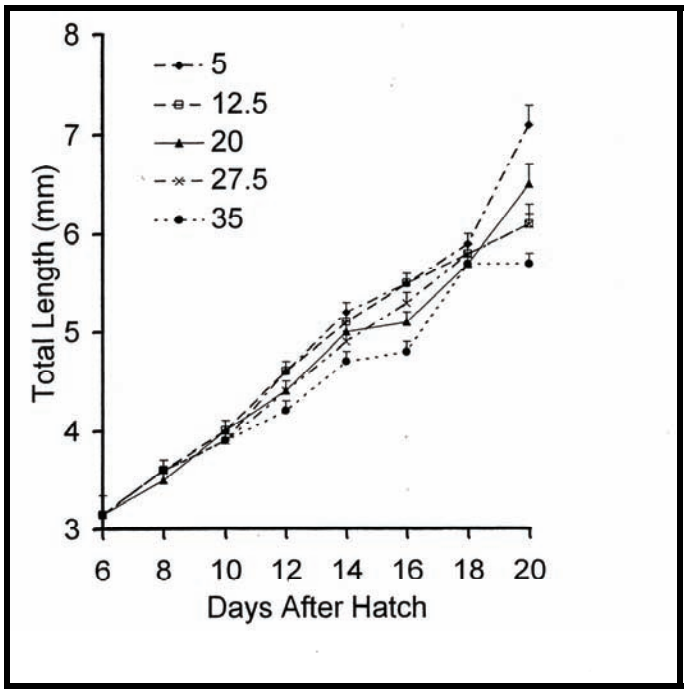


FIGURE 59: Effect of salinity on the growth (means ± sd) of 20 dah mulloway larvae at 23 ±1°C. (Source: Fielder, Bardsley and Allan,1999).

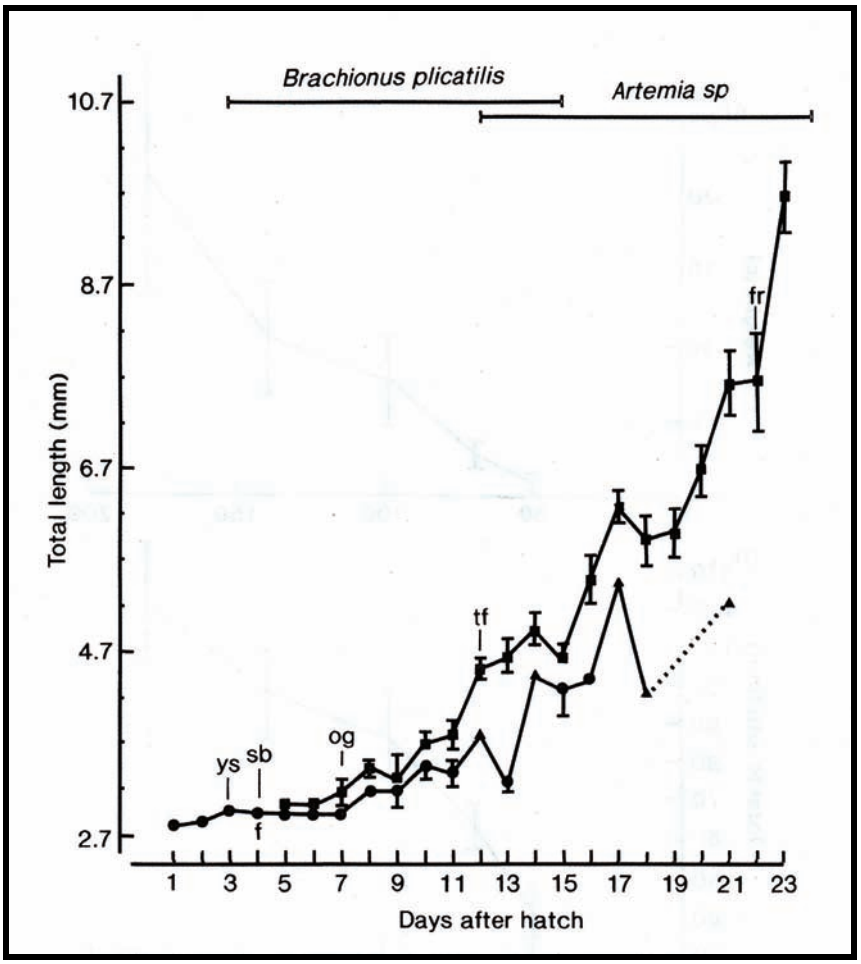


FIGURE 60: Growth and development of larval mulloway with (■) and without (●) swim bladders. Feeding regimes and important stages of larval development are indicated. f = feeding started; fr = fin rays present; og = oil globule absorption; sb = initial swim bladder inflation; tf = tail flexion; ys =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

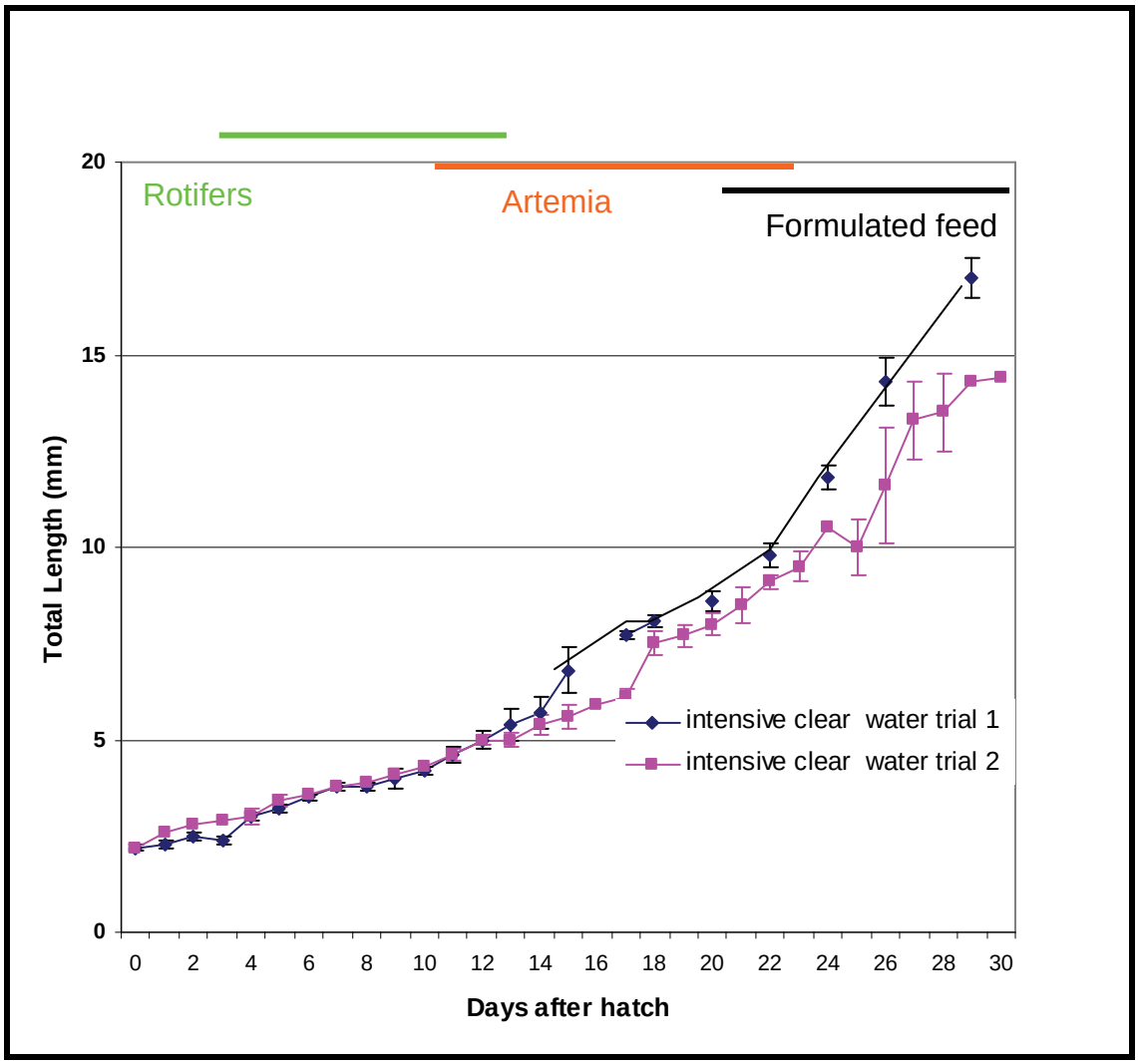


FIGURE 61: Growth of mulloway larvae in 2000L clear water recirculation tanks. Data are mean Total Length ± s.d. (trial 1: n=3 tanks into 20 dah and n=2 tanks 20-30 dah; trial 1: n=2 tanks throughout). (Source: Fielder, Bardsley and Allan, 1999).

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* metanaulplii 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 n*auplii 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 of larvae to juveniles), mulloway 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).

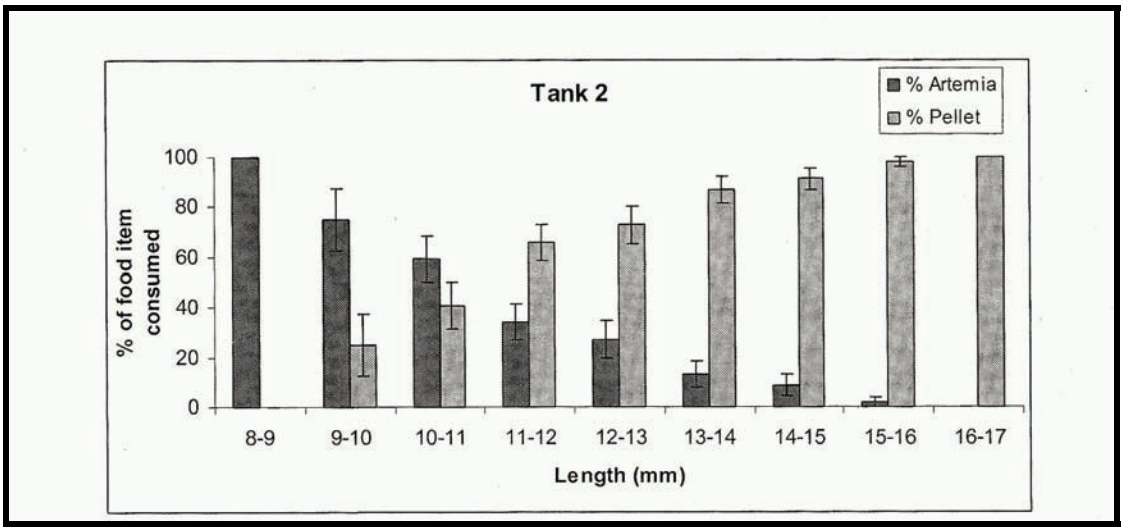


FIGURE 62: Percentages (mean ± s.e.) of *Artemia* and artificial particulate diet consumed by mulloway larvae (Source: Ballagh et al. 2010).

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 µm. This size can be increased within about 5 days to 400-600 µm and over the next 7 days up to 1400 um 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 mulloway 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 mulloway grow faster and more efficiently under medium light intensity (≈130 lux) than at low (≈0.3 lux) or high (≈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^3 than at a low density of 250/m^3 (Fig. 65; Fielder et al., 2010). The optimal rearing parameters and feeding schedule for mulloway larval rearing used at PSFI is summarized in Table 6.

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

Species: Mulloway (*Agyrosomus japonicus*)

Parameter	Target	Dah	Adjustment
pH	7.6 - 8.2	0+	
Dissolved Oxygen (mg/l)	>6.00	0+	Use compressed oxygen diffuser to maintain saturation level
Temperature (°C)	22	0+	Increase post SB inflation
Salinity (ppt)	5 to 35	0+	5-12.5 ppt optimal
Water Exchange (%/day)	100 - 200	0+	Increase exchange as larvae develop
Surface Skimmer (hrs/day)	24	4+	Monitor skimmer to ensure larvae at water surface are not affected
Photo-period (L:D)	(12:12) (18:06)	(0+) (6+)	Increase post SB inflation
Light Intensity (Lux)	225-400	0+	Start with light at lower intensity
Green-water (cells/ml)	1.4 x 10^6	0+	Pro-Aqua* concentrate 57x10^9 per ml
Rotifer (R/ml)	20.0 - 5.0	4+	Initial 20/mL until feeding and then increase frequency of reduced concentration (e.g. 4x5/ml/d).
***Artemia* (A/ml)**	0.2 - 2.0	12+	0.2/mL until weaned, then increase concentration and frequency. Start at 5.4 mm TL
Weaning Diet size (µm)	200 - 800	22+	Commence weaning at 10.5 mm TL

*Algae concentrate used Rotifer Diet-3600 (*Nannocholoroosis/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 ≈100,000 lux ≈ 10,000 foot candle ≈ 500 µmol/m2/sec (microeinsteins/square metre/second)

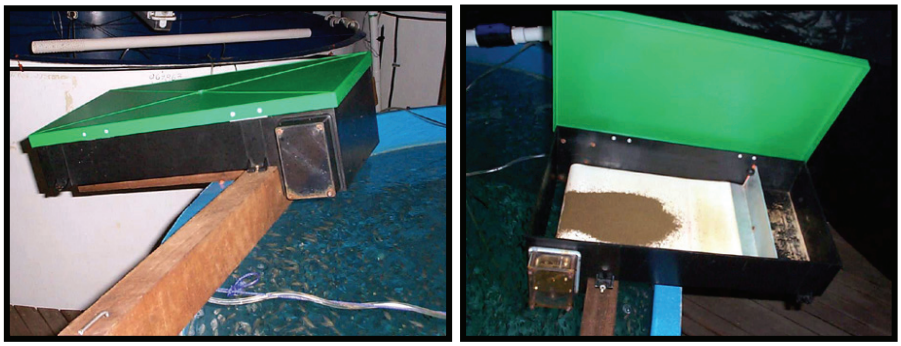


FIGURE 63: Belt feeder used to dispense formulated feeds during weaning and beyond. Source: Partridge et al., 2003.

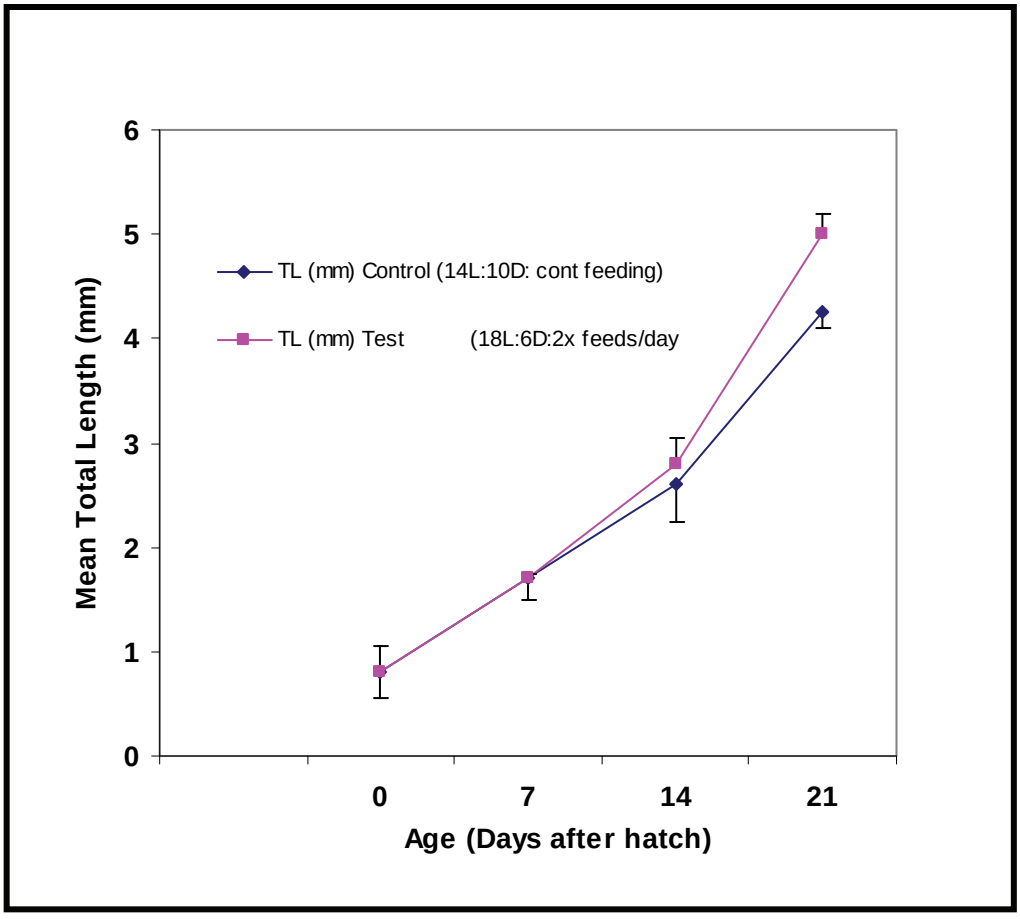


FIGURE 64: Mean ± sd live-weight of juvenile mulloway grown under 2 regimes, the first (Control) involving continuous feeding and a 14h light: 10h dark cycle and the other "test regimen" involving twice daily feeding coupled with an 18h light :6 h dark regimen. (Source: Fielder et al., 2010).

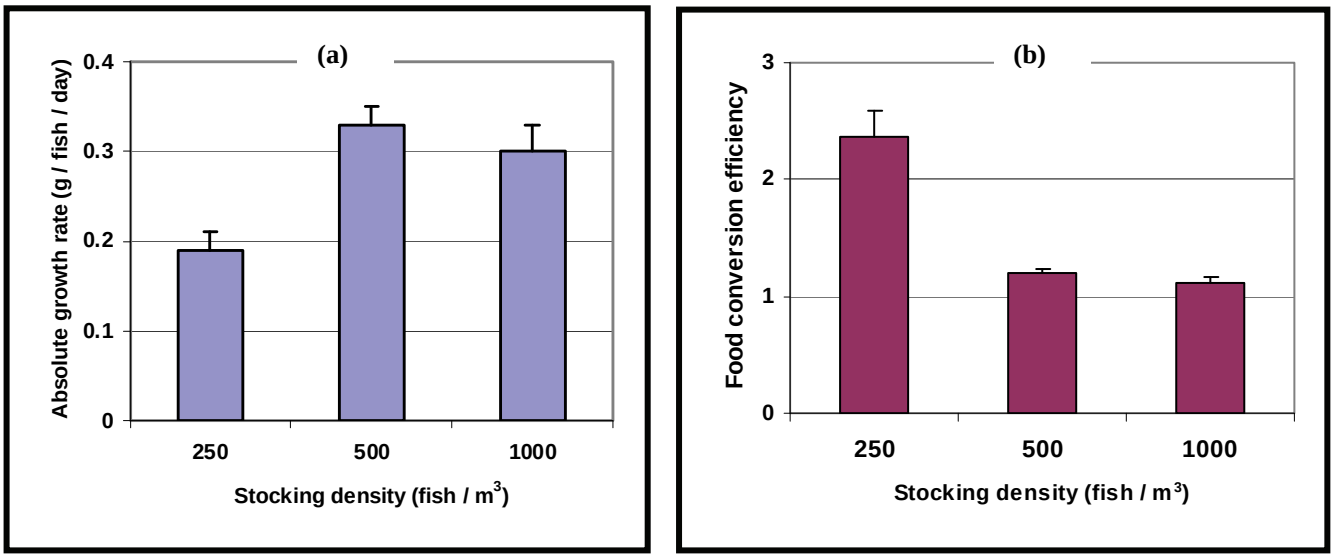


FIGURE 65: Effect of stocking density on: a) absolute growth rate (mean ± sd) and b) food conversion efficiency, (Food Conversion Ratio [FCR] = weight of dry food/wet weight gain of fish), of juvenile mulloway (initial mean weight per fish 17 ± 3.5 g , final mean weight 17 to 29 g) over 37 days. Stocking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

2.8.3 Semi-intensive greenwater larviculture of mulloway

System design and general operating specifications

Greenwater hatchery technology for mulloway 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 mulloway can either be conducted indoors or in outdoor green houses. Outdoor greenhouse culture in southern Australia is effective in summer under ambient temperatures of 20-30ºC, 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 22-26ºC. 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 mulloway 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.

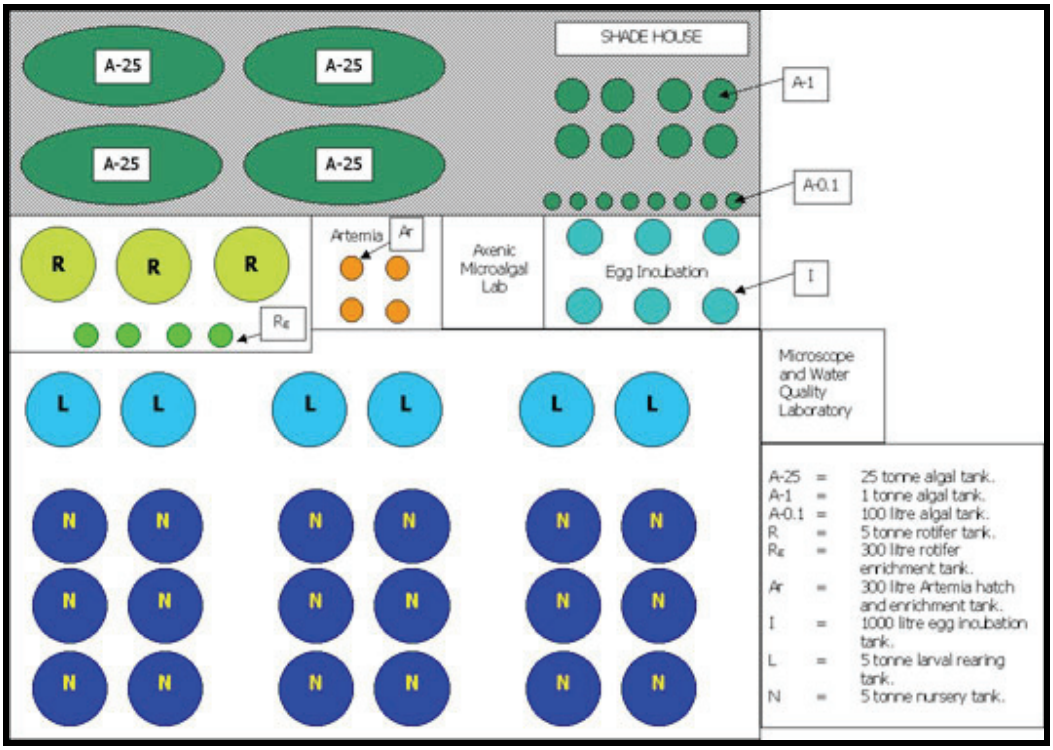


FIGURE 66: Schematic layout of greenwater marine fish larval rearing unit. (Source: Partridge et al., 2003).

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 x 10^6 per day.
- Peak rotifer demand; 800 x 10^6 per day (on each day a new tank is stocked)
- Maximum enriched *Artemia* requirement; 80 x 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 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 µm 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 H_2S 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 1µm 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.

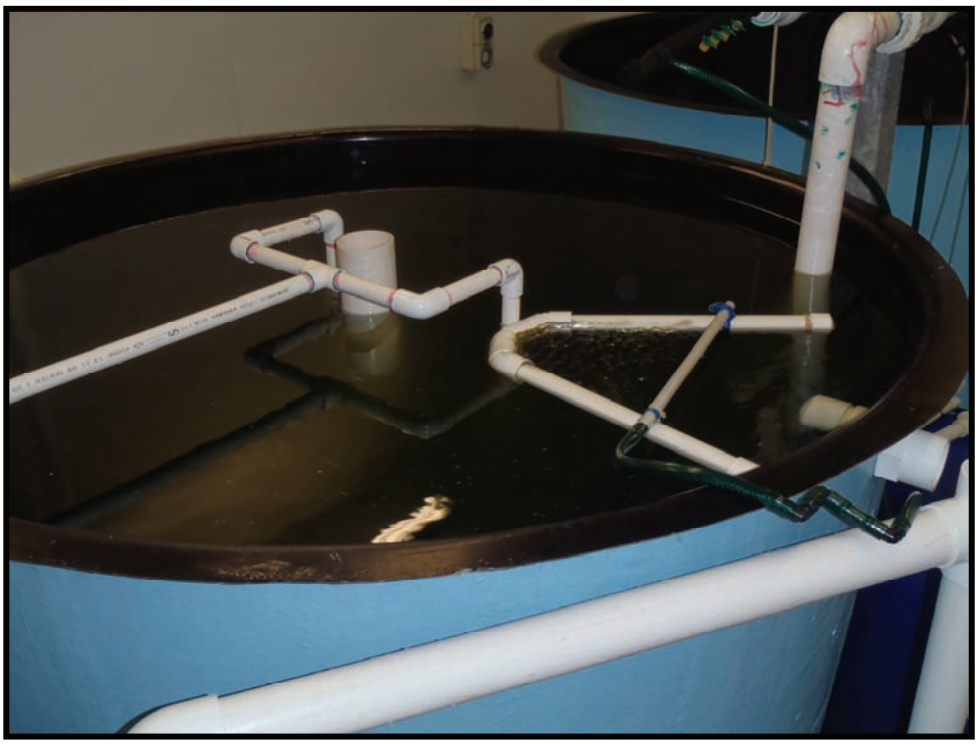


FIGURE 67: 2000-L larval rearing tanks at PSFI.

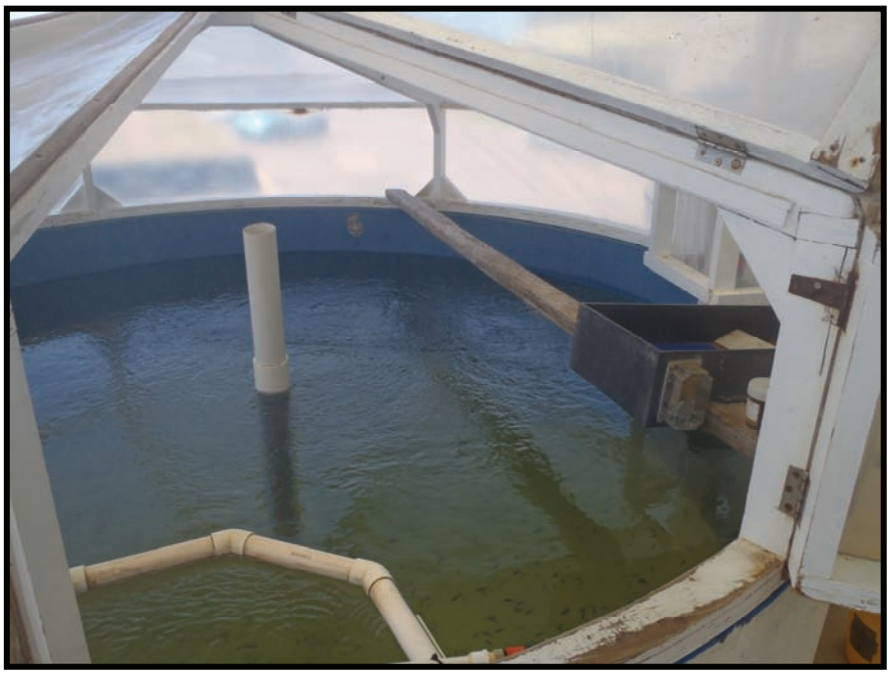


FIGURE 68: 10,000-L larval rearing tanks at PSFI. Tanks are covered with translucent, polyethylene sheeting.

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 to 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.

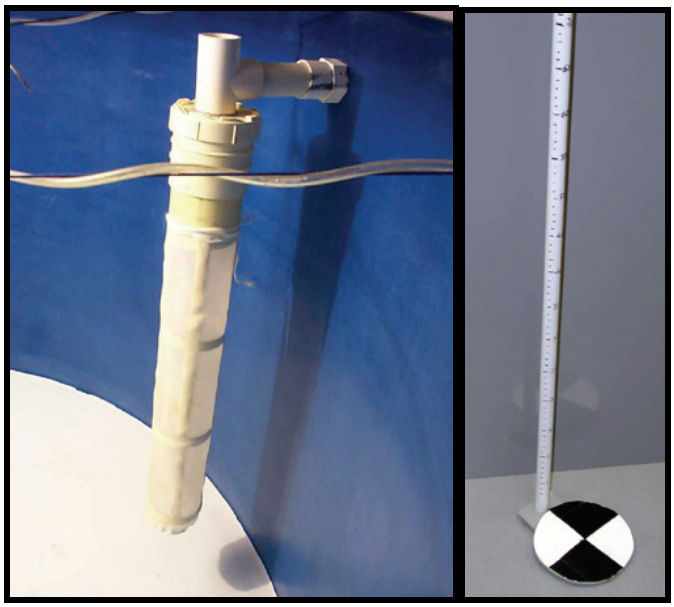


FIGURE 69: Cylindrical screen outlet and Secchi Disc used to assess microalgal cell density. (Source: Partridge et al., 2003).

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 $200x10^6$ (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)

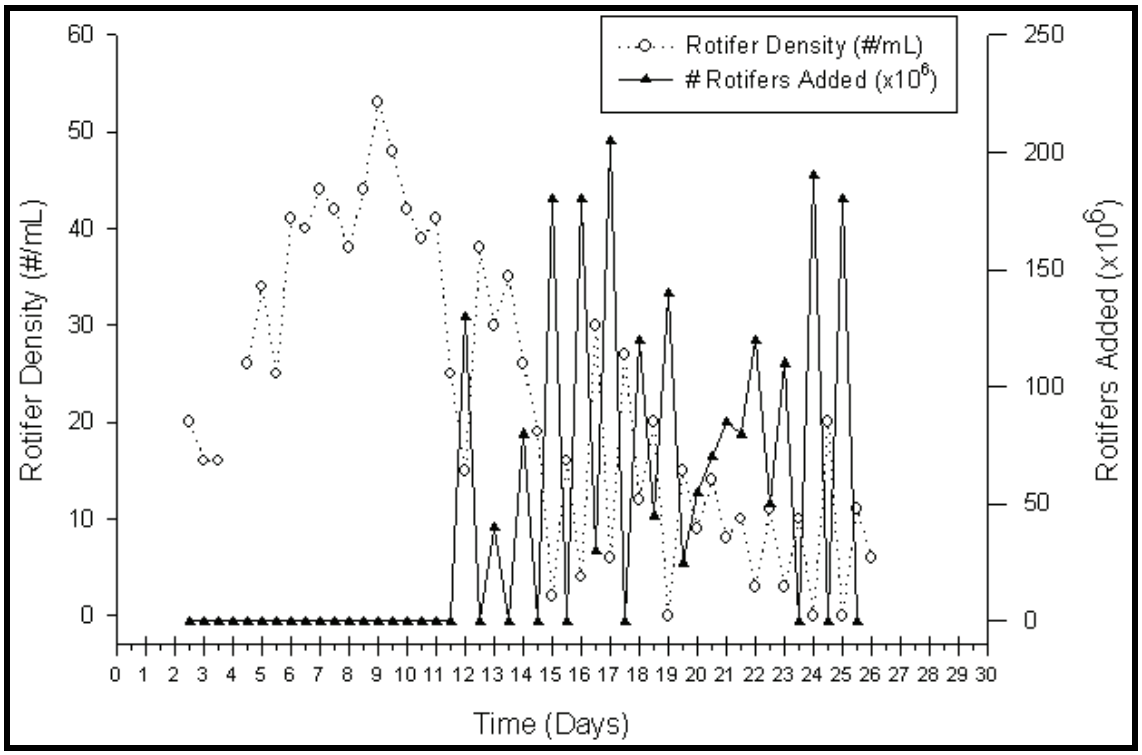


FIGURE 70: Rotifer densities and additions made to a semi-intensive snapper culture. (Source: Partridge et al., 2003).

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).

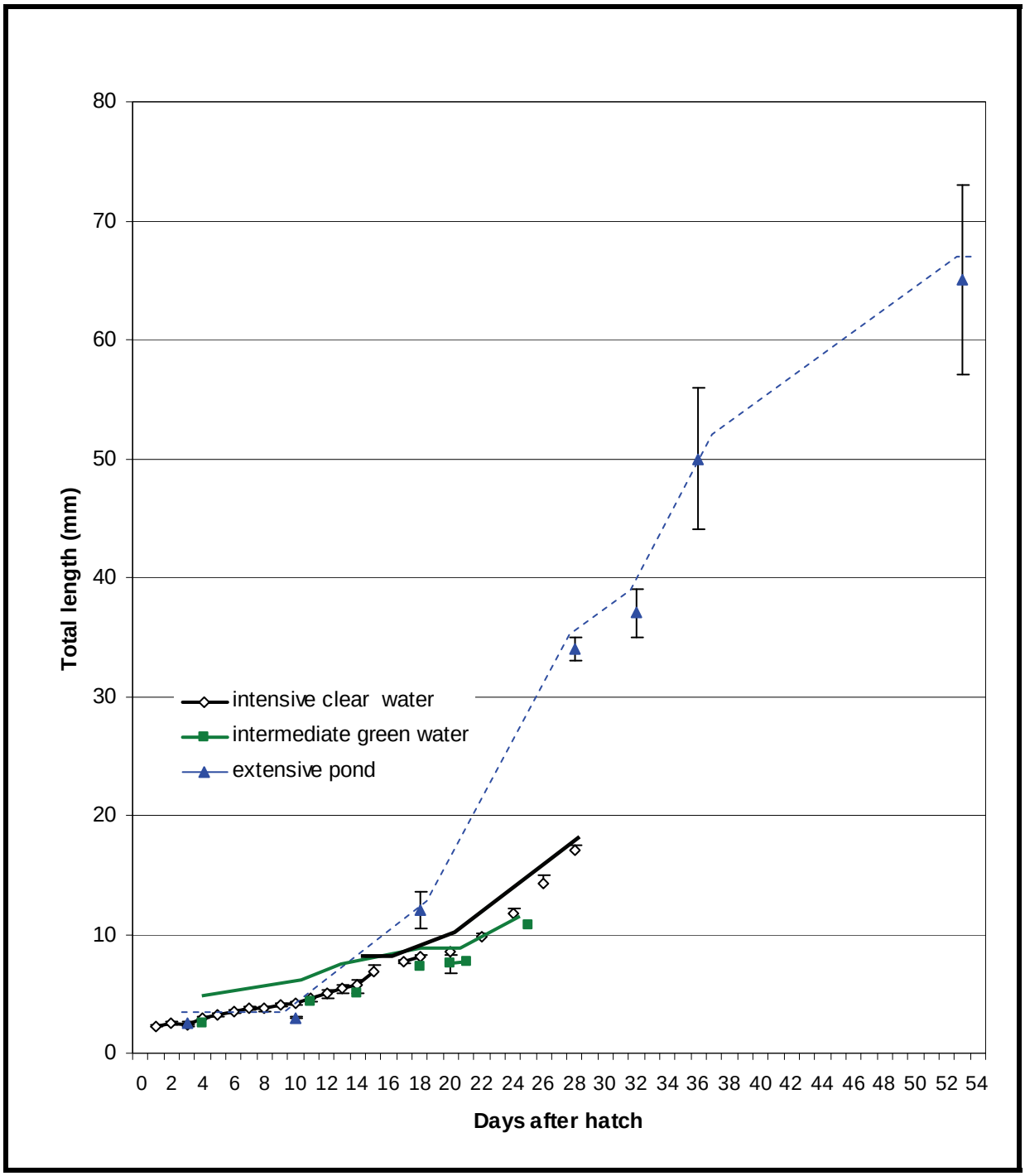


FIGURE 71: Comparative growth of mulloway larvae reared intensively indoors in clear water tanks, semi intensively in outdoor green-water tanks and in extensively in earthen or plastic lined ponds. (Source: redrawn from data provided in Fielder, et al., 1999).

Reported growth rates of mulloway reared from larvae to fully metamorphosed juveniles reared extensively in large (250–10,000 m^2) 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.

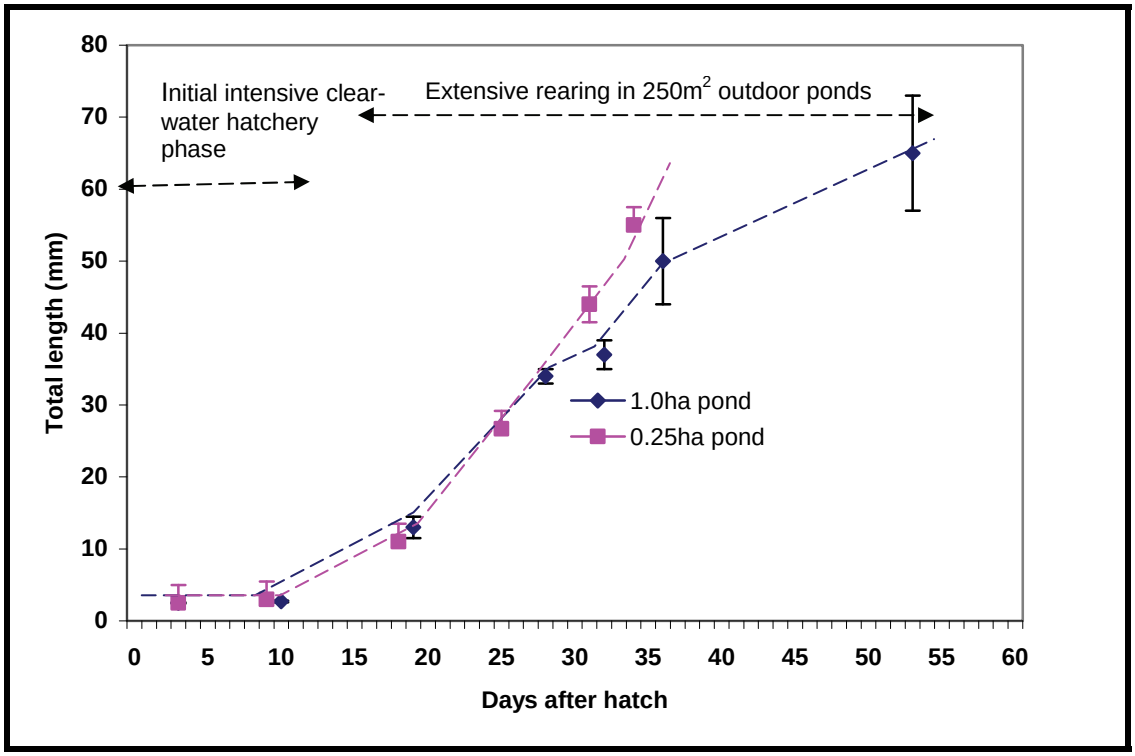


FIGURE 72: Growth of mulloway larvae reared extensively in 0.25 and 1.0 ha ponds at Yamba NSW. (Source: Fielder et al., 1999)

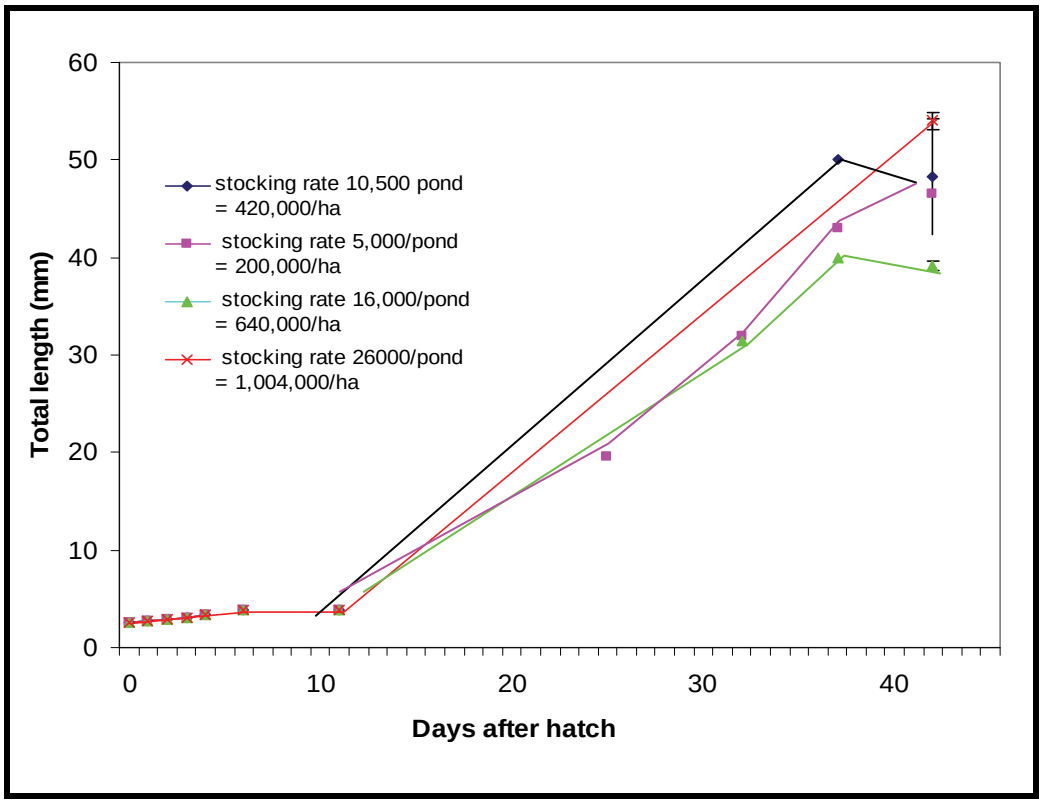


FIGURE 73: Effect of stocking density on growth of mulloway stocked after 11 days of initial intensive hatchery rearing into replicate 250 m^2 ponds at Yamba in northern NSW. (Source: Fielder et al.,1999).

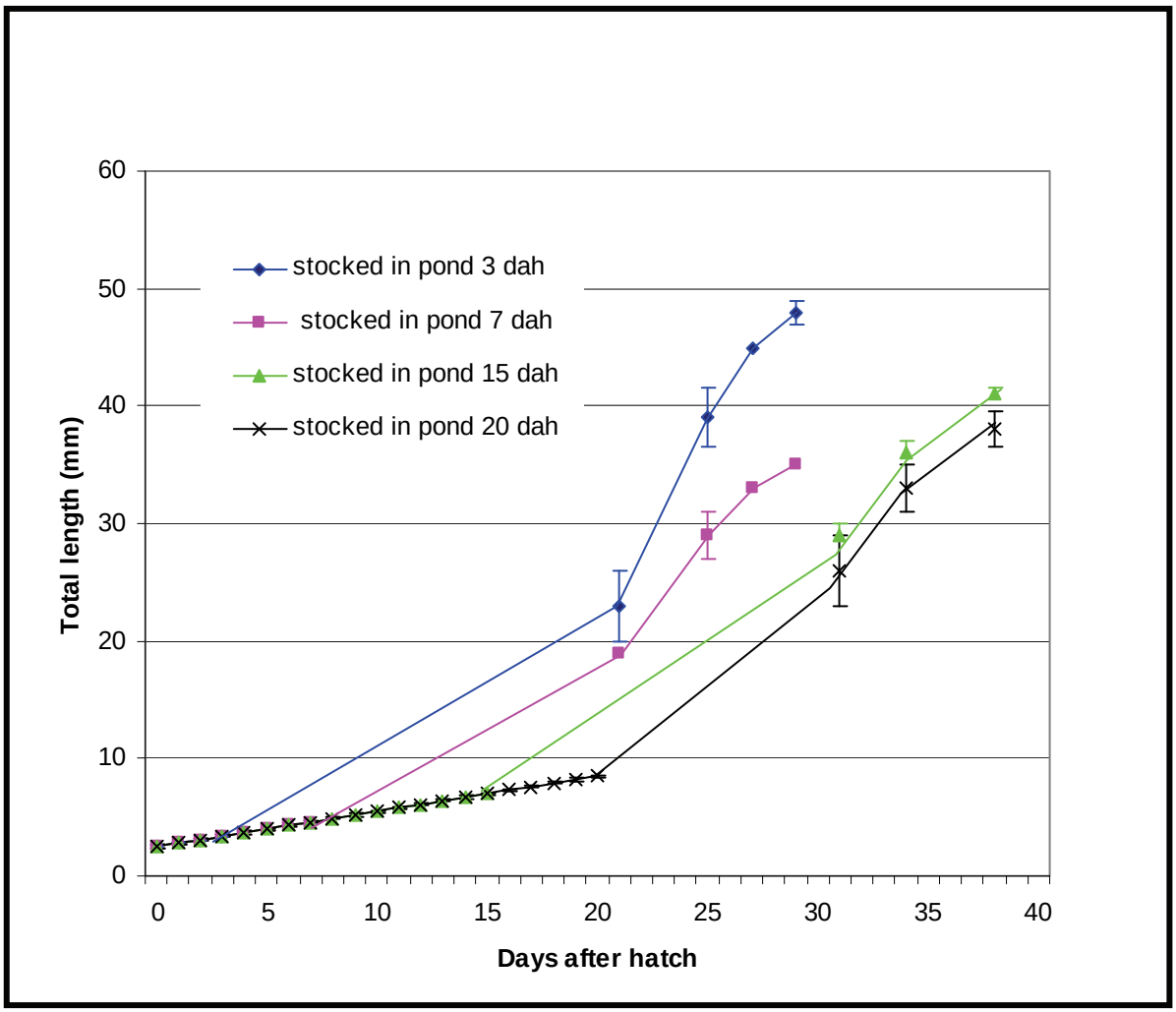


FIGURE 74: Effect of age at stocking on growth of mulloway stocked into replicate 250 m^2 ponds at Yamba in northern NSW. (Source: Fielder et al., 1999).

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 indispensible 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 indispensible 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 mulloway 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 Mulloway Fingerlings

Table 7 summarises "best-practice" larval rearing regimes for mulloway at PSFI.

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

SPECIES: MULLOWAY (*ARGYROSOMUS JAPONICUS*)			
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		
SPAWNING INDUCTION	HCG		1000 IU/KG NECESSARY FOR WILD-CAUGHT FISH. 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 ^{O}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^{O}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^{O}C
LARVAL FIRST-FEEDING	3-4 DAH		AT 23 ±0.5^{O}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**
PH	7.6 - 8.2	0+	
DISSOLVED OXYGEN (MG/L)	>6.00	0+	USE COMPRESSED OXYGEN DIFFUSER TO MAINTAIN SATURATION LEVEL
TEMPERATURE (°C)	22	0+	INCREASE POST SB INFLATION
SALINITY (PPT)	5 TO 35	0+	5-12.5 PPT OPTIMAL
WATER EXCHANGE (%/DAY)	100 - 200	0+	INCREASE EXCHANGE AS LARVAE DEVELOP
SURFACE SKIMMER (HRS/DAY)	24	4+	MONITOR SKIMMER TO ENSURE LARVAE AT WATER SURFACE ARE NOT AFFECTED
PHOTO-PERIOD (L:D)	(12:12) (18:06)	(0+) (6+)	INCREASE POST SB INFLATION
LIGHT INTENSITY (LUX)	225-400	0+	START WITH LIGHT AT LOWER INTENSITY
GREEN-WATER (CELLS/ML)	1.4 X 10^{6}	0+	PRO-AQUA* CONCENTRATE 57X10^{9} PER ML
LARVAL FEEDING SCHEDULE	**TARGET**	**DAH**	**ADJUSTMENT**
ROTIFER (R/ML)	20.0 - 5.0	4+	INITIAL 20/ML UNTIL FEEDING AND THEN INCREASE FREQUENCY OF REDUCED CONCENTRATION (E.G. 4X5/ML/D).
***ARTEMIA* (A/ML)**	0.2 - 2.0	12+	0.2/ML UNTIL WEANED, THEN INCREASE CONCENTRATION AND FREQUENCY. START AT 5.4 MM TL
WEANING DIET SIZE (µM)	200 - 800	22+	COMMENCE WEANING AT 10.5 MM TL

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

3. YELLOWTAIL KINGFISH (YTK)

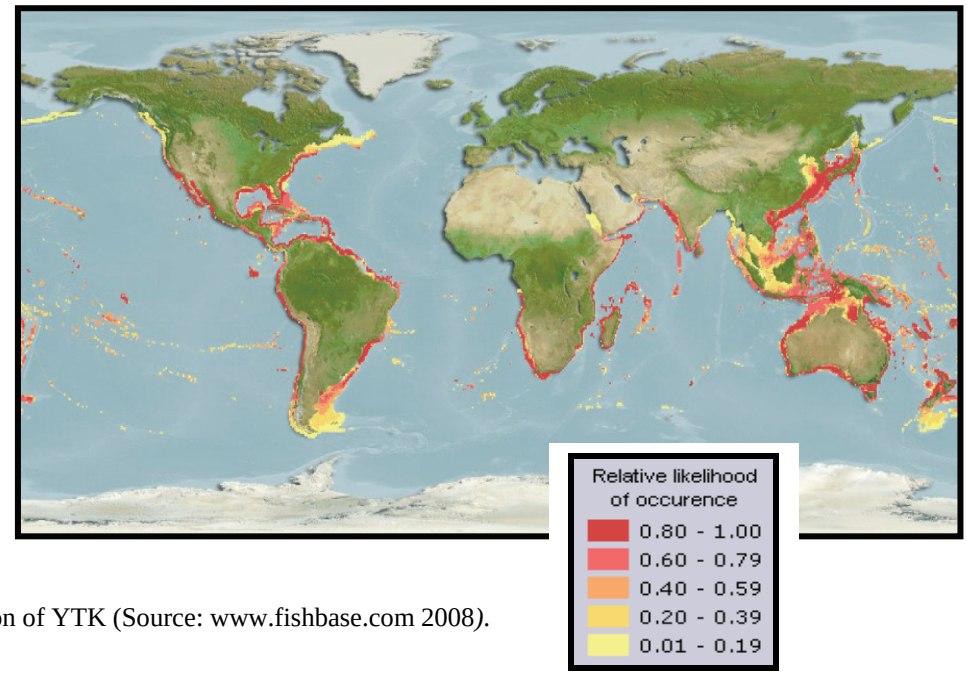
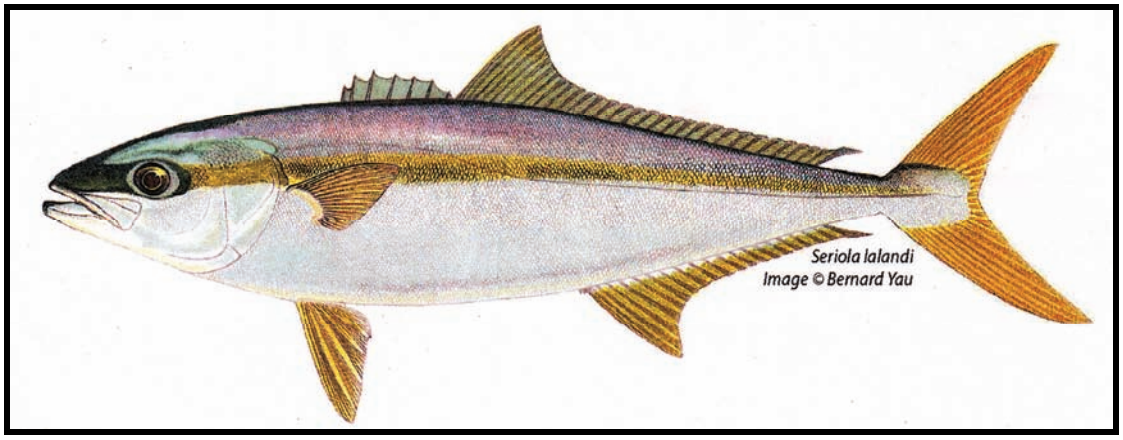


FIGURE 75: Global distribution of YTK (Source: www.fishbase.com 2008).

3.1 Appearance, Distribution and Movements

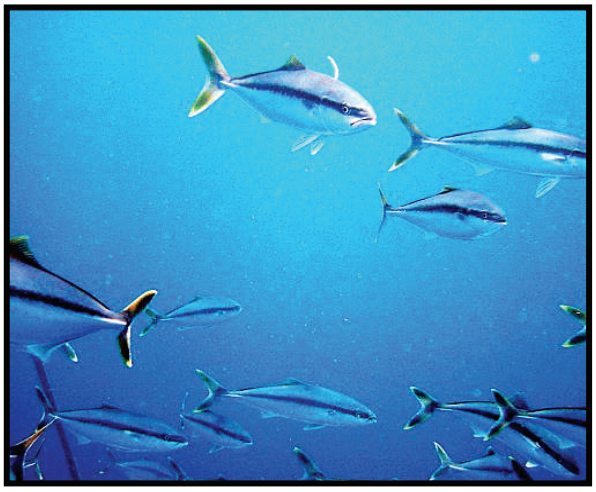
Yellowtail kingfish (*Seriola lalandi*), also known as gold striped amberjack, is a member of the family Carangidae, commonly referred to as jacks and pompanos. The body tapers posteriorly to a narrow tail stem (caudal peduncle) then broadens to a large forked yellow tail (caudal fin). The "scooped-out" centre of the tail presents a small surface area with respect to the large span resulting in little lateral displacement of water and hence low turbulence and drag. The powerful and very swift mode of swimming of YTK, involves very little movement of the head but considerable movement of the tail. The upper surface of the torpedo shaped body of YTK is generally blue or blue-green providing camouflage against the ocean depths when viewed from above. Likewise the white-silver underside provides camouflage when viewed from below against the mirror like sea surface.

Juveniles have distinctive black and bright yellow lateral bands and fins but these fade as the fish ages. By about 30 cm, YTK have assumed adult colouration.



Juvenile YTK (Source: I&I NSW website)

YTK are found circum-globally mainly in high salinity (marine) waters but prefer temperate and subtropical waters (18-24°C) (Fig. 75). Populations are disjunct occurring in the Indo-Pacific (South Africa, Walter Shoals, Amsterdam Island, Japan, Australia, New Zealand, New Caledonia, Hawaii, Rapa, Pitcairn Island, and Easter Island) and the Eastern Pacific (British Columbia, Canada to Chile. Eastern Atlantic: St. Helena, South Africa). Within Australia YTK occur from North Reef, Queensland (23°11'S) to Trigg Island, Western Australia (31°52'S), as far south as Tasmania and around Lord Howe and Norfolk island. (Australian Museum Fish-site 2008).



Schools of juveniles commonly comprising hundreds of fish up to 7 kg are generally found close to the coast. YTK occasionally enter brackish estuarine waters in pursuit of prey comprising small fish, squid and crustaceans, while larger fish are more common around deep reefs and offshore islands out to the edge of the continental shelf. Schools are commonly associated with floating debris or weed that provides a focus for gathering (Fig. 76).

FIGURE 76: School of juvenile YTK in NSW.
Note tag on uppermost fish (Source: I&I NSW Website Nov 2008)

Tagging programs have shown widespread movements of YTK. These include trans-Tasman (from Australia to New Zealand and vice versa) crossings and many large scale (>500 km) movements along the coast of New South Wales (Fig. 77).

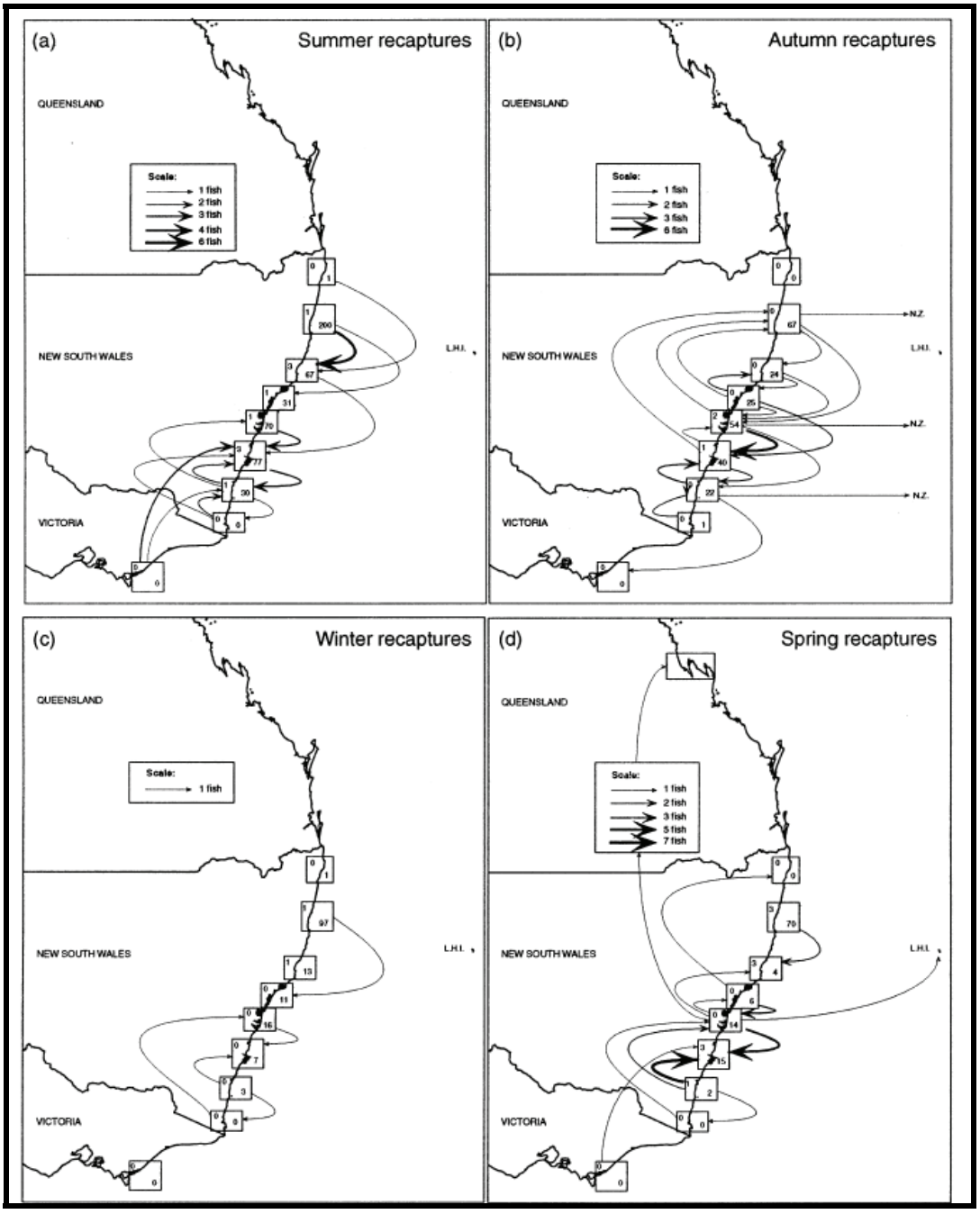


FIGURE 77: Movement of YTK by season in which they were recaptured. (Source: Gillanders et al., 2001). Only fish moving >50 km are shown. Movements shown to the right of the coast are southerly movements; those to the left represent northwards movement of fish, with the exception of one fish moving to Lord Howe Island (L.H.I.) and three fish moving to New Zealand (N.Z.). Numbers in the left corner of the boxes are fish that moved >50 km but were recaptured in the same general area as they were tagged; numbers in the right corner are fish recaptured <50 km from their release point. The size of the arrow is proportional to the number of fish.

3.2 Breeding and Early Life History

YTK are serial spawners, breeding in summer and/or autumn (Gillanders et al., 1999; Poortenaar et al., 2001) depending on seasonal sea temperature ranges of particular locations (Figs. 78 and 79). Spawning in captive YTK held under ambient temperatures and photoperiods in New Zealand by Moran et al., 2007 was consistent with that of wild fish being temperature limited to the range 17 - 24°C and occurred either just prior to dawn during the first half of the spawning season and 1 h either side of dusk in the latter half. No mass spawning events (those involving more than one female) were recorded, although two or three individual females spawned within an hour of each other on several occasions. This suggests that female YTK may spawn close together, but not necessarily at the same time.

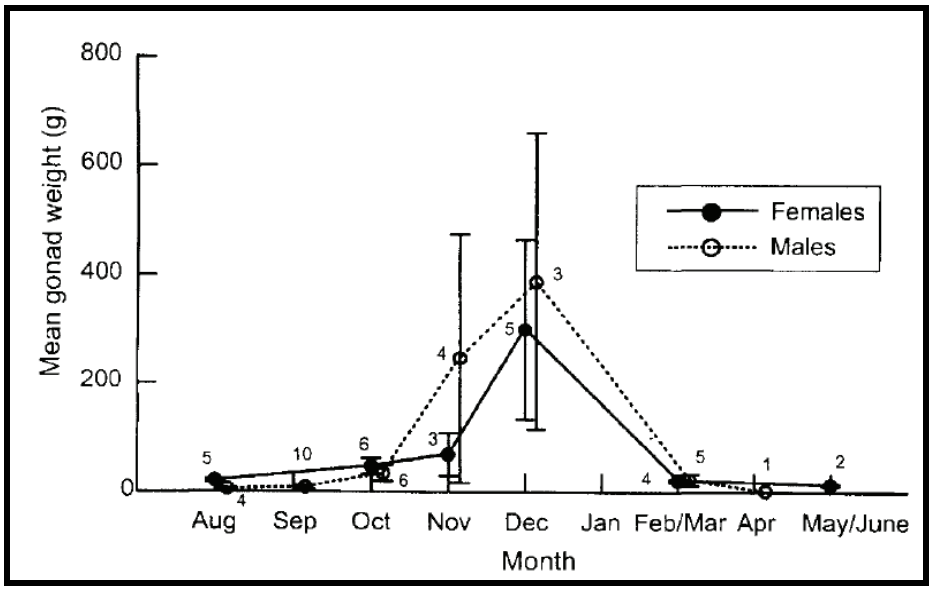


FIGURE 78: Mean gonad weight (±SE) of female and male YTK for monthly samples from along the coast of New South Wales. (Source: Gillanders et al., 1999).

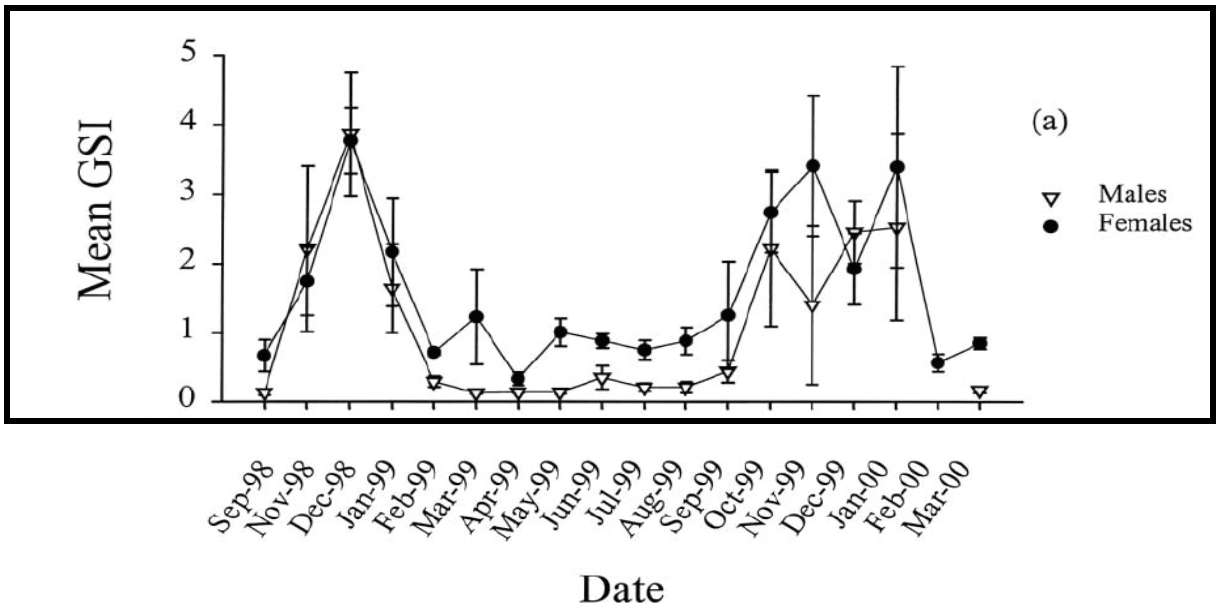


FIGURE 79: Seasonal variation in breeding condition (mean gonadal-somatic index value) of YTK *in* New Zealand (Source: Poortenaar et al., 2001).

The estimated mean size and age of onset of sexual maturity in YTK varies with gender and geographic location. In New South Wales (Fig. 80) the mean size and age at maturity recorded for female YTK was 834 mm and 3+ years, respectively and for males 471 mm and 0.9 years, respectively (Gillanders et al., 1999). Corresponding sizes and ages reported for YTK stocks in New Zealand (Fig. 81) are 944 mm and 7- 8 years for females and 812 mm and 4 years for males. The large differences in size and age of sexual maturity between NSW and NZ populations may be due to different growing conditions, e.g. warmer water temperatures in NSW, or behavioural and physiological differences between populations. Although no fixed genetic differences have been identified between NZ and NSW populations, large scale movements between these populations are uncommon (Poortenaar et al., 2001).

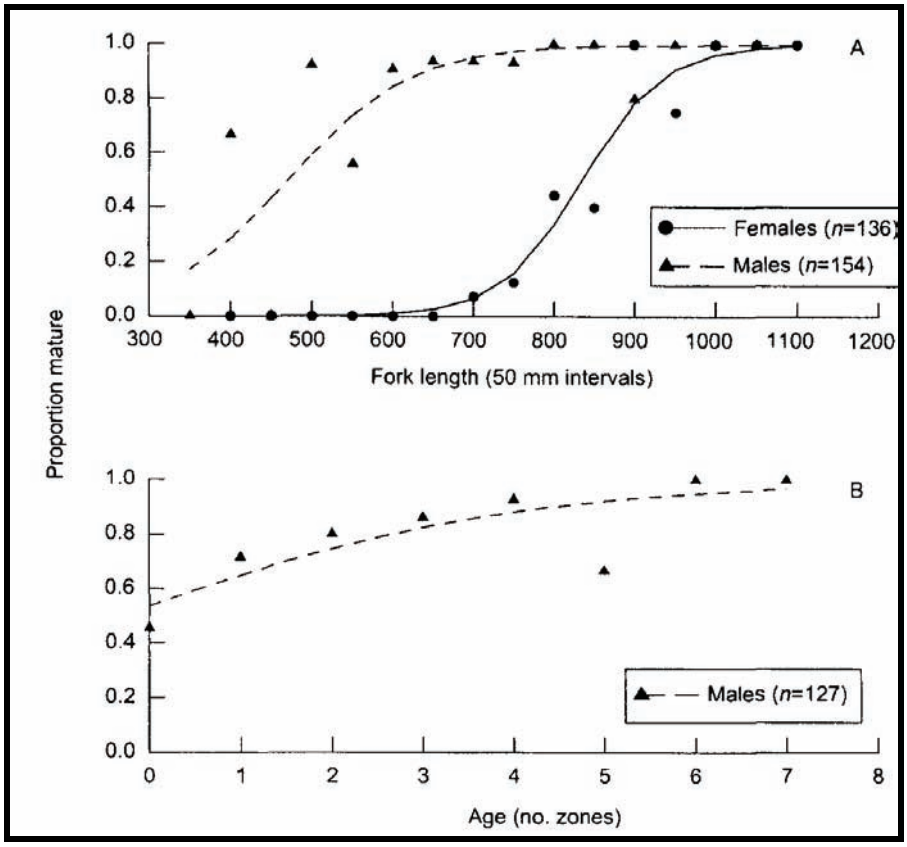


FIGURE 80: Distribution of mature *Seriola lalandi* in New South Wales: A, size; and B, age. There were insufficient age data to determine age at maturity for females. (Source: Gillanders et al., 1999).

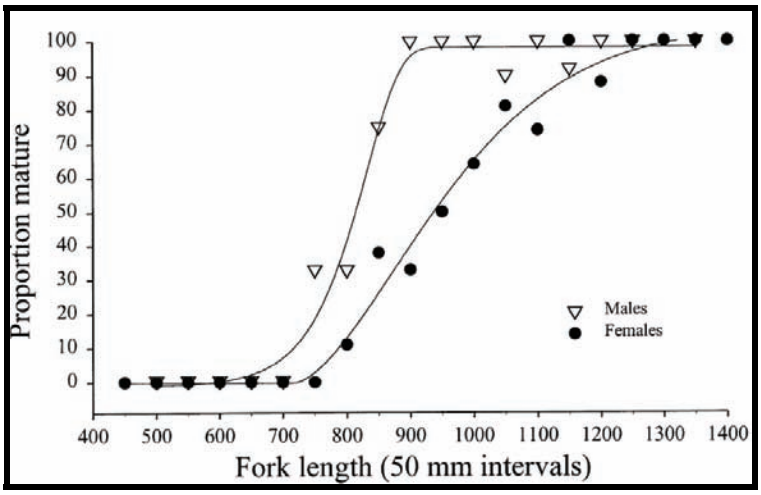


FIGURE 81: Size distribution of mature *Seriola lalandi* YTK in New Zealand. (Source: Poortenaar et al., 2001).

3.3 Food and Feeding

Predation by YTK on small schooling pelagic fish (e.g. sardines, anchovies, jack mackerel and Pacific mackerel) and cephalopods has been reported off California, Australia, New Zealand and in the Gulf of Mexico. There have been fewer studies on the diet of YTK food in the south-western Atlantic, with reports the diet comprises juvenile Argentine anchovy (81%), jack mackerel (7%) and chub mackerel (1.9%). In Argentina, YTK tend to be restricted to rocky reefs, which have a scarce and patchy distribution in the region, and this affinity to reef habitats may be related to their feeding behaviour. Observations on the feeding tactics of YTK in the Gulf of California showed a high degree of cooperation among individuals while performing well-coordinated foraging behaviour that involved fish enclosing schooling prey against the rocky reefs prior to feeding, (Vergani et al., 2008).

3.4 Growth, Longevity and Mortality

YTK have a maximum recorded length, weight and age of 250 cm, 96.8 kg and 21 years, respectively. In Australia, the largest recorded YTK was about 200 cm in length and 70 kg in weight but as indicated by size and age frequency catch data for NSW (Figs. 82, 83 and 84), fish considered large are commonly around 100 cm and 10-15 kg and 10-12 years old.

FIGURE 82: Typical haul of YTK Photograph: Paul Jennings, (Source: http://www.sardi.sa.gov.au)

NSW stocks of YTK grow to a mean length of about 450 mm in their first year. Thereafter to an age of about 11 years annual growth increments are essentially constant, progressively diminishing to about 90 mm in year 6. Growth of NSW stocks of YTK under relatively warm temperature regimes is considerably faster but terminal size considerably smaller than counterparts in colder New Zealand and the United States waters. Annual growth rates of 144 mm/y for 500 mm TL fish in NSW compare with 98 mm/y in New Zealand and a range of 34 -10 mm/y in the USA for similar size fish (Gillanders et al.,1999; Stewart et al., 2004).

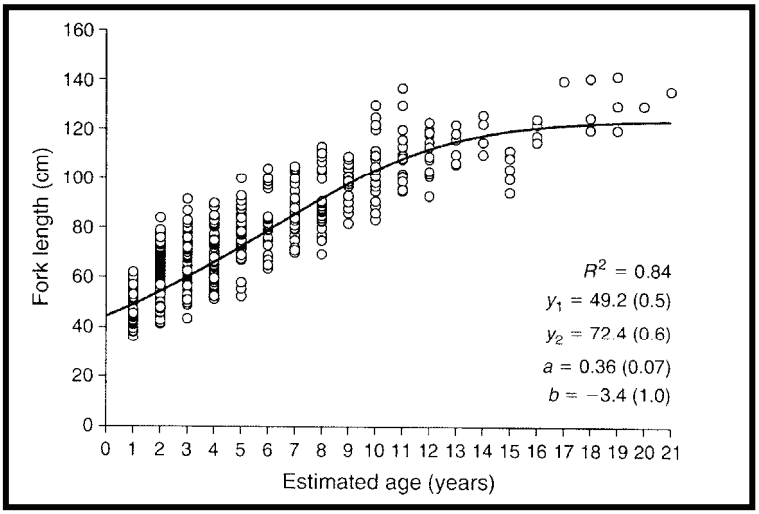


FIGURE 83: General growth curve for YTK off New South Wales (Source: Stewart et al., 2004).

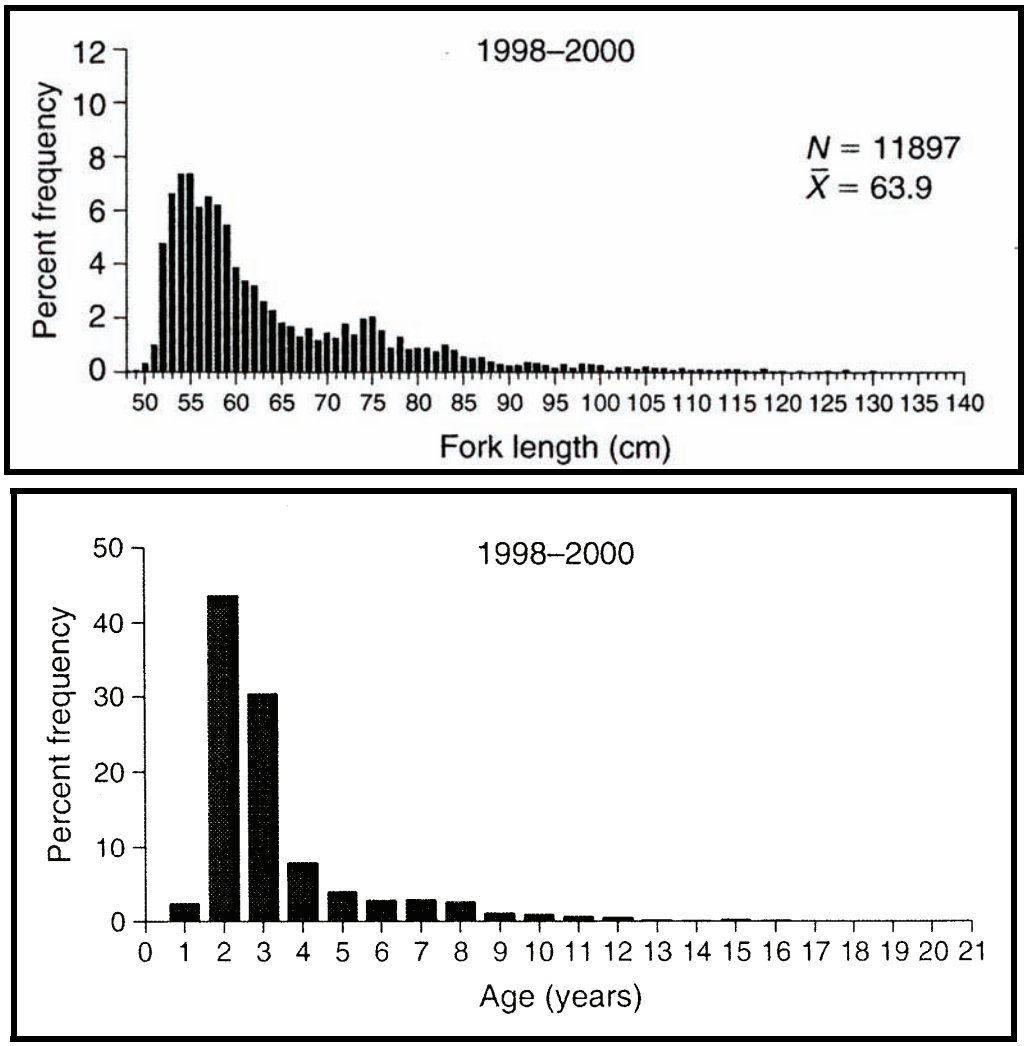


FIGURE 84: Size and estimated age composition of commercial landings of YTK between 1998 and 2000. (Source: Stewart et al., 2004).

Combined natural and fishing mortality rates of YTK on the east coast of Australia (NSW) has been estimated in the range from 35 to 55% per year for 3-14 year old fish with the major losses being due to fishing rather than natural (predation and disease) factors.(Stewart et al., 2004)

3.5 Hatchery Protocols - Yellowtail Kingfish (YTK) -

3.5.1 Introduction

For the most part broodstock, larval and nursery rearing equipment and operating protocols already described for mulloway in this document (see Chapter 2), and for snapper in good detail by Partridge et al., 2003 also apply to YTK. What follows is an account of variations on equipment and operating protocols that apply generally to kingfish (*Seriola* spp.,) and in particular to YTK (*Seriola lalandi lalandi*) as described in Moran (2007), Poortenaar et al., (2001) and PIRSA (2002).

3.5.2 Husbandry of captive broodstock

As with many other fish including Australian Bass and mulloway (see Chapters 1 and 2) viable gametes can be obtained from either wild, captive or cultured YTK broodstock. However wild, YTK broodstock suffer severe stress at capture and offshore spawning sites are relatively inaccessible. Breeding condition regresses while the fish are held in interim quarantine holding facilities. Quarantined fish are typically held for a fortnight before being transferred to breeding tanks. During this period they are rid of parasites by bathing in specialised chemo-theraputant or formalin baths followed by a freshwater bath and, ideally, tested for carrier status in relation to VNN (see Chapter 4), although no reliable, non-destructive test is available as yet for nodavirus detection.

Breeding tanks are typically 20-70 m^3 or occasionally larger, and at least 2 m deep with reported stocking rates of 5-14kg/m^3. Although captive broodstock are usually fed fresh or frozen premium quality diets e.g. chopped fish, squid, it is recommended that they are weaned onto a semi-moist or dry pellet (50.5% protein, 24% lipid) with vitamin and mineral supplements. Reason for the latter is that in kingfish (*Seriola* species), a diet of soft-dry pellets may produce 2-5 times more eggs and larvae of superior quality (higher fertilization rates and fingerling yields). While the majority of research on broodstock nutrition in marine fish concerns the levels of essential fatty acids (EFA's) required to support normal larval development, the optimal amounts of various EFA's for *Seriola* broodstock are not known. It does however appear that the ratio of EFA is likely to be more important than the absolute quantities of the individual EFA's. Several studies on the broodstock nutrition of congeners of YTK *(S. quinqeradiata)* have shown that certain carotenoids, in particular astaxanthin, improves egg and larval quality. In one study, astaxanthin improved egg buoyancy, fertilization and hatching rate and prolonged the period of egg production. However not all carotenoids appear beneficial to broodstock. For example β carotene is very poorly absorbed and high levels of β carotene in broodstock diets have little effect on the subsequent egg and larval quality. Feeds are administered at 1 to 3 % or l0 % of total fish weight daily for pellet and wet diets, respectively.

Wild caught YTK broodstock spawn spontaneously in captivity within 1 to 2 breeding seasons of domestication. If in the shorter term, hormone treatments are required to stimulate maturation and spawning, the timing of treatments need to coincide with critical stages of reproductive development. Females with oocytes less than 650pm in diameter do not spawn, females with oocytes around 700pm spawn but subsequent fertilization and hatch rates are poor, whereas females with oocytes greater than 800pm in diameter produced vast quantities of good quality eggs. As with Australian Bass (Chapter 1) and mulloway (Chapter 2), samples of oocytes can be collected from anaesthetised broodstock by inserting a biopsy tube (catheter) into the genital opening. Three different hormonal treatments have been successfully used to induce oocyte maturation and ovulation in kingfish, including a single injection of hCG at 500 IU/kg (as described for Australian Bass (see Chapter, 1 pp 12 to 18), priming injections of HCG one day after the single hCG, and injection and single implantation of LHRHa at 220-400 pg/kg in a cholesteropl pellet. While the latter (LHRH implantation) is superior in terms of egg quality and yields of eggs, a single injection of hCG is a much cheaper procedure.

As discussed above (Chapter 3.2), YTK are serial spawners, breeding under ambient conditions beginning in late spring or early summer and continuing to autumn over temperatures in the range 17-24°C. Photoperiod at the start of the natural spawning season (November) is about 13.5 h light :10.5 h dark, and increases to a maximum day length in mid December (14 h light:10 h dark) before decreasing to 11.5 h light:12.5 h dark at the end of the spawning season (April). In the southern hemisphere, when held under constant temperature 20 ±1°C and natural photoperiods, YTK spawning and larvi-culture occurs from November until the end of February, after which time egg production and quality tends to decrease to a degree that makes it unviable for commercial purposes. At PSFI, year-round, on-demand spawning of captive YTK broodfish has been achieved from a single tank of fish (see Chapter 2 for description of tanks) containing 7 pairs of wild collected fish. After initial exposure to a truncated phototherm (Table 8), fish have

been held at a constant 10h:14h light: dark regime and at 16^{o}C. Spontaneous spawning can be induced to occur 3-4 days after the water temperature is increased from 16 to 22^{o}C within 24-48h.

Courtship behaviour involves one male and female, and consists of a high-speed pursuit punctuated by stalling, nipping and touching. This lasts for approximately 0.5–1.5 h until, immediately prior to spawning, males nip at the female gonoduct, presumably to induce spawning. An additional male becomes involved at this stage in 50% of spawns. Release of gametes involves frenzied circling behaviour near the bottom of the tank and lasts 20-25 seconds.

Spawning occurs either just prior to dawn during the first half of the spawning season and 1 h either side of dusk in the latter half. In commercial hatchery operations it is often desirable to extend the natural spawning season or stimulate out-of-season spawning to increase production. This is achieved with YTK as in mulloway (see Chapter 2 for detailed description of equipment and protocols) by controlling the temperature and photoperiod into abbreviated seasonal cycles called photo-therms. Optimum spawning temperature for YTK is 2l.5ºC. As discussed above, at the start of the breeding season spawning occurs early in the morning around dawn or the time of the simulated sunrise in controlled photo-therm systems, meaning eggs could be collected very close to the spawning event. However after December spawning tends to occur at night, precluding the immediate collection of eggs.

TABLE 8: Compressed seasonal photoperiod and temperature regime used in the phototherm rooms at PSFI. This regimen is suitable for mulloway and yellowtail kingfish as well as snapper, for which it was originally designed.

Compressed (15 min) regime						
Date	**Daylength**	**Light ON**	**Light OFF**	**CHANGED**	**Temperature ^{o}C**	**Day**
19-Dec	10.5	6 45	17 15		16	1
24-Dec	11.25	6 15	17 30		16.5	5
29-Dec	11.75	6 00	17 45		16.5	10
3-Jan	12.5	5 30	18 00		16.7	15
8-Jan	13	5 15	18 15		16.95	20
13-Jan	13.5	5 00	18 30		17.2	25
18-Jan	13.5	5 00	18 30		17.65	30
23-Jan	14.25	4 45	19 00		18.1	35
28-Jan	14.25	4 45	19 00		18.95	40
2-Feb	14.25	4 45	19 00		19.8	45
7-Feb	14.25	4 45	19 00		20.4	50
12-Feb	13.25	5 15	18 30		21	55
17-Feb	12.75	5 30	18 15		21.4	60
22-Feb	12.25	5 45	18 00		21.8	65
27-Feb	11.75	6 00	17 45		22	70
4-Mar	11.25	6 15	17 30		22.2	75
9-Mar	10.75	6 30	17 15		21.85	80
14-Mar	10.25	6 45	17 00		21.5	85
19-Mar	10.25	6 45	17 00		20.5	90
24-Mar	10	7 00	17 00		19.5	95
29-Mar	10	7 00	17 00		18.85	100
3-Apr	10	7 00	17 00		18.2	105
8-Apr	10	7 00	17 00		16	110
13-Apr	10	7 00	17 00		16	115
18-Apr	10.5	6 45	17 15		16	120

3.6 Larviculture (Based on PIRSA, 2002; Moran et al., 2007; Benetti et al., 2002; Carton 2005; Kolkovski, 2005 and Kolkovski and Sakakura, 2004.)

3.6.1 Introduction and background

Spawned eggs from well nurtured YTK broodstock are positively buoyant, have a high fertilisation rate (90- 99%), range from 1.33 to 1.50 mm in diameter and have a single oil droplet 0.30–0.33 mm in diameter. In the case of natural spawning, fertilized eggs are collected from the surface of broodstock tanks with nets or screens or using automatic skimmers as described for mulloway hatchery production (see Chapter 2). Collected eggs are rinsed and treated with l00 ppm formalin or preferably with ozone to disinfect them of bacteria, fungi and viruses. Disinfected fertilised eggs are placed in sloping bottom tanks and maintained under 12:12 photoperiod conditions. As illustrated in Figure 85, incubation (time to hatch) is 2-4 days depending on water temperature in the range 16 and 24°C. Mean ± s.d. egg viability within the floating fraction over a complete spring / summer reproductive season has been reported as 74% ±17% (approximate range 50-90%).

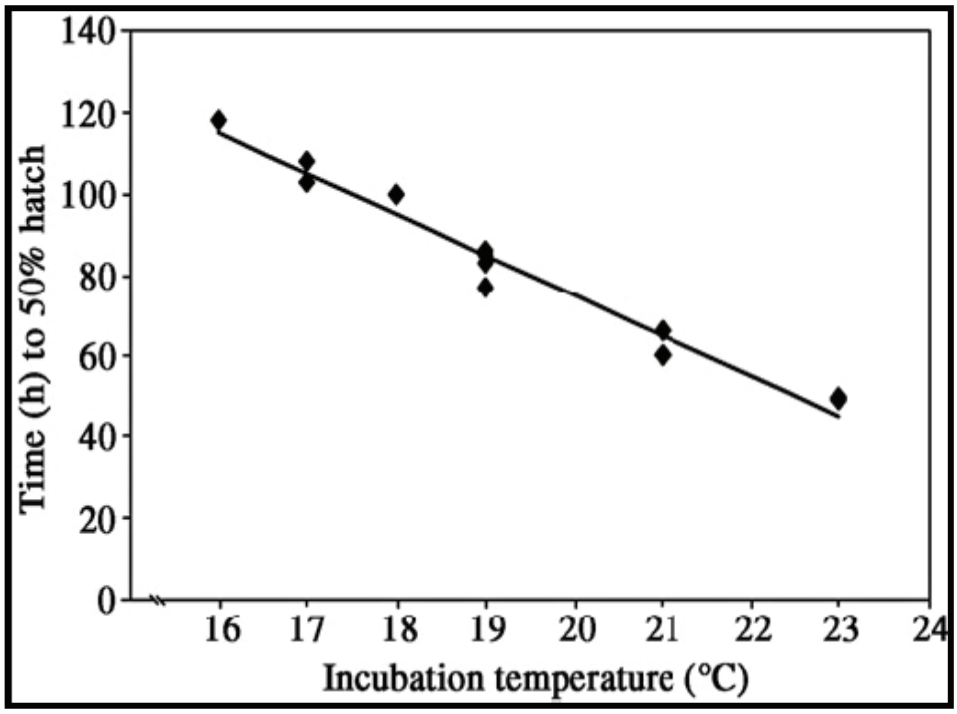


FIGURE 85: Correlation between incubation temperature and time to 50% hatch for YTK . The regression function is represented by y=−9.99 x+274.84, r=−0.98, P≤0.01. (Source Moran et al., 2007).Correlation between incubation temperature and time to 50% hatch for YTK. The regression function is represented by y=−9.99 x+274.84, r=−0.98, P≤0.01. (Source Moran et al., 2007).

Indistinct cell margins and asymmetrical cleavage during blastomere formation (Fig. 86g) are the most common deformities observed. Egg and oil droplet volume may decrease by 15–20% over the spawning season, though no associated fall in egg and larval viability has been reported. For instance while larvae hatch at a smaller length with a larger yolk sac and oil droplet at warmer incubation temperatures, there is no substantial difference in the maximum larval length reached at the onset of first feeding across incubation temperatures in the range 16 to 24°C.

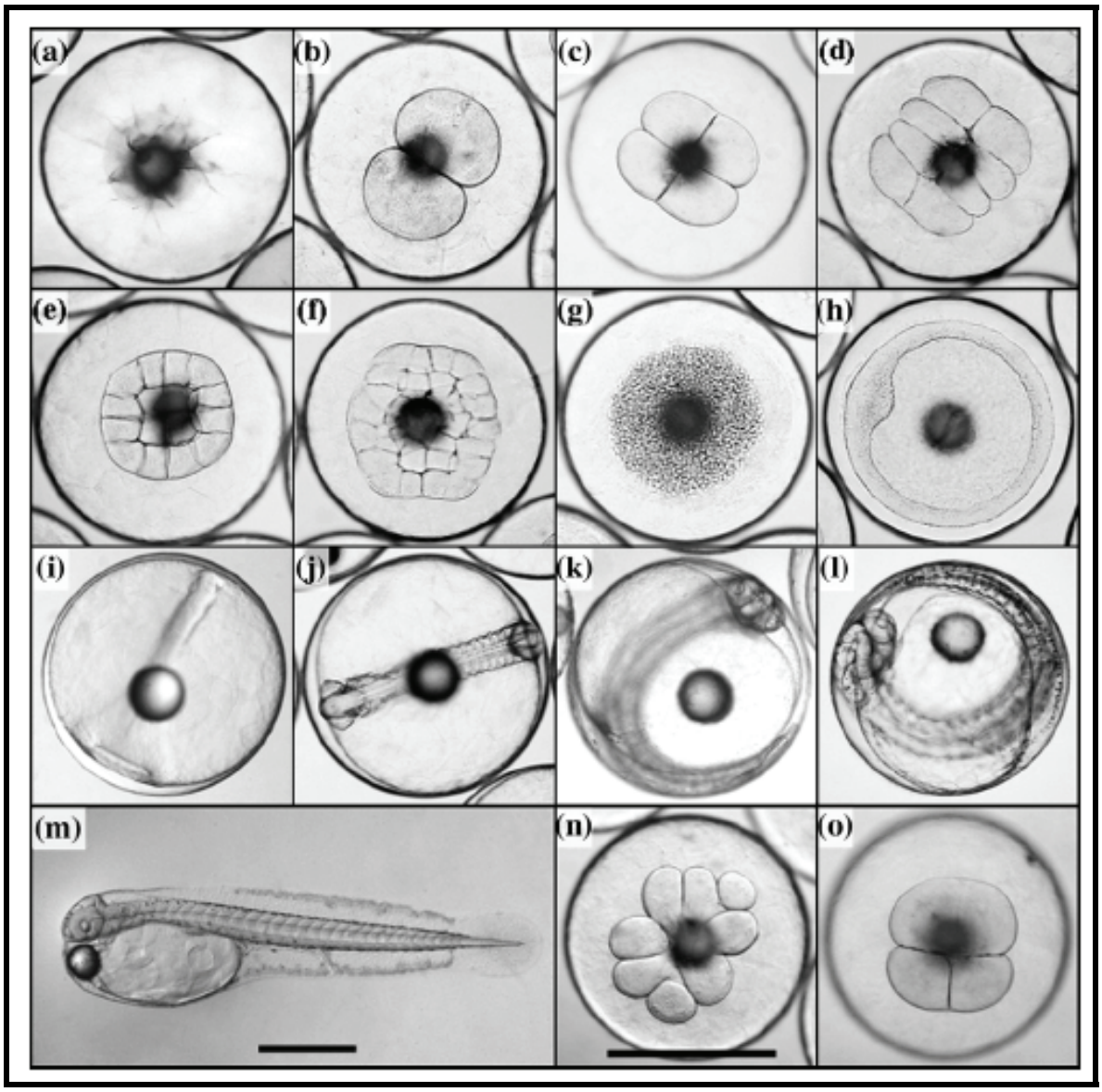


Figure 86: Developmental stages and cleavage abnormalities of YTK: (a) pre-cleavage; (b) 2 cell; (c) 4 cell; (d) 8 cell; (e) 16 cell; (f) 32 cell;(g) mid-stage blastula; (h) gastrula; (i) appearance of embryo; (j) 20 myomere embryo; (k) advanced embryo; (l) pre-hatch embryo; (m) larva 4 h posthatch; (n) asymmetrical cleavage in blastula; (o) indistinct cell margins in blastula. Scale bar for (a)–(l) and (n)–(o) shown in (n); scale bars represent1 mm. (Source: Moran et al 2007).

3.6.2 Larviculture protocols

YTK (*Seriola* spp) larvae grow faster than many other marine finfish including those of Australian Bass (see Chapter 1) and mulloway (see Chapter 2) (Figs. 87, 88 and 89). The eggs and first feeding (Day 3-4) larvae are relatively large averaging about l.l mm diameter and 4.5 mm TL, respectively. Therefore relatively standard hatchery equipment and rearing protocols as already described in detail for mulloway (see Chapters 2) can be followed (Table 7). YTK larvae are stocked into rearing vessels commonly in the range 1 to 5 m^3 as previously described for mulloway (see Chapter 2) at densities ranging from 20-100/l into water conditioned with algae, and aeration is used to maintain the larvae in gentle rolling suspension to help reduce the incidence of deformities and early mortalities (see Chapter 4 below). As for mulloway larvae (see Chapter 2), surface skimmers fitted to larval rearing tanks are essential to ensure normal swim bladder inflation. YTK larvae begin feeding 3-4 days after hatch once the yolk-sac has been absorbed and jaw development completed.

Feeding performance of YTK larvae increases with age and light intensity under both clearwater and greenwater rearing conditions, demonstrating that visual feeding proficiency increases with larval stage. Feeding intensity remains low over the first 3 days of feeding regardless of light intensity. On days 6 and 7 after hatch, larvae show considerably higher feeding intensity particularly at light intensities in the range 8 and 17 mu mol/ sec/ m^2 (≈1600 to 3400 lux). This improvement indicates an ontogenetic shift in sensory acuity and/or swimming competence. First-feeding larvae perform equally well in clear-water and green-water up to algal cell densities of 8 x 10^4 /mL, although at a low light intensity of 0.1 mu mol/sec/m^2 (≈ 20 lux), feeding performance is significantly constrained. The ability of YTK larvae to capture and consume free-swimming prey during the first-feeding window is also impeded under high algae cell densities above 16 x 10^4 cells/mL, thereby undermining the suitability for large scale production in outdoor greenwater ponds.

Hatchery reared YTK larvae are initially fed enriched small or large strain rotifers at 10-30 rotifers mL and, if available, wild collected or pond produced copepods. Enriched *Artemia* meta-nauplii are subsequently added to the diet l0-14 days after hatch. Feeding protocols are in fact essentially identical to those already described in detail for mulloway (see Chapters 2.81 and 2.8.2 for details). Metamorphosis occurs approximately 20 days after hatching coincident with weaning onto inert formulated foods and is usually completed 40-50 days after hatching.

TABLE 9: Optimal rearing parameters and feeding schedule for YTK larvae used at PSFI.

Species: Yellowtail Kingfish (*Seriola lalandi*)			
Parameter	**Target**	**dah**	**Adjustment**
pH	7.6 - 8.2	0+	
Dissolved Oxygen (mg/l)	>6.00	0+	Use compressed oxygen diffuser to maintain saturation level
Temperature (°C)	22	0+	Increase post SB inflation
Salinity (ppt)	25 - 35	0+	
Water Exchange (%/day)	100 - 200	0+	Increase exchange as larvae develop
Surface Skimmer (hrs/day)	24	2+	Monitor skimmer to ensure larvae at water surface are not affected
Photoperiod (L:D)	(12:12) (18:06)	(0+) (6+)	Increase post SB inflation
Light Intensity (Lux)	225-400	0+	
Green-water (cells/ml)	1.4×10^6	0+	Pro-Aqua* concentrate 57×10^9 per ml
Rotifer (R/ml)	20.0 - 5.0	4+	Initial 20/mL until feeding and then increase frequency of reduced concentration (e.g. 4x5/mL/d).
***Artemia* (A/ml)**	0.2 - 2.0	12+	0.2/mL until weaned, then increase concentration and frequency
Weaning Diet size (µm)	200 - 800	18+	Commence weaning at 10 mm TL

*Algae concentrate used Rotifer Diet-3600 (*Nannocholoropsis/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 ≈100,000 lux ≈ 10,000 foot candle ≈ 500 µmol/m2/sec (microeinsteins/square metre/second)

Water temperature is typically maintained at the higher end of the optimum range (20–28 ºC), although recent data suggest that 21-23°C may be optimal for survival and low incidence of deformities (pers. comm., SARDI, 2010). The water exchange rate is gradually increased from 4 L/minute (1 – 2 x exchanges per day) at the time of stocking, up to 20 L/minute (4 - 8x exchanges per day) immediately prior to weaning.

As in other finfish larvae, such as those of Australian Bass (Chapter 1) and mulloway (see Chapter 2), first feeding in YTK larvae is a major hurdle and adequate nutrition is critical to the success of this phase. Also in common with larvae of most other marine finfish, essential fatty acids (EFA's), in particular docosahexaenoic acid (DHA), are critical for normal development. DHA is accumulated in the central nervous system of YTK larvae and is essential not only for activity and vigour but also for the development of schooling behaviour in juveniles. Studies on the effect of the different EFA's on the growth and survival of kingfish larvae such as those of *S. quinqueradiata* have shown that growth and survival rate of those fed DHA-enriched *Artemia* at 2.1- 2.5% dry wt./day is up to ten times better (88%) than larvae fed *Artemia* enriched with other EFA s including eicosapentaenoic acid (EPA), arachidonic acid (AA) or oleic acid (OA).

Generally there are 2 peaks of mortality in rearing kingfish (*Seriola* spp) larvae. The first is the so-called 'critical period' of high mortality from hatch to first feeding especially during the mouth-opening phase/first feeding stage (day 3 or 4 after hatch), during which larvae sink to the bottom of rearing tanks. This phenomenon can be mitigated by imposing strong upwelling currents that are also of substantial benefit for retaining the inert particles longer in the water column especially if live feeds are partially or totally substituted by inert micro particulate diets. The second mortality peak is caused by cannibalism. The first obvious signs of aggressive interactions becomes evident as early as 12 days after hatch (6-7 mm total length), with both the large and medium size individuals displaying threatening (aiming) behaviour at smaller siblings. The onset of cannibalism does not occur in small larvae but coincides with increased growth rate and size heterogeneity and the onset of metamorphosis 18 to 22 days after hatch at a mean size of 10 mm, cannibals being able to successfully prey on fish up to half of their own body size.

Fortunately cannibalism is a relatively fleeting phenomenon in YTK progressively waning from around day 30 after hatch (>l2mm TL) as schooling behaviour takes over. A clear dominance hierarchy exists within schools of post-larvae although it is likely that the ranking order changes with time. The aggressive behaviour of larvae is affected by temperature and light intensity, stocking density, feeding level, starvation, and the size-difference between fish (heterogeneity). Aggression in general, and cannibalism in particular, is exacerbated by increasing temperature over the range 15–30ºC, starvation (food deprivation) periods exceeding 12 hours and is highest at medium light intensity. Numbers of aggressive encounters decreases as density is increased probably due to the inability of predators to single out and attack individual smaller prey at very high densities. Aggression in dominant fish also increases at higher densities and based on practical experience with other highly aggressive carnivorous fish species, it is likely that the overall level of cannibalism at intermediate densities will increase with increasing density. Although the above factors exaggerate the level of aggression in larvae, aggression still persists under low density, well-fed conditions amongst individuals of the same size. In order to reduce the level of cannibalism in cultured conditions the recommended optimum stocking density is 3 fish /L. Fish should also be size-graded regularly (e.g. weekly) beginning at about 10 mm TL (Fig. 90). At night time, juveniles cease swimming and drift at the surface in dense aggregations and are therefore easy to handle and grade. Indeed night time grading, has shown to increase survival yields by 1.5 to 3 times and is therefore recommended .

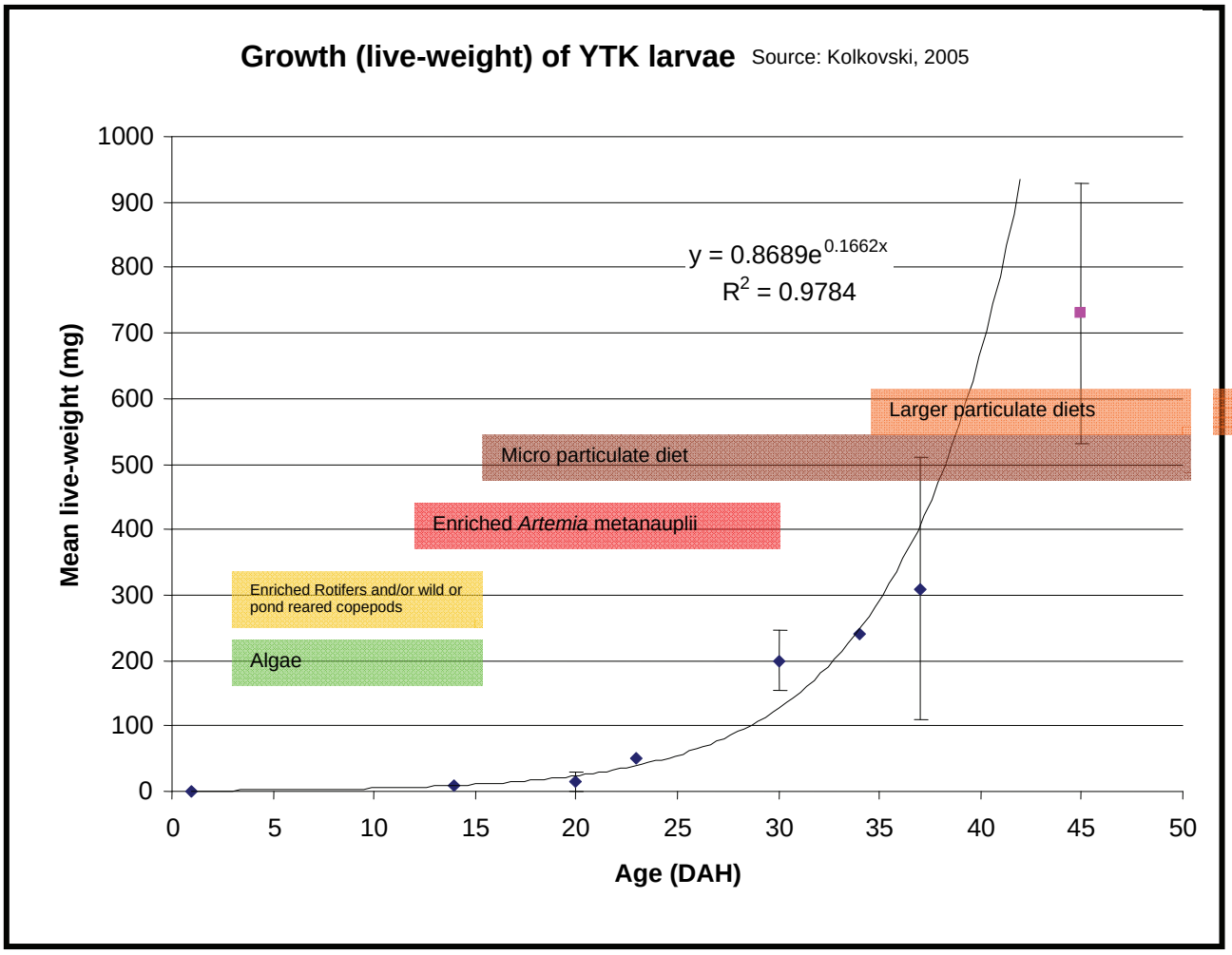


FIGURE 87: Typical exponential growth curve and feeding regimen for YTK larvae and post-larvae. (Source: redrawn from Kolkovski, 2005).

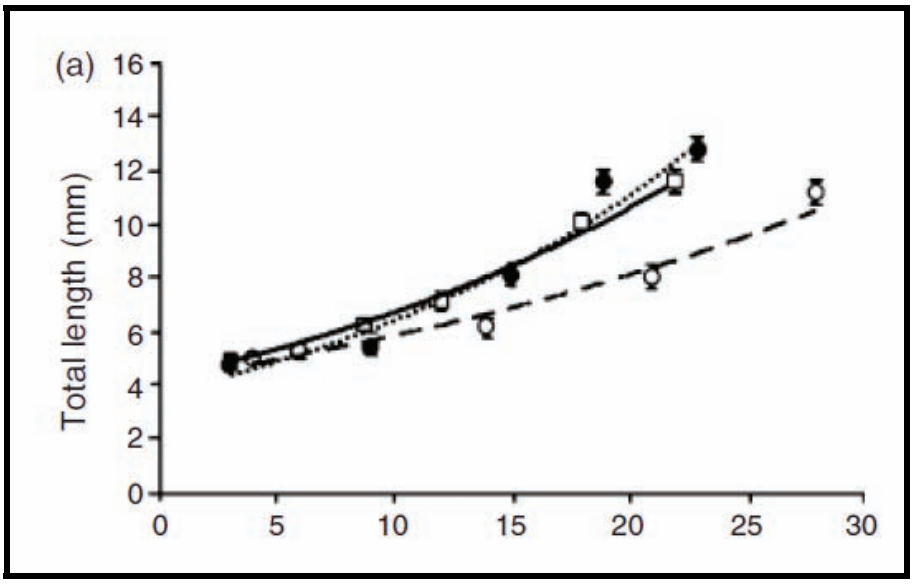


FIGURE 88: Typical exponential growth curves for three commercial batches of YTK larvae and post larvae cultured between 21 and 23 ºC. (a) Total body length during development (mean ± SE), n = 20 for each data point): Batch 1 (□) y = 3.68 $e^{(0.055x)}$, r = 0.99; Batch 2 (○) y =4.18 $e^{(0.033x)}$, r =1.00; Batch 3 (●) y = 54.22 $e^{(0.048x)}$, r = 0.99, (Source: Moran et al., 2007).

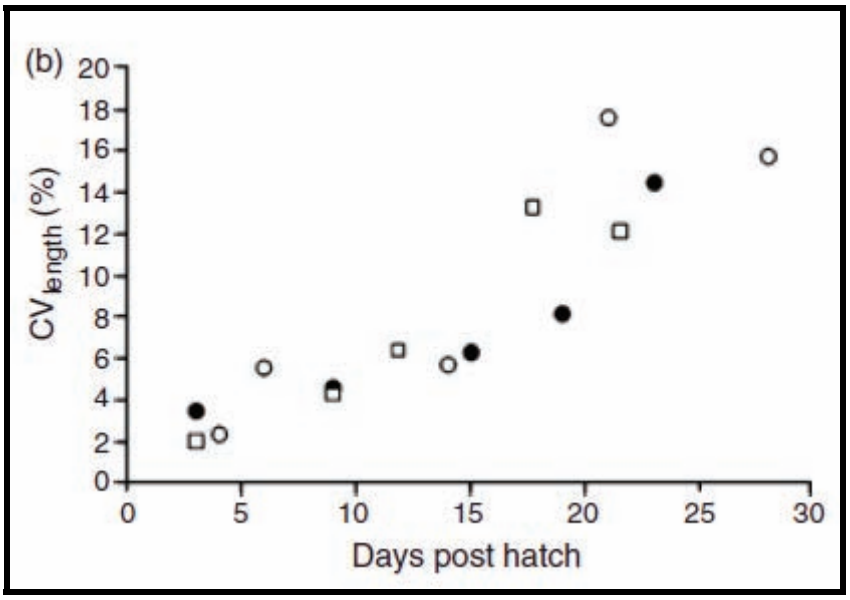


FIGURE 89: Co-efficient of variation of body length during development of YTK larvae Symbols as in Figure 90. (Source: Moran et al., 2007).

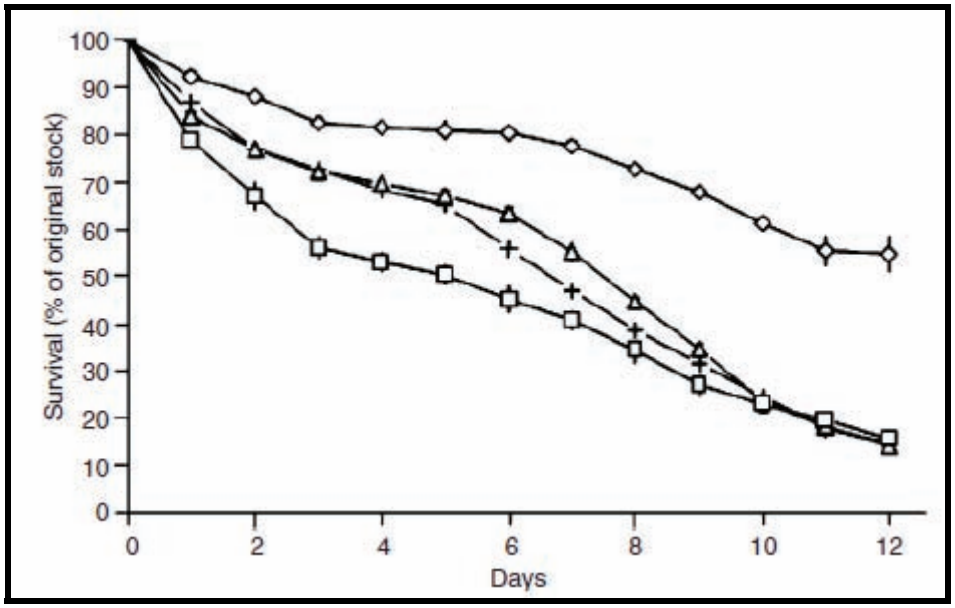


FIGURE 90: Larval survival during the grading trial. Survival is shown as the mean ± SE survival from three replicate tanks (+) = control ungraded; (◊) = large grade; (Δ) = medium grade; (□) = small grade. (Source: Moran et al., 2007).

While relatively low rates of survival are to be expected with YTK larval as with those of other species of kingfish, survival rates to metamorphosis and weaning have nevertheless been progressively improved from initial rates of 0.1 to 2 % up to 5 to 10% currently with adoption of the measures discussed above.

An important lingering problem with hatchery production of YTK and other kingfish is the level of deformities. This problem is common to fish cultured in different places including Japan, Australia and New Zealand. These deformities range from fused vertebrae and scoliosis, bent and/or shortened lower jaws, incomplete or absent gill covers (opercula) and compacted body and tails (Figure 91a-d). Although the full array of factors causing deformities are yet to be determined and negated, significant progress in mitigating these deformities has been made through improved nutrition. Feeding *Artemia* metanauplii (but not rotifers) enriched with 'mega' doses of vitamins E and C to YTK larvae is effective in significantly lowering the incidence of these deformities.

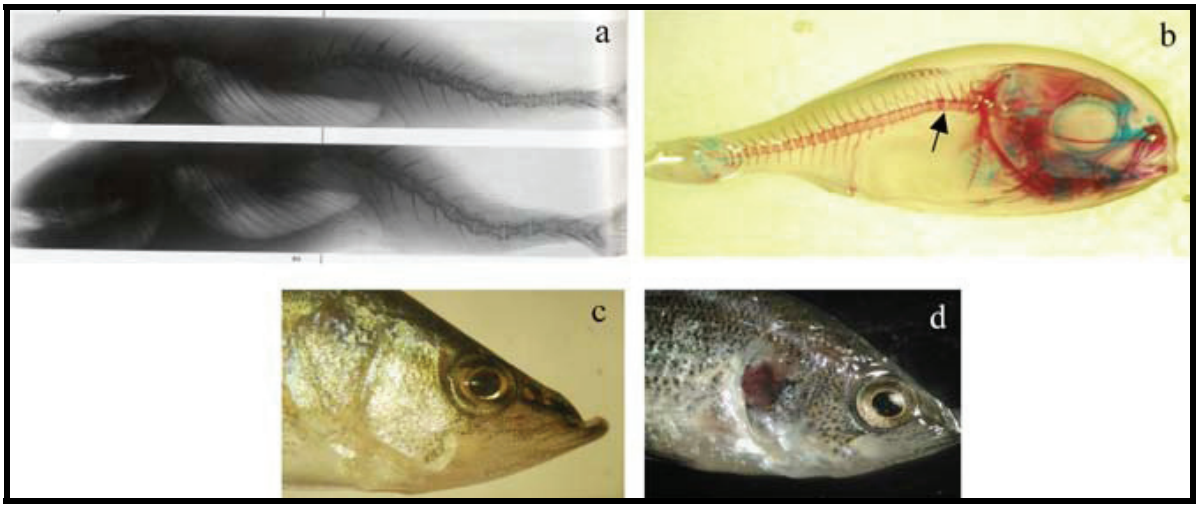


FIGURE 91: Photomicrographs of common deformities of hatchery-reared YTK. (Source: Kolkovski, 2005).

3.6.3 Weaning

Recent dietary research on greater amberjack (*Seriola dumerilli*) showed that there may be considerable advantages to growth performance and body condition factor in applying the following dietary protocols to nursery rearing and subsequent on farming of YTK (Fig. 92):

- Feed juveniles from the point of weaning through to a weight in the range 300 to 400g on soft pellets comprising a 50:50 blend of fish and formulated pellets (35 % moisture)
- change the diet to a 40% moisture soft pellet for a short interim period of 1 month
- change the diet again to an intermediate 20% moisture soft pellet for the next 3 months
- Adopt a standard dry diet (7% moisture) thereafter.

3.7 Summary of "best-practice" Rearing Regimes for YTK

"Best-practice" rearing regimes for YTK larvae at PSFI is summarized in Table 10.

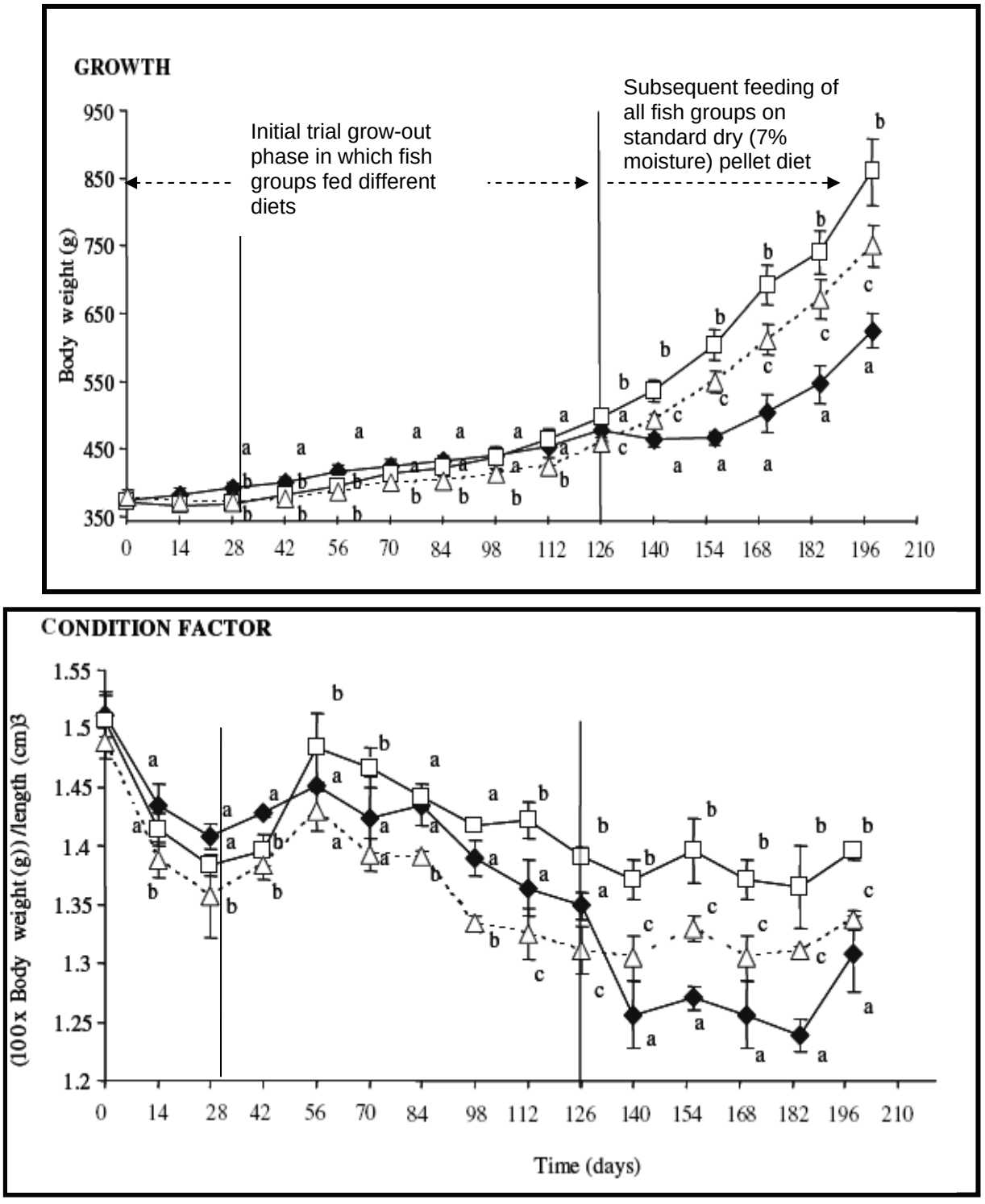


FIGURE 92: Effect of formulated diet moisture content (♦ 40%, □ 20%, Δ 7%) on: (a) growth and (b), condition factor of juvenile amberjack (*Seriola dumerilli*). Values are mean ± SD (of three replicate groups). Statistical differences within sample days are represented by different letters (a, b, c) All fish fed moist diet comprising a blend of 50% fish flesh and 50% dry crushed pellet from weaning to commencement of experiment (Source: Papadakis et al., 2008).

TABLE 10: The "best-practice" regime for yellowtail kingfish larval rearing used at PSFI.

SPECIES: YELLOWTAIL KINGFISH (*SERIOLA LALANDI*)			
BREEDING & DEVELOPMENT	**UNIT**		**COMMENT**
BROODSTOCK ORIGIN	WILD-CAUGHT		CAPTURED FROM INSHORE COASTAL AND DEEP OFFSHORE OCEAN SITES
BROODSTOCK TANK SIZE	22,000 L		
SPAWNING INDUCTION	PHOTOTHERM MANIPULATION		INCREASE WATER TEMPERATURE FROM 16 TO 22^{O}C OVER 24 -48 H INDUCES SPAWNING
TANK SIZE FOR SPAWNING	22,000 L		1 : 1 MALE:FEMALE (14 FISH)
LATENCY PERIOD TO SPAWNING	72 – 96 H		AFTER TEMPERATURE REACHES 22 ^{O}C
METHOD OF FERTILISATION	SPONTANEOUS SPAWNING		FERTILISATION OCCURS WITHIN SPAWNING TANK
EGG INCUBATION TANK SIZE	500-1000 L		
TIME TO HATCH	60 H		AT 22 ±0.5^{O}C
LARVAE TANK SIZE	2000-10,000 L		INTENSIVE GREENWATER CULTURE
LARVAL YOLK-SAC PRESENT	0-3 DAH		AT 22 ±0.5^{O}C
LARVAL FIRST-FEEDING	2-4 DAH		AT 22 ±0.5^{O}C
LARVAL SWIMBLADDER INFLATION	3-7 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**
PH	7.6 - 8.2	0+	
DISSOLVED OXYGEN (MG/L)	>6.00	0+	USE COMPRESSED OXYGEN DIFFUSER TO MAINTAIN SATURATION LEVEL
TEMPERATURE (°C)	22	0+	
SALINITY (PPT)	25 - 35	0+	
WATER EXCHANGE (%/DAY)	100 - 200	0+	INCREASE EXCHANGE AS LARVAE DEVELOP
SURFACE SKIMMER (HRS/DAY)	24	2+	MONITOR SKIMMER TO ENSURE LARVAE AT WATER SURFACE ARE NOT AFFECTED
PHOTOPERIOD (L:D)	(12:12) (18:06)	(0+) (6+)	INCREASE POST SB INFLATION
LIGHT INTENSITY (LUX)	225-400	0+	
GREEN-WATER (CELLS/ML)	1.4 X 10^{6}	0+	PRO-AQUA* CONCENTRATE 57X10^{9} PER ML
LARVAL FEEDING SCHEDULE	**TARGET**	**DAH**	**ADJUSTMENT**
ROTIFER (R/ML)	20.0 - 5.0	4+	INITIAL 20/ML UNTIL FEEDING AND THEN INCREASE FREQUENCY OF REDUCED CONCENTRATION (E.G. 4X5/ML/D).
***ARTEMIA* (A/ML)**	0.2 - 2.0	12+	0.2/ML UNTIL WEANED, THEN INCREASE CONCENTRATION AND FREQUENCY
WEANING DIET SIZE (µM)	200 - 800	18+	COMMENCE WEANING AT 10 MM TL

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

4. HEALTH MONITORING AND DISEASE PREVENTION, DIAGNOSIS AND TREATMENT

4.1 Introduction

Australian bass and mulloway are relatively hardy fish with relatively few disease problems when raised in good quality water in combination with other good husbandry practices. Both species are euryhaline and are capable of tolerating a wide range of naturally occurring water conditions, including variations in salinity, turbidity and temperature (see Chapter 1, Table 2 and Chapter 2, Table 6).

By contrast Yellowtail kingfish, as is typical of marine species, are stenohaline and ill equipped to deal with large environmental variations including fluctuating salinity, high turbidity, or pH outside the narrow range 7.5 to 8.5. They are however surprisingly tolerant of large, short to intermediate term declines in dissolved oxygen.

While many fish health problems can be avoided by careful attention to water quality and other good husbandry practices, once a disease problem occurs the cause must be identified as soon as possible. Once the cause of the disease is known, specific treatments can be used to reduce further losses and the economic impact of the disease. Constant routine measurement of water quality variables is essential as is vigilance in continually maintaining variables within tolerable limits. The maintenance and frequent reference to water quality records will help managers identify underlying factors that pre-disposed fish to disease and to take early action that may prevent recurrence or at least reduce the severity of the disease.

The severity of any disease outbreak depends on the interaction of three groups of predisposing factors illustrated in Fig. 93.

1. The fish themselves, especially their genetically determined natural susceptibility /resistance to particular disease agents. Disease susceptibility may vary substantially with the species, age or life cycle stage of fish and their previous exposure to the disease, including vaccination.
2. The culture environment. Provision of a poor or suboptimal diet and/or exposure to poor or suboptimal physiochemical environmental conditions, overcrowding and handling stress all influence susceptibility to disease.
3. The type and virulence/toxicity of the disease organism/agent

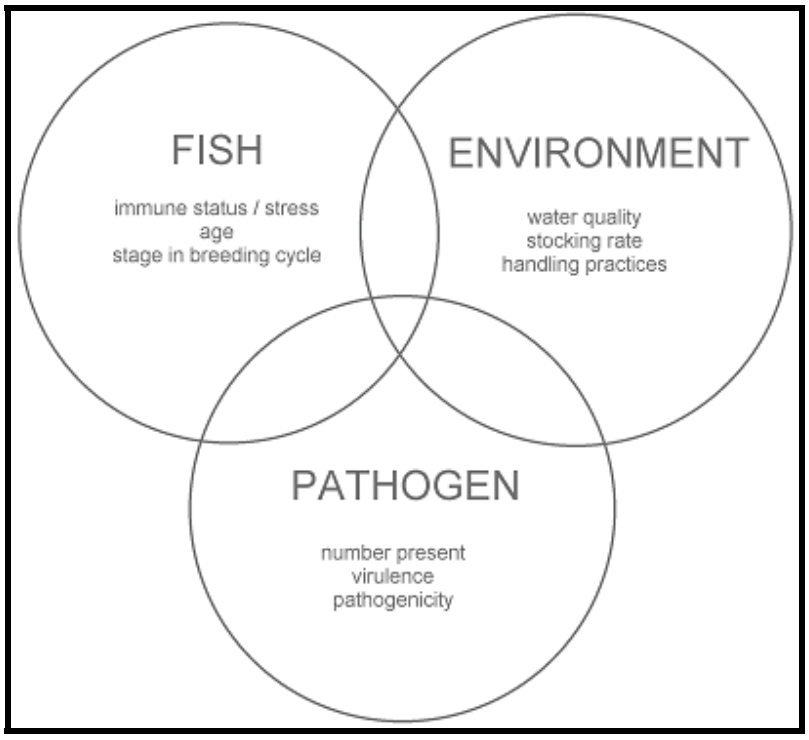


FIGURE 93: Diagram illustrating the interaction between the fish, the pathogen causing disease and the environment. (Source: Partridge et al., 2003).

Apart from a few catastrophically virulent diseases such as nodavirus infections of young immune-incompetent larval and juvenile fish (see Section 4.5 below), it is the interaction between these three factors that determine the severity of a disease or whether it occurs at all. For example, in the summer months, high water temperatures increase growth and food intake leading to increase waste production and thence reduced oxygen levels that compromise the general health and disease resistance of fish. Such increases in disease susceptibility are often coupled with increases in the replication rates of potential pathogenic microorganisms (bacteria, fungi and protozoans) thereby further exacerbating the likelihood of disease outbreaks (epizootics).

Important components of a successful health management strategy within fish hatcheries including those producing Australian bass, mulloway and yellowtail kingfish are as follows:

1) Take all reasonable measures to exclude disease agents but be prepared with a health management plan if disease occurs to limit the impact and spread of disease within and beyond the hatchery.

2) If hatchery breeding stock need to be brought in, they must be *health certified* which means they must have a documented disease-free history and/or have tested negative when checked for disease carrier status in respect to specific pathogens where suitable tests are available.

3) If disease-free status cannot be established prior to receipt, incoming broodstock must be quarantined in a secure isolated area for 4 to 12 weeks of observation and subjected to prophylactic disease disinfection. (Quarantine is also recommended for health-certified broodstock brought in to the hatchery but a reduced period is satisfactory).

4) Risks of introducing disease with outsourced stock can be further reduced by separating different batches of fish. This precaution can be augmented by clever hatchery design, layout and operating protocols that minimise the probability of disease transmission via water and aerosols. Likewise, ensure that other potential routes (portals) of transmission such as on the skin and clothes of staff, on the surfaces of fish handling and treatment equipment such as buckets or water quality monitoring devices, are minimised using appropriate hygiene protocols. The latter must include mandatory routine cleaning, rinsing and disinfection of all facilities and equipment between successive uses and/or providing separate fully replicated (batch-dedicated) facilities, equipment, utensils and staff.

Disease prevention - Keep fish in optimum health and under minimum stress.

Healthy, well-fed fish kept in good quality water are much less likely to suffer from disease outbreaks. Many disease-causing organisms are commonly present in low numbers on the fish or in their environment. Healthy fish are more likely to withstand increases in the number of these potential pathogens in their environment. For broodstock held in controlled environment recirculation systems, disease prevention also entails disinfection of all new (incoming) seawater. The most appropriate of alternative seawater disinfection protocols, including ultra-filtration, chemical chlorination, UV treatment, ozonation or pasteurisation, depends on the disease agents being excluded, the susceptibility of host fish to such agents and the quality of source water and its accessibility in time, space and volume. As with seawater, pre treatment, including disinfection, of freshwater will vary in accordance with its source and quality, For example:

- Reticulated mains water should be routinely monitored for residual chlorine and de-chlorinated.
- Water sourced from streams or dams should be settled and /or pre-filtered to 1um nominal and sterilised while degassing.
- Ground water must be regularly checked for, pH, excessive alkalinity, DO and other potentially toxic dissolved gases, especially carbon dioxide and hydrogen sulphide.

Frequently observe and become familiar with the normal appearance and behaviour of the fish.

Early recognition of disease often results in decreased losses as treatment and management practices can be fine tuned at an early stage in the disease process. Recognition of normal behavioural patterns and healthy appearance of fish is very important for early recognition and prevention or mitigation of impending health problems.

In juvenile and adult fish, altered feeding behaviour, particularly reluctance to feed vigorously, is often the first sign of trouble. Other behavioural signs of stress and onset of ecto-parasitic diseases include signs such as ‘flashing' or a rapid rubbing movement on a surface of the tank; and in the case of gill parasites, gaping opercula, increased ventilation rates, “loitering” of the fish near the water inlets, ‘hanging' over an air source. Other warning signs are changes in physical appearance such as dark coloured skin lesions and a ‘hollow' or distended abdomen.

4.2 Larval Fish

As soon as the larvae start feeding, the following monitoring should be performed to assess health status. Quantitative evaluation of:

- predatory activity and feeding performance
- stress
- swim bladder development

4.2.1 Predatory activity and feeding performance

Presence and quantity of ingested food is the most important single indicator of fish health especially over the first 10 days of feeding. To do this a sample of 30-50 larvae should be sampled and microscopically examined according to the following schedule each morning an hour or so after the introduction of fresh live food.

- Pipette the larvae on a cavity or Sedgewick rafter slide
- Remove excess water with the pipette and put under the microscope
- Take a first look for body deformities not caused by handling.
- Observe the gut contents at x100 magnifications
- Look for whole rotifers or for their components such as mastax, lorica, or eggs.
- Look for other ingested material if any, and for internal/external parasites
- Look for the presence of calculi in the urethra and in the urinary bladder
- Record all findings on a specific form

Try not to damage the larvae with the pipette. Morphological condition assessment should be made as quickly as possible since heat irradiated by the microscope lamp causes shrinkage within minutes. The presence of ingested rotifers can be easily recognized by the presence of their masticatory mouth-parts (mastax), left undigested in the larval gut. Under a 100x magnification they clearly appear amongst rotifer egg and other debris. In practice only the number of mastax found is recorded to estimate the total number of rotifers eaten. The number of prey per larva ranges from 2-3 (early feeding) to over 50.

To check the ingestion rate of *Artemia* nauplii in older larvae, it is sufficient to visually estimate the percentage of repleted (fully fed) larvae sampled in a 100 ml transparent beaker by checking digestive tracts of the larvae for the presence of the deep orange nauplii that are visible through the transparent larval skin. Observation on prey ingested should be integrated with an assessment of fish behaviour as described below.

4.2.2 Quantitative evaluation of stress

Stress in fish larvae induces both morphological and behavioural changes that can be detected by the hatchery operator in order to improve culture conditions or to replace as soon as possible a poor larval batch. The main criteria for stressed fish larvae as described by Moretti et al. (1999) are:

- starvation
- calculosis (presence of deposits of calcium [usually as Ca oxalate] within the urinary duct)
- abnormally passive behaviour
- absence of "schooling ("in the first two weeks of life")
- frenzied crowding at the tank surface perimeter (meniscus)

Starvation is an obvious indication that something has gone wrong. lt is a general response to stress and often is impossible to link with a single cause. With the sole exception of acute toxicity to a chemical, all rearing parameters alone or more probably in association may stop larval feeding. As a starved fish will not survive for long, it is also important to monitor the onset of first feeding (see above).There is some scientific evidence that a direct correlation exists between environment-induced stress and the appearance of calculi in the urinary system of gilthead seabream and seabass larvae.

Although there is no confirmed correlation between calculi and death, calculi are often associated with starvation and consequently are present at a higher rate in dead larvae. The early appearance of calculi in a larval population is therefore considered as a stress indicator.

Calculosis can be easily detected by examining the lower part of the larval urinary duct (urethra and urinary bladder) under a microscope at 100 x magnification. This condition becomes evident by the appearance of a single stone-like corpuscle or a chain of smaller ones, reddish or grey in colour. Sometimes they completely obstruct the urethra. The count of renal calculi may be done when the repletion rate is being evaluated and can be recorded on the same sheet. When a large calculosis, say in more than 40% of the fish examined, is observed it can be interpreted as a sign of poor rearing conditions which typically will result in a low survival rate. ln this case environmental and feeding parameters such as the following should be checked:

- excessive water currents due to wrong aeration or water inflow;
- disproportionate prey size;
- insufficient light intensity;
- dangerous levels of some water quality parameters such as dissolved oxygen and/or
- total ammonia nitrogen (TAN) content

Another useful indicator of stress is a high proportion of fish that do not actively swim and attack prey. At the mercy of water currents, the passive behaviour of stressed fish prevents efficient hunting of live food. Such abnormal behaviour can be easily detected as glimmering points of light throughout the rearing tank. This effect is caused by the retinas of the inverted passively drifting and tumbling fish as they catch and reflect artificial light sources. Such events may lead to the loss of a substantial part of a brood. An unhealthy rearing environment, together with a possible congenital factor is considered as the most probable causes of this syndrome.

By contrast, healthy broods of fish display the following normal predatory behaviour:

- Searching behaviour - continuous swimming with side to side head movement in search of food;
- Pointing and close approach to prey;
- strike preparation marked by tail flexion;
- attack marked by rapid tail straightening and forward thrusting to capture prey;
- Prey gulping;
- Resumption of searching

The exact location and density of larval shoals depends on:

- Water currents induced by aeration and water inflow;
- tank shape and size and water depth;
- active counter-current swimming of the larvae as a reaction to an external stimulus such as avoidance of high light intensity or high concentrations of live food.

Tanks of healthy fish comprise one or more dense shoals of larvae, typically in the calmest places of the tank or slowly moving about. Samples to monitor the size and health status of the population are taken inside these shoals. A particular type of behaviour such as an erratic swimming at the water meniscus should also be considered as a possible response to stress. This syndrome is characterized by a frenzied activity of larvae which seem to be attracted by the water meniscus where they get stuck, beating the tank wall head-on or being shaken by spasmodic head-up movements. This impairs their feeding rate and consequently survival. Usually only a small or insignificant fraction of larvae show these symptoms but over a protracted period, suggesting chronic stress or unsuitable rearing conditions as the cause.

Mass mortality can however occur with significant proportions of larvae exhibiting the "meniscus-stress syndrome" leading to death most commonly in the age range of between 10 and 30 days. lf measures cannot be taken to counteract the stress inducing factors hatchery managers should consider the option of euthanizing the compromised population sooner than later and starting a new batch.

4.2.3 Monitoring and control of swim bladder development

At 18 -22ºC, swim bladder formation begins within 3 to 4 days after hatch. The first sign of inflation clearly visible at 20-40x magnification after 5 to 7 days, is a single small air bubble within a tissue vesicle. A few days later, a second bubble develops and joins the first to form an almost spherical body that will gradually expand into an elongated vesicle.

Initial activation of the swim bladder relies on air gulping and swallowing ingestion at the tank surface. A temporary duct between the swim bladder area and the mouth (typical of physoclistic fish such as Australian bass, mulloway and Yellowtail kingfish) makes this process possible. This active air swallowing is crucial to swim bladder development and if impaired, prevents proper swim bladder formation, as the duct remains open only for a few days. As experienced during the first 10 years of hatchery production of Australian bass (see Chapter 1) no or incomplete inflation of the swim bladder has severe consequences for developing larvae. These include spinal deformities, limited or negative buoyancy and abnormal swimming behaviour and hence greatly impaired feeding and growth. Even if the deformed fish reaches marketable size, its marketable value will be greatly diminished. Therefore an early correct determination of the percentage of

swim bladder inflation is vital to proceeding with a hatchery brood. These factors are important for normal swim bladder activation:

- physical barriers (scum) at the air- water interface;
- physio-morphological abnormalities in newly hatched larvae;
- early disease outbreaks;
- insufficient and irregular feeding;
- poor suitable water quality

Any scum or fine particulates at the air–water interface that prevents larvae from gulping air will impair swim bladder inflation. The presence of an oily layer originating from rotifers being fed an enrichment diet is a particular problem. Use of "surfaces skimmers" (see Chapter 1) largely mitigates this risk provided they are cleaned and checked that they are operating correctly regularly at least three times daily. Daily quantitative monitoring and recording of swim bladder development in 30-40 larvae should be done in conjunction with that of feeding status described above.

Monitoring should be continued until completion of inflation which varies according to fish species (from days 4 to 11 in AB, from days 3 to 7/8 in mulloway and from days 3 to 6/7 in YTK).

4.3 Diagnosis of Infective Disease – systematically investigate the cause of worrying changes in appearance, behaviour or increased mortalities.

The first step in identifying an infective disease agent in the hatchery is to observe and record disease symptoms and signs especially aberrant behaviour and appearance such as external colour or textural changes and presence and appearance of lesions. If practicable these recorded macroscopic observations should be supported with digital photographs and video recordings of affected fish in situ in rearing tanks of smaller vessels after capture and removal. Removed fish must also subjected to a skin smear and gill biopsy. After being anaesthetised and or euthanized. The samples are smeared onto a glass microscope slide with a drop of water and examined under a microscope. For a comprehensive description of these biopsy procedures and identification of the likely pathogenic causes and organisms refer to the following manuals:

1. Herford A., and Rawling G. (1999) *Australian Aquatic Animal Diseases Identification Field Guide* - Publ. Department of Agriculture, Fisheries and Forestry, Canberra, Australia
2. Munday, B. (1996) *Treatment of finfish diseases. Fish Health Workshop* Post Graduate Foundation in Veterinary Science, the University of Sydney.
3. Noga, E. J. (1996) *Fish Disease: diagnosis and treatment,* Mosby, St Louis.
4. Read P., Landos M., Rowland S.J., & Mifsud C. (2007) *Diagnosis, treatment and prevention of the diseases of the Australian freshwater fish Silver Perch (Bidyanus bidyanus)* Publ. NSW Department of Primary Industries ISBN 978 0 7347 1792
5. Thorne T. J. (1995) "*Fish Health for Fish Farmers in Western Australia"* Fisheries Department of Western Australia.
6. Untergasser, D. (1989) *Handbook of Fish Diseases,* TFH Publications Inc.

Whether or not on-site examination reveals a possible causative agent, the relevant state department Aquatic Health Unit should be contacted and live or moribund fish, plus suitable preserved samples, delivered to them as soon as possible for a definitive diagnosis and recommended courses of treatment, eradication and future prevention.

4.4 Disease Treatment (refer to item of references listed above and Table 11 below)

4.4.1 General

Once the cause of an infective disease has been positively identified, an appropriate course of treatment (see Table 11) can be initiated.

Many different treatments are often suitable for the treatment of a specific disease problem. The final choice of treatment will depend on many factors including the pattern of mortality and condition of remaining fish, water temperature, stocking density, type of filtration system, value of the fish and the cost and availability of the treatment chemical. Many fish with early stages of disease do not eat, making "in feed" treatments less effective. In addition, many fish with early stages of severe diseases will die irrespective of which treatment is used. The priority is to prevent more fish from becoming infected and to treat those with mild disease. Until confidence and experience has been gained in identifying and treating disease problems commonly occurring in a hatchery, it is wise to consult a more experienced person or organisation before starting treatment. It should be noted that many chemotherapeutics require prescription from a registered veterinary surgeon.

Some treatments used successfully at finfish hatcheries for various disease-causing agents are summarised in Table 11. As some treatments are stressful to fish, especially diseased fish held in warmer water, it is advisable to test the treatment on a small group of fish before treating the larger group. Good aeration, preferably in the form of pure oxygen should always be administered during treatments. Tanks should be vacuumed to remove various infective stages of pathogens after each treatment.

All chemicals should be used with great care when administering to diseased fish. Read instructions pertaining to the use of these chemicals prior to application and ensure that appropriate protective clothing and equipment is available and used.

In Australia, the Australian Pesticides and Veterinary Medicines Authority (APVMA) is responsible for regulating chemicals used in agriculture, including aquaculture. Other relevant legislation in NSW includes the *Stock Medicine Act 1989* and the *Food Act 2003*. Hatchery operators should be aware of the rules and regulations of these Acts and any amendments in order to use chemicals legally, responsibly and safely. Information regarding the use of chemicals in aquaculture can be obtained at www.apvma.gov.au (Rowland et al., 2007).

A small number of chemicals are registered for use in aquaculture in Australia. To be approved for use in food animals, a drug must usually undergo rigorous testing of its efficacy in treating specific diseases in each species at specific dosages and routes of administration. Information must be obtained on residue dynamics, safety for the operator and consumer, and any environmental effects. This can be time consuming and expensive. Once completed, the registered drugs must be used only in accordance with the label to treat the species on the label, at the directed dose rates.

The APVMA, can allow the use of unregistered chemicals, or registered chemicals off-label, under a minor use permit (MUP). When no alternative registered chemical is available, an MUP can be issued temporarily following the APVMA completing a risk assessment. It is essential to confirm with the APVMA the applicability and validity of any MUP (via the APVMA website) and/or I&I NSW (or equivalent department in other states) the conditions under which a chemical can be used.

Salinity change or a formalin bath has controlled the majority of the fish health problems that have been encountered with Australian bass and mulloway. A freshwater bath for euryhaline fish is the treatment of choice for some disease agents such as metazoan and protozoan parasites as it is cheap and does not leave toxic residues in the tissue. For AB and mulloway held in full strength seawater (35 ppt), a fresh water bath of less than 2 ppt for 90 minutes is sufficient to kill or remove the majority of external parasites on the fish. Freshwater baths are not however effective in treating fungal or bacterial diseases and can't be used at all for high salinity marine (stenohaline) species such as YTK.

For Australian bass and mulloway held in low salinity, a bath in high salinity water will be more effective than fresh water. A detailed description of pathogens and treatments is available in many texts as listed above and these should be consulted prior to treatment. Understanding the lifecycle of the disease agent and factors that can be exploited to reducing infection levels help when deciding the type and frequency of treatment options that may be used.

TABLE 11: Infectious diseases of hatchery held, AB, mulloway and YTK and recommended control and prevention measures (Source: OIE Manual of Diagnostic Tests for Aquatic Animals, 2006).

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Viral nervous necrosis (VNN)	Encephalitis virus (LeEV) – a beta-noda-virus	Virus	Encountered in hatchery production of Australian bass, mulloway larvae and advanced YTK. Pale or dark colouration; erratic swimming behaviour; spiral swimming; boating; 'fainting', extensive vacuolation of the brain & spinal cord; generally encourntered during hatchery phase. Asymptomatic carriers also common.	No treatment developed to date. Prevention by screening of broodstock; low larval rearing densities; optimal larval nutrition; improved broodstock nutrition; improved hatchery hygiene including sterilisation of incident seawater and full dry-out and disinfection of plant and equipment between successive hatchery broods especially once a disease outbreak has been experienced.
Lymphocystis	Lymphocystis virus	Virus	Wart-like growths on skin & fins; generally only fatal if infection severe & associated with very poor environmental conditions.	Removal of infected fish; improved environment.
Vibriosis	*Vibrio harveyi; Vibrio* spp.	Bacteria	Marine fish with darkening; lethargy; anorexia; reddened ulcerations on body; reddened abdominal fluid; associated with nursery systems, poor environment & skin trauma.	Improved environment; antibiotic treatment as prescribed by a qualified beterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100-200 mg/L) otherwise trimethoprim or oxolinic acid.
Bacterial haemorrhagic septicaemia	*Aeromonas hydrophila; A. sobria; A. Caviae; Aeromonass spp.; Pseudomonas spp.*	Bacteria	Freshwater fish with irregular reddened skin ulcerations; lethargy; anorexia; reddened abdominal fluid; pale gills; associated with poor environment & skin trauma.	Improved environment; antibiotic treatment as prescribed by a qualified beterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100-200 mg/L) otherwise trimethoprim or oxolinic acid.
Integumentary bacteriosis	*Aeromonas sobria*; *Aeromonas hydrophila*; *Vibrio harveyi*; *Vibrio alginolyticus*	Bacteria	Irregular reddened skin ulcerations; loss of scales; associated with poor environment & skin trauma.	Improved environment; increased water exchange.
Streptococcosis	*Streptococcus iniae*	Bacteria	Darkened fish; anorexia; pale gills; reddened abdominal fluid; reddened abdominal organs & inner wall.	Antibiotic treatment. Vaccines are not available for Australian bass, mulloway or YTK, but are being developed for Striped bass.
Columnaris disease	*Flavobacterium columnare*; *Flavobacterium johnsoniae*; & *Flavobacterium* sp. (gliding forms) in freshwater *Tenacibaculum marinimum* in seawater	Bacteria	Pale skin patches on dorsal surface behind dorsal fin & on caudal peduncle; lethargy; most commonly occurs in nursery phase; in older juveniles a mouth form with erosion of skin around upper & lower jaws has been seen; associated with overstocking, tank rearing, poor hygiene & skin trauma.	Treatment in potassium permanganate or copper baths 0.28 mg/ L Cu++ 24h or 0.1 to 0.2 mg/L Cu++ 10 days may help in early disease NB Use chelated copper rather than CuSo4 on fish in seawater; Otherwise antibiotic treatment as prescribed by a qualified veterinarian or fish pathologist
Bacterial gill disease	Various bacteria, *Flavobacterium* spp., *Cytophaga* spp.	Bacteria	Swimming at water surface; gulping; rapid opercular movement; excess mucus on gills; white patches on gills; most commonly occurs in nursery phase.	Improve water quality; treatment with salinity reversal, or under veterinary advice use of potassium permanganate or quaternary ammonium baths; increase water exchange; reduce stocking density.
Bacterial peritonitis	Various Gram-negative & Gram-positive bacteria including *Vibrio harveyi* & *Aeromonas hydrophila*	Bacteria	Darkened fish; lethargy; swollen abdomen; adhesions & bad smelling fluid in abdomen; abdominal fistulas; more common in recirculation systems.	Cull affected fish; antibiotic treatment prescribed by a qualified veterinarian or fish pathologist e.g. Oxytetracycline (in feed at 75 mg/kg biomass or in water at 100 -200 mg/L) otherwise trimethoprim, or oxolinic acid.

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Bacterial enteritis	Various Gram-negative bacteria	Bacteria	Acute disease in intensive larval rearing systems; anorexia; pin heads; darkened fish & death.	Cull affected larval batch
Fin and tail rot	*Aeromonas* spp.; *Pseudomonas* spp.; *Vibrio* spp.; *Flavobacterium* spp.; *Cytophaga* spp.	Bacteria	Erosion of soft tissue in fins and tail; may extend to involve entire tail & caudal peduncle.	Improve environment; reduce stocking density
Epitheliocystis	Epitheliocystis organism – a *Chlamydia*	Bacteria	Swimming at water surface; rapid opercular movements; disease rare but seen in marine fish & in recirculation systems.	None known
White spot	*Ichthyophthirius multifiliis* in freshwater *Cryptocaryon irritans* in marine	Protozoa	'Flashing'; rubbing skin on surfaces; anorexia; swimming at water surface; white spots on skin & fins.	Treatment with salinity reversal, formalin baths at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for combinations; treatment in copper bath for marine fish 0.28 mg/ L Cu++ 24h or 0.1 to 0.2 mg/L Cu++ 10 days NB Use chelated copper rather than $CuSO_4$ on fish in seawater
Chilodonelliasis	*Chilodonella* spp.; *Chilodonella hexasticha*	Protozoa	Swimming at water surface; rapid opercula movement; flared opercula; seen in poor environmental conditions & in weakened fish.	Treatment with salt, formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or potassium permanganate bath or combinations
Trichodiniasis	*Trichodina* complex spp.	Protozoa	Swimming at water surface; rapid opercular movements; excess gill mucus; typically follows cold water temperatures, high organic loads & high stocking densities.	Increase water exchange; treatment with salt or formalin bath at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24 h.
Ichthyobodosis (costiasis)	*Ichthyobodo necator*	Protozoa	'Flashing'; rubbing skin on surfaces; opaque patches on skin; raised scales; swimming at water surface; rapid opercular movements; flared opercula.	Treatment with salinity reversal; or formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or potassium permanganate bath.
Piscinoodiniasis	*Piscinoodinium* sp.	Protozoa	Found on AB and Mulloway in freshwater: In young fish opaque patches or a greenish discolouration of the skin; patches of skin lifting of surface & ulcers in older fish; rapid opercular movements; excess gill mucus; dark green gill colour.	Treatment with salt bath for AB and mulloway currently held in fresh water.
Velvet Disease = Amyloodiniasis	*Amyloodinium ocellatum*	Protozoa	Found in marine conditions: In young fish opaque patches or a green discolouration of the skin; patches of skin lifting of surface & ulcers on older fish; rapid opercular movements; excess gill mucus; dark green gill colour More common in broodstock and in raceways; associated with low water temperatures or rapid drops in temperature.	Fresh water bath for AB and mulloway held in seawater. Otherwise hydrogen peroxide (75g/L) bath for 75minutes (see further treatment details below) .

DISEASE	CAUSATIVE AGENT	TYPE OF DISEASE AGENT	SYNDROME & SYMPTOMS	CONTROL & PREVENTION MEASURES
Gill fluke	*Diplectanum* sp.; *Dactylogyrus* sp.	Monogean trematodes	Rapid opercular movements; anorexia; white areas on gills.	Treatment with salinity reversal for AB and mulloway, or formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h or praziquantel bath 1-2 ppm for 24h.
Skin fluke	*Neobenedinia melleni*; *Gyrodactylus* spp.	Monogean trematodes	Marine fish with opaque cornea; white patches on skin; skin ulcers; associated with high salinity & cool water temperatures.	Treatment in freshwater for AB and mulloway or praziquantel bath 1-2 ppm for 24h.
Myxosporidiosis	*Henneguya* sp.; *Kudoa* sp.	Spore-forming protozoa	Disease uncommon but histologically spore cysts seen in gill filaments (*Henneguya* sp.) & brain (*Kudoa* sp.).	None known
Microsporidiosis	*Pleistophora* sp.	Spore-forming protozoa	Raised lumps on skin; soft white nodules in muscle.	None known
Integumentary mycosis	*Saprolegnia* spp.; *Achlya* spp.	Fungi	Raised, fluffy growths on skin & fins; associated with low water temperatures & skin trauma.	Salinity reversal and formalin baths formalin at 100-200 ppm for 1 h, or Formalin at 75g/l for 2h or 25g/l for 24h ; do not handle fish when water temperatures low.
Branchiomycosis	*Brachiomyces* sp.; *Achlya* spp.	Fungi	Swimming at water surface; rapid opercular movements; white & red patches (mottled appearance) on gills; associated with cold water temperatures & high organic loads.	No treatment known; reduce organic load & increase water exchange.
Fish louse	*Argulus* sp.	Copepod	Disc-shaped parasite visible on skin; red foci; darkening.	Treatment in organophosphate especially Trichlorphon at 0.5 ppm bath Leave fish in dose water where active ingredient will fully denature after about 24 hours.
Anchor worm	*Lernaea* sp.	Copepod	Thin body of female parasite visible on skin with small red ulcer where parasite penetrates skin.	Treatment in organophosphate especially Trichlorphon at 0.5 ppm bath Leave fish in dose water where active ingredient will fully denature after about 24 hours.

4.4.2 Management of velvet disease and viral nervous necrosis

Two diseases that present continuous high level and widespread risk to hatchery production of finfish including AB, mulloway and YTK are velvet disease and viral nervous necrosis (VNN).

- Velvet Disease - *Amyloodinium ocellatum* (based on Fielder et al., 2008)

By far the most common disease of AB, mulloway and YTK and indeed of all farmed fresh, brackish-water and marine finfish globally, velvet disease, is caused by the single celled (protozoan) *Amyloodinium ocellatum*. In common with other parasitic "Dinoflagellates", *A. ocellatum* is distinguished by a motile life stage possessing 2 flagella, one a regular backward beating form, the other ribbon-like that beats to the cell's left.

As illustrated in Fig. 94, *A. ocellatum* has 3 life stages**:**

1. The trophont (2 in Fig. 94). This is a pear shaped (pyriform) parasitic feeding stage commonly averaging 100-150 µm with individuals occasionally up to 350 µm). Trophonts attach to and feed mainly on the epithelial (skin) cells of the gills but also of the scales and eyes of infected fish via root like structures (rhizoids) on the base. Duration of infection in the trophont stage increases from 1 to 5 days with temperature over the range 14-27ºC. At this point the rhizoids are retracted and the trophonts drop from the host fish to the substrate on the floor of ponds and rearing vessels.
2. The tomont (3 to 6 in Fig. 94), This is an external stage that after changing from the previous pear shape to spherical, begins cell division on the substrate after about 12 hours and continues Fig. 95).
3. Dinospores (7 in Fig. 94), these are a free swimming infective stage released from sub-cells that have undergone sporulation

4.4.3 Modes of entry and symptoms of A. ocellatum disease outbreaks

In aquaculture systems, where stocking density is high fish are usually more susceptibility.. Entry of the pathogen into a hatchery can lead rapidly to high intensity infections.. Entry pathways of the pathogen into the facility may be introduced stock, contaminated equipment, influent water or even aerosols.

Clinical signs of infection usually manifest as parasite intensity increases rapidly. Loss of appetite (anorexia) is a key indicator of chronic *A. ocellatum* infection at low intensity levels. Other signs of infection include flashing, irregular or rapid opercular beat and uncoordinated movement. Unfortunately, these clinical signs are often encountered in many other parasitic infections and are therefore not diagnostic for *A. ocellatum.* The gills are considered the primary attachment site for *A. ocellatum* infections. However, infections can also occur on skin and eyes. Attachment to the host epithelia with rhizoids can cause physical damage to several cells surrounding each trophont. In, heavy infestations this damage to the gills can result in anoxia and impaired osmoregulation and heavy mortality, within as little as 12 hours.

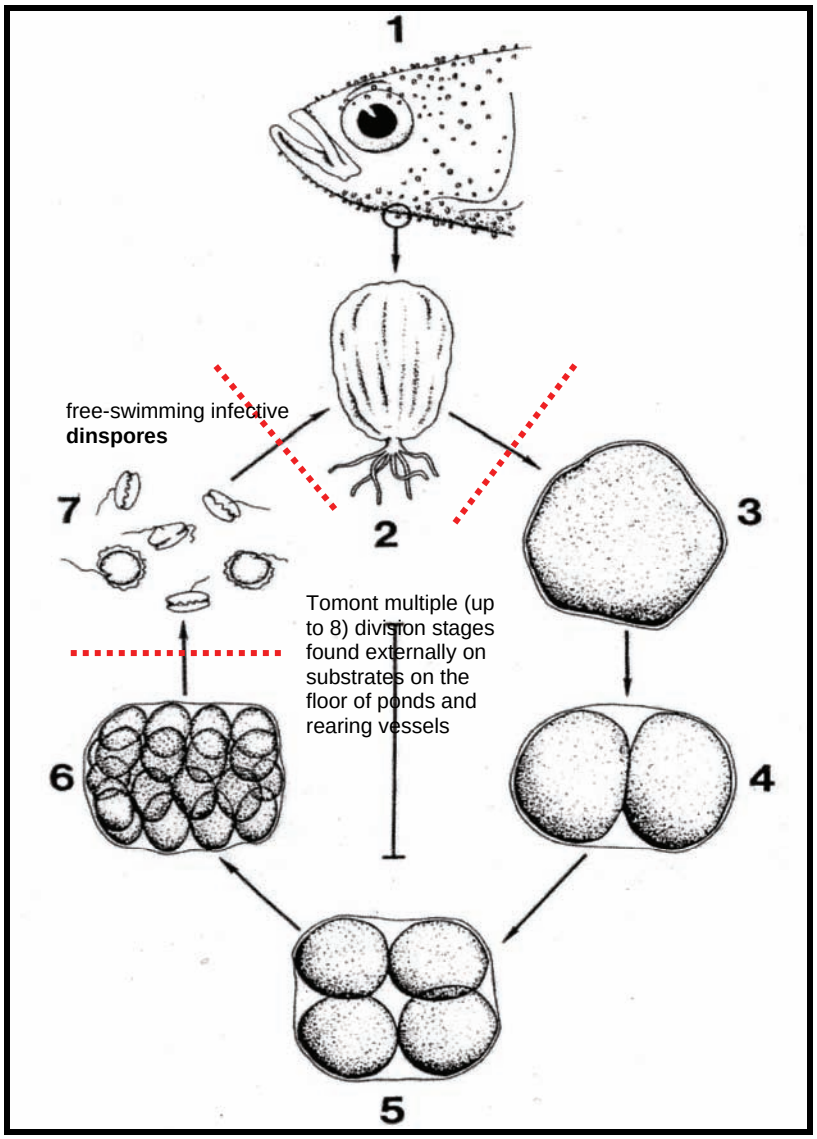


FIGURE 94: Life stages of *Amyloodinium ocellatum*. 2 attached trophont stage; 3-6 multiple division tomont stages ; 7 free swimming dinospores. (Source : Lom and Dykova, 1992).

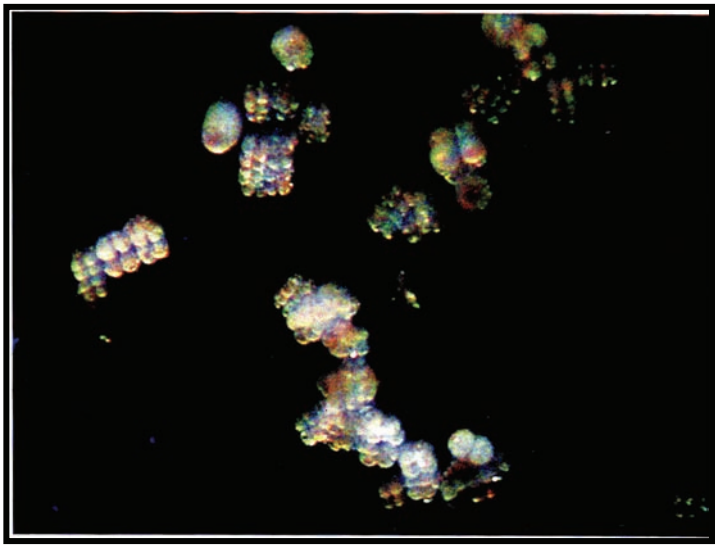


FIGURE 95: Photomicrograph of Tomont stages of *Amyloodinium ocellatum* in various stages of division. (Source: Roberts-Thomson, 2008).

4.4.4 Management (prevention, control and treatment) of Velvet disease

Management of *A. ocellatum* in aquaculture facilities is a difficult task. The parasite itself is extremely hardy being able to reproduce in salinities ranging from 3 ppt up to 45 ppt and temperatures from 17^{o}C up to 40^{o}C, making it more resistant to environmental change than many of its hosts including AB, mulloway and especially YTK. Under optimum temperatures of 17-23 ^{o}C, the life cycle can be completed in as little as 5 days. Treatment also becomes problematic when targeting the tomont stage. This is insulated from the environmental conditions by its cyst wall, making it near impervious to chemicals. Despite these hurdles, a measure of success has been reached in the implementation of chemotherapeutic treatments.

Chemicals affecting the trophont stage are generally non-specific for *A. ocellatum*. These include formalin, copper compounds, hydrogen peroxide, and fresh water baths. While these treatments are effective in shocking the parasite from its host they do not generally arrest development and trophonts through to tomonts. Many of these compounds are also toxic to the fish, making them generally ineffective as control measures. Copper compounds can be useful in dinospore elimination. Chloroquine, a common human anti-malarial drug, has also been found effective however, its high cost and long half-life in fish flesh limit its cost effectiveness to hatcheries in the treatment of broodstock and intensively reared juveniles. While malachite green, acriflavine, furanace and nitrofurazone have been found to act on the tomont stage, none are acceptable treatments due to their adverse affects to human health, including hatchery staff.

Securing hatchery facilities against intrusion of *A. ocellatum* therefore remains the best method of diseases prevention and control. This entails:

1. quarantining and regular prophylactic treatment of broodstock
2. physical separation of larval and juvenile rearing systems and associated utensils and equipment and replicate sets thereof
3. routine cleaning and chlorine disinfection of rearing tanks and equipment between successive uses
4. disinfection of all incoming freshwater and seawater

A large array of alternative treatments (Fig. 96 and Table 12) are effective in the short-term management of *A. ocellatum* infections. Freshwater bathing is particularly cheap and effective for treating euryhaline fish species such as the bass and mulloway. However, freshwater does not eliminate all trophonts from the gills and therefore remaining trophonts can potentially re-infect if the original salinity is restored, even if fish are moved to a different tank or pond.

Hydrogen peroxide added to rearing water at 75 g/L for 30 minutes is the treatment of choice in hatcheries as it is relatively cheap, leaves no flesh residues and is very effective for treating both euryhaline fish, such as AB and mulloway, and marine fish such as YTK. It is especially effective due to its ability to shock tophonts from fish gills and skin and its suppression of dinospore production and release from tomonts when used over a protracted period of 4 days. Repeat dosing may however be needed in such protracted treatment due to the rapid break down of hydrogen peroxide in the environment. Caution needs to be exercised when using hydrogen peroxide due to its potential negative impacts of treated fish including reduced feeding and growth for up to three weeks.

The overall efficacy of agents that simply remove trophonts from infected animals such as freshwater, formalin and short duration hydrogen peroxide bath, will ultimately be determined by bio-security procedures in place within a facility. If treatment is not coupled with movement of treated fish into new tanks, or tank brushing and vacuuming to remove the released tomonts from the system, then the lifecycle of the parasite will not have been effectively disrupted and re-infection is almost certain.

TABLE 12: Tomont chemotherapeutic results including tomont division, dinospore emergence and motility from vitro trials.

Treatment	Dosage (mg/l)	Treatment time (mins)	Tomont Division	Maximum divisions	Dinospore Emergence	Dinospore Motility
Bronopol	50	30	Y	6	Y	Y
Bronopol	50	4 days	Y	6	N	N/A
Hydrogen Peroxide	200	30	Y	7	Y	N
Hydrogen Peroxide	200	4 days	Y	2	N	N/A
Hydrogen Peroxide	75	30	Y	7	Y	N
Hydrogen Peroxide	75	4 days	Y	2	N	N/A
Formalin	200	60	Y	6	Y	Y
Formalin	25	4 days	Y	5	Y	Y
Marine Oodinium and Whitespot Treatment	150	4 days	Y	4	N	N/A
Control	-	4 days	Y	8	Y	Y

Recommended treatment highlighted. (Source: A. Roberts-Thompson, 2008).

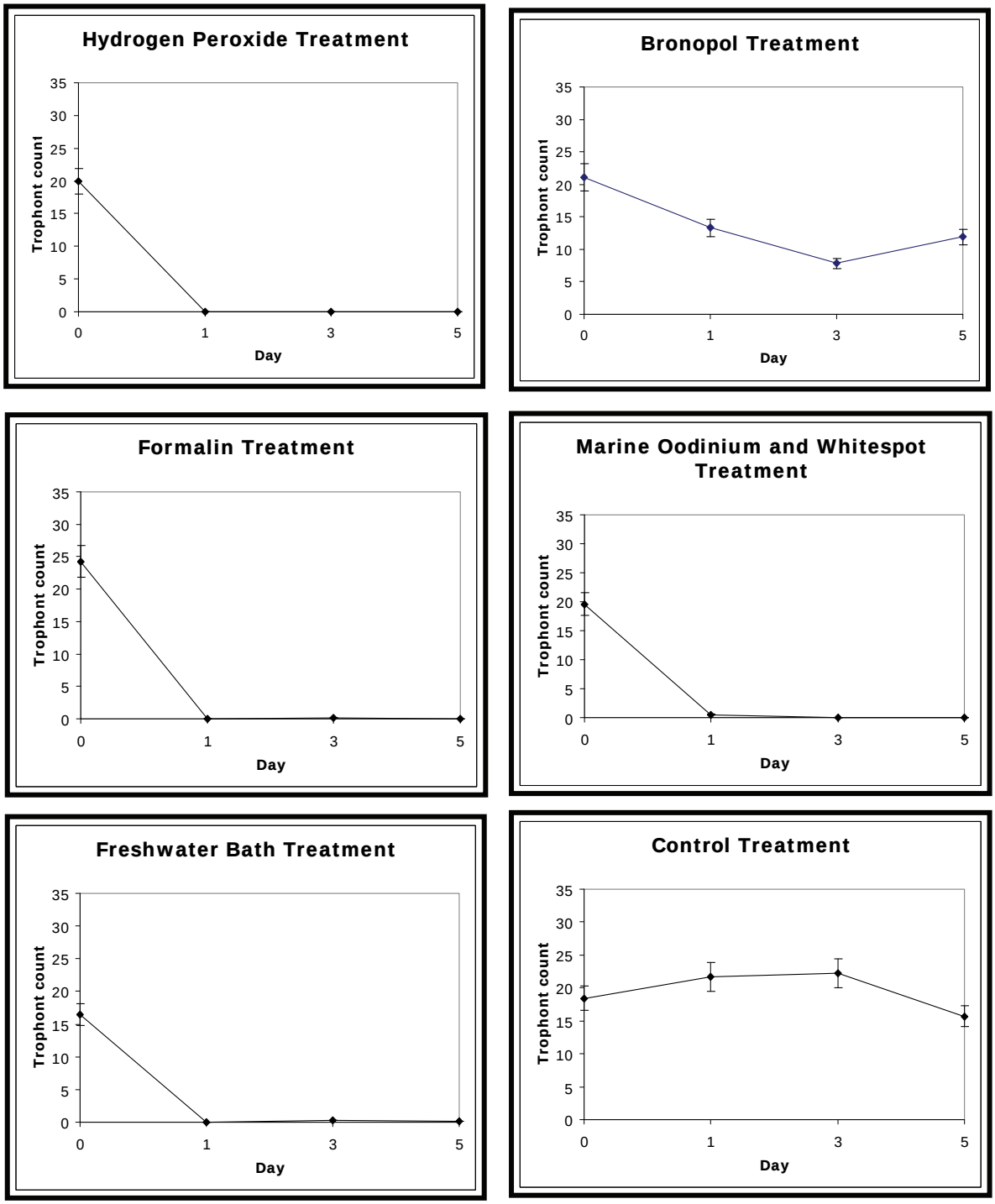


FIGURE 96: Trophont treatment trials with a range of chemotherapeutic chemicals of barramundi infected with *A. ocellatum.* (Source: A. Roberts -Thompson, 2008 in Fielder et al., 2008).

4.5 Viral Nervous Necrosis (VNN = Viral encephalopathy and retinopathy)

4.5.1 Introduction

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by a betanodavirus was formerly called barramundi picorna-like virus when first encountered in Australia in 1989 (Glazebrook, Heasman & de Beer, 1990). VNN is a serious disease of finfish that has been reported in over 40 fish species from most tropical to temperate parts of the world, with the exception of Africa. Most reports of disease have been from farmed fish, with disease observed most commonly in larval and juvenile fish although disease in adult fish has been reported. Mortality rates of up to 100% are observed in larval fish populations with rates tending to decrease as the size/age of infected fish is increased. Fish surviving infection can become sub-clinical carriers and vertical transmission is suspected (although little evidence is available to support the suspicion) to occur via spawning products from sub-clinically infected broodstock to progeny during spawning.

The aetiological agent causing VNN is a virus of the genus *Betanodavirus* (is the target for detection of viral RNA by PCR). The viral coat protein is the target for detection by immunological methods. There are four recognised genotypes all the other known Australian isolates are members of the RGNNV genotype. However, the isolates form two distinct groups within this genotype; one group comprising isolates from New South Wales and South Australia and a second group comprising isolates from Queensland, the Northern Territory and Tasmania.

Clinical signs of disease include:

- colour change - affected AB become lighter
- abnormal swimming behaviour, including spiral and/or looping swim pattern, uncoordinated darting, belly-up at rest and pinpoint flashes of light reflected by the retinas of afflicted fish as they are roll ,twist and turn
- overinflated swim bladder
- lethargy and anorexia leading to emaciation
- blindness resulting from vacuolation lesions in eyes
- abrasions

These may vary depending on the host species but result from damage to the brain and retina, i.e. anorexia (a secondary effect of blindness), abnormal swimming and changes in body colour.

Gross pathological lesions are uncommon, but over-inflation of the swim bladder in infected sevenband grouper and red drum has been reported. In larval epizootics, mortalities of up to 100% cause problems for hatchery productivity and the regular supply of fingerlings for on-growing. While high level mortalities in intensive larval cultures are most common, there are also records of disease in older juveniles. Such reports indicate that nodaviruses can cause significant mortalities in any class of cultured marine finfish.

In the absence of chemotherapy for viral diseases of fishes there is the potential for significant economic loss. More recently around the world reports of VNN in fish reared in freshwater have occurred: European sea bass, sturgeon, European eels and Chinese catfish and freshwater guppy. These reports confirm the possibility that natural outbreaks of VNN and nodavirus infections can occur in farmed freshwater fishes. Also there is the suggestion that spread of nodavirus can occur between different species of fish in the fresh water ecosystems. The lack of epidemiological information on the prevalence of nodavirus in wild populations remains a constraint on the development of appropriate policy for managing real risks in fisheries.

In Australia, disease was first reported from barramundi (*Lates calcarifer*) larvae in the late 1980s (Glazebrook, Heasman & de Beer, 1990). In the following years, high mortalities were seen in the first few weeks of experimental, intensive larval culture. VNN epizootics had a significant effect on the one commercial barramundi hatchery operating at that time, but were rare in government hatcheries. Changes in husbandry and improved hygiene practices in the hatchery resulted in prevention of any further VNN epizootics. Subsequently, in 1999, one commercial barramundi hatchery experienced mass mortality of barramundi around 15 days of age in larval rearing ponds. This was the first time any significant VNN was seen outside of intensive larval rearing systems. Since then nodavirus has been reported from an increasing number of species including Australian bass (*Macquaria novemaculata*), mulloway (*Argyrosomus japonicus*), yellowtail kingfish (*Seriola lalandi*), barramundi cod (*Cromileptes altilevis*), goldspotted rockcod (*Epinephelus coioides*), flowery cod (*Epinephelus fuscoguttatus*), sleepy cod (*Oxyeleotris lineolatus*) and striped trumpeter (*Latris lineata*) from marine and freshwater facilities in New South Wales, Northern Territory, Queensland, South Australia and Tasmania. As other species are evaluated for aquaculture potential, the range of species found to be susceptible to infection is likely to increase.

Nodaviruses have been detected in juvenile fish surviving experimental and natural infections and while the duration of viral persistence is unknown, virus has been reported in one fish species that survived acute infection for at least 12 months after the initial disease outbreak. Virus has also been detected in healthy juvenile and adult fish from susceptible species and from species in which disease has not been observed. Antibodies have been detected in broodstock yet transmission via spawning products from infected broodstock to progeny is still considered the most common mode of transmission. Thus, the relationship between immune status and infectivity remains to be determined.

Because the effect of Australian nodavirus on native fish species is unknown, strict controls are in place to reduce the risk of translocation of virus with commercial stock or stock used for restocking programs and to reduce the risk of escape of virus from aquaculture facilities into the environment. Exclusion of the virus from aquaculture premises, good hygiene in these premises and reduced stocking densities have decreased the incidence of VNN outbreaks. It is worth noting that while reduced stocking densities can undoubtedly help they are not necessarily an essential component of a risk reduction strategy (Schipp, pers. comm.). It should be noted that currently no reliable non-destructive test is available for screening broodstock fish for nodavirus status. Blood and even gonad tissue have proven to be poor samples to determine nodavirus status of AB, as indicated by many negative results from blood from fish which were confirmed infected by nodavirus in brain and retina tissue sample.

Betanodavirus infection has a significant economic, social and environmental impact in Australia through direct losses due to disease, inhibition of trade for established and emerging aquaculture industries, restriction on locations suitable for aquaculture expansion, and suspension of fish restocking programs due to concerns of the impact on native fish species due to translocation of the virus with stock.

4.5.2 Management (Prevention control and eradication) of VNN:

Preventative Strategy comprises 5 components (Chapter 1. Viral Diseases FAO Gilda D. Lio-Po and Leobert D. de la Pena)

- Screening of all broodstock for VNN free status. (This is currently not possible for Australian bass, mulloway and YTK. The development of reliable, non-destructive tests such as PCR and / or ELISA is essential to allow broodstock screening)
- strict hygiene coupled with isolation of broodfish from hatchery and nursery rearing areas.
- ozone disinfection of all incident seawater used to incubate eggs and to rear larvae and early juveniles
- ozone disinfection of embryos
- vaccination

Eradication Strategy - Total disinfection of hatchery - in response to an initial major catastrophic VNN disease outbreak or subsequent succession thereof.

Preventative Strategy

1. Screening for VNN free status and quarantining of all broodstock.(see above comments regarding inadequate test and the need for caution accepting results from blood samples).

   Pre- and post-spawning screening of broodstocks for VNN using a PCR test is very important. Only VNN-negative broodstocks should be retained and allowed to spawn. This precaution is very effective in preventing vertical transmission of the virus when combined with disinfection of the fertilized eggs preferably using ozone.

2. Strict hygiene coupled with isolation of broodfish from hatchery and nursery rearing areas.

   Strict hygiene is very important in the management of VNN infection. Betanodaviruses are quite resistant to some environmental parameters, thus it is highly possible that the virus could be easily translocated via contaminated rearing water and other hatchery paraphernalia. The use of non-recycled, chemically treated rearing water and decontamination of tanks after every hatching cycle were effective in preventing VNN infection in Australian barramundi hatcheries.

   Contrary to earlier fears that live feeds, rotifers and brine shrimp were a potential source of infection they in fact appear to be insusceptible to betanodaviruses. Another potentially important issue in managing this disease is that some species of fish such as grouper have been shown to be more susceptible to VNN at higher water temperatures, i.e temperature may differentially affect disease and host in this case favouring the virulence of the disease (VNN) over the resistance of the host (finfish larvae and early juveniles)

3. Ozone disinfection of all incident seawater used to incubate eggs and to rear larvae and early juveniles.

   The amount of ozone required to inactivate NNV and other potential viral diseases such as infectious pancreatic necrosis virus (IPNV) is only 0.1-0.2 mg/ liter of seawater for 1 - 2.5 minute. However the recommended protocol based on the recent work of Battaglene and Morehead, 2006) to disinfect Day 3 post-fertilisation embryos of striped trumpeter is 1 mg ozone/L seawater for 1 minute (CT = 1). Similarly, research by Ballagh et al. (2010) has shown that ozone at a CT of 1 is the maximum concentration that mulloway eggs can tolerate without causing damage to the eggs which prevents successful hatching. This protocol not only safeguards against betanodavirus but reduces bacterial loads and improves incubator hygiene. Since adopting the practice of ozone disinfection of embryos, and other control measures, there have been no mortalities attributed to betanodavirus in cultured striped trumpeter.

4. Other measures in the control of VNN are:

    a. Cleaning, chemical disinfection and dry out of all hatchery buildings plumbing and other equipment and utensils with caustic soda, gluteraldehyde or chlorine ;

    b. rearing of each batch of larvae and juveniles in separate tanks supplied with UV or ozone-sterilized seawater; and

    c. isolation of larvae and juvenile from broodfish

    d. Vaccination of juveniles and non carrier status broodstock. This is a promising method of preventing VNN in groupers. Immunization of groupers with recombinant coat proteins prepared from RGNNV genotype strains induced virus-neutralizing antibodies that resulted in high protection against experimental infection of the virus. Moreover a multivalent vaccine reputedly providing total protection from infection by different genotypic variants of piscine nodavirus has been recently developed in the USA (US patent application #: **20080286294**). Reputedly this simple vaccine **i**s suitable for administration to fish via the intramuscular or intraperitoneal route, or by bath and/or via the oral route.

Detailed description of eradication procedures for VNN and other highly-virulent infectious diseases of fish, as recommended by The World Organisation for Animal Health (OIE), is shown below (Table 13).

TABLE 13: Eradication protocols for VNN and other highly virulent -infective disease (Based on *OIE Manual of Diagnostic Tests for Aquatic animals* 2006) episodes

Introduction
Disinfection may be used for a range of reasons
1. In response to a major (catastrophic) disease episode or as a pre-emptive prophylactic sanitary practice within bio-security programmes designed to exclude or reduce the incidence of the disease
2. Total eradication of an infective disease to prevent its possible spread into the wild
The specific reason for disinfection, will determine the disinfection strategy used and how it is applied. When a notifiable disease such as VNN or an important but unlisted emerging disease occurs for the first time at a particular farm, at a particular site (i.e. at a quarantine facility), or within a region or state y previously believed to be free of that disease, it may be advisable, if not required, to eradicate the disease by depopulating the facility and performing a thorough disinfection of all or part of the facility. Fallowing of the affected facility for a defined period of time may be warranted in some situations
Successful disease eradication protocols generally employ a number of the following disinfection methods in accordance with particular applications as discussed separately below
• chlorine (as calcium hypochlorite, HTHT or a bleach solution containing a sufficient concentration of hypochlorite);
• formaldehyde gas (from sublimated paraformaldehyde or concentrated formalin/potassium permanganate reaction);
• iodine (as contained in iodophors);
• lime (as calcium oxide or calcium hydroxide);
• UV light (from natural sunlight);
• ozone;
• steam;
• hot water (60ºC);
• concentrated acids;
• desiccation;
• detergents (for general cleaning, with some degree of disinfection capability for many products).

1. Disinfection of hatcheries and of broodstock rearing/holding facilities in response to a major (catastrophic) disease episode or as a pre-emptive prophylactic sanitary practice within bio-security programmes designed to exclude or reduce the incidence of the disease

Virtually all marine finish hatcheries and broodstock holding/rearing facilities use seawater that has been disinfected to remove potential pathogens, pests, and disease-carrying agents via mechanical filtration, UV irradiation, and/or chemical disinfection. This may be by passive source water filtration (i.e. by the use of seawater wells or well points) or by mechanical filtration using high pressure pumps and a variety of water filtration devices and pore sizes. Some facilities use filtration coupled with UV light disinfection of source water, while others use chemical disinfection methods, using either chlorination and de-chlorination or high doses of ozone and subsequent removal of residual oxidants. Chemical disinfection of source water typically requires the use of one or more water storage reservoirs in which the water is treated and detoxified before use in the hatchery or broodstock facility.

a) Disinfection of eggs and larvae

Vertically transmitted diseases, due to viruses such as VNN, and to bacterial and fungal disease agents, can be eliminated or have their incidence reduced through the routine use of disinfection protocols when used to surface disinfect eggs. A recommended method using Ozone on developing embryos is given above

b) Disinfection of tanks, equipment, pipes, air stones, etc.

For routine sanitation, hatchery and broodstock tanks (i.e. tanks for broodstock maturation, matting, spawning, larval rearing and indoor nursery) should be cleaned, disinfected and dried between use. Tanks used for the above-named purposes in hatcheries are typically precast fibreglass tanks or they are constructed of concrete coated or painted with resin-based coatings (e.g. epoxy or fiberglass resin) or lined with plastic liners manufactured for that purpose. After harvest of the stock from the tank, all loose objects and large-sized organic debris such as algae, faeces and left-over feed should be removed. With relatively small tanks, it is advisable after harvest of the stock to fill the tank to capacity, immerse all nonporous corrosion resistant equipment (i.e. airlines, air stones, stand pipes, screens, sampling containers, etc.) in the tank, and then add calcium hypochlorite to provide a minimum of 200 ppm of free chlorine. This should be allowed to set overnight. After the proper chlorinated soak-time, the tank can be drained and freshwater rinsed. Before draining the system, the treated water should be dechlorinated (see specific subsections on chlorination described below), unless appropriate effluent collection and treatment systems are in place. After the tank has been rinsed it should be allowed to completely dry. In the case of large tanks, an initial cleaning to remove loose debris should be followed by disinfection with a concentrated (~1600 ppm as chlorine) solution of calcium hypochlorite. All inside and outside surfaces should then be sprayed with this chlorine solution. The tank should then be allowed to set for several hours and then rinsed, filled and flushed. Surfaces should then be scrubbed free of all remaining material. After disinfection with chlorine, small or large tanks should be rinsed with clean water, then filled and flushed to ensure that no chlorine residues remain before the tank is restocked for another crop.

Disinfection of growout ponds

Following the routine harvest of a crop from a growout pond (or from a large tank or raceway used for growout of a crop), the pond (tank) bottom should be inspected. Large deposits of organic debris should be treated or removed. This is easily accomplished in lined tanks, raceways, or ponds (i.e. by flushing with a high pressure hose), but poses more of a challenge in large earth bottom ponds. Many methods of pond bottom disinfection and treatment between crops are practiced.

a) Chlorination

This disinfectant may be used for routine treatment of ponds between crops or when disease eradication is the goal. After draining the pond, remove (and dispose of [see section on carcass disposal in Section C.6]) as many animals from the system as is possible (this may be difficult in pond systems where the removal of large numbers of dead fish would not be practical). Partially refill the pond (or fill to capacity if required), discontinue the addition of new water, stop the discharge of effluent water, and remove any internal or external sources of aeration or aeration devices, which might be subject to corrosion. Then evenly distribute sufficient granulated calcium hypochlorite (such as Olin HTHT) to provide a minimum residual free chlorine concentration of 10 ppm within the entire system s water. (NB: The person(s) applying the chlorine should wear waterproof outer ware to protect their skin, an approved chlorine mask, and goggles or a face shield for eye protection.) Redistribute additional calcium hypochlorite as often as required to maintain the residual concentration at near or 10 ppm. Allow the system to set for a minimum of 24-48 hours (especially if applied to large systems) at this minimal chlorine concentration. The chlorine will kill all fish and most, if not all, of the other organisms occupying the water column or resident in the pond. After the pond has been treated with chlorine for the required minimum time and before any water is discharged, neutralise the chlorine either passively by exposure to sunlight and air for approximately an additional 48 hours (without the addition of new chlorine) or by the addition of sodium thiosulphate at a rate of five (5) molecules of sodium thiosulphate for each four (4) molecules of chlorine (or the weight of sodium thiosulphate being 2.85 times the weight of chlorine in the water, see example table below).

Pond size	Average depth	Volume	Chlorine dose	Chlorine required	HTH (65% active Cl)	Thiosulphate required
1 hectare	1 m	10,000 m^3	10 ppm	100 kg	154 kg	285 kg

Periodic testing should be done for residual chlorine; water should not be discharged until it has reached 0 ppm (below detection level). Once the chlorine levels have been ascertained to be at 0 ppm, the system water can be safely dumped into the effluent system. In some culture systems, in particular raceways, tanks and small lined ponds (i.e. those systems in which the majority of the fish are not removed prior to disinfection), the dead fish should be collected for proper disposal (see section on carcass disposal in Section C.6).

b) Liming

The lime, as calcium oxide or calcium hydroxide, should be applied to a very moist bottom at a rate of 5000 kg/ha or 1500 kg/ha, respectively. Great care should be taken to assure that the lime is spread evenly over the soil surface. The pond should then be allowed to set for at least a week, or at least until the soil has dried to the point of cracking to a depth of approximately 10-20 cm. Additional lime may be applied after ploughing (see below) at a rate of 50% of that normally prescribed. The pond should again be dried for at least a week, depending on the weather.

c) Drying and ploughing

Whether or not a pond is treated by chlorination or liming or left to dry untreated, ploughing is a commonly used method of treating a pond bottom to reduce its organic content, improve nutrient recycling, buffer pH, eliminate pests, and achieve disinfection through a combination of microbial degradation, exposure to sunlight, aeration, and desiccation. In some regions, drying and ploughing of dry pond bottoms may only be possible during the 'dry season'. When pond drying is an option, the pond bottom should be allowed to dry until the surface has cracked to a depth of approximately 10 cm. Once this level of drying has been reached, the soil should be broken up to a depth of approximately 20 cm with a plough, tiller, disk harrow, tine harrow or other similar farm implement. Ponds treated in this manner should be left for at least a week before being refilled and restocked.

Disinfection of source water

Because VNN and a number of important diseases, can be introduced into hatcheries with source water when it contains vectors or carriers, biosecurity plans should include provisions for the disinfection of source water. This may be accomplished by a variety of means which may include one or some combination of the following strategies:
a) Filtration of source water - source water is pumped into a supply/settling canal where it first passes through coarse bar screens to remove large aquatic animals and debris. Then the water is passed through a series of progressively finer screens, and final filtration is accomplished by passing source water through a fine mesh (150-250 µm mesh size) bag screen before being introduced into a culture pond or storage reservoir.
b) Instead of using mesh nets, filtration structures can be placed in the supply canal system. A series of compartments within these structures are filled with filter matrixes, beginning with coarse gravel for initial removal of large aquatic animals and debris, an intermediate section which contains a finer matrix of sand and gravel, and the end section which contains fine sand.
c) Chlorination and de-chlorination - source water is pumped to a supply canal or directly into culture ponds or reservoirs (with or without filtration) and treated with sufficient chlorine to kill any potential vectors or carriers in the source water.
d) 'Zero' or reduced water exchange: Some fish hatcheries and farms use supplemental aeration and re-circulation of water in culture ponds and within the supply and discharge systems to reduce source water requirements. This reduces the volume of source water that must be disinfected before use, as well as reducing nutrient loss from farms with effluent.
Disease eradication and total facility clean-up
This action may be necessary for disease control when significant, untreatable diseases occur at sites where eradication is an option. The confirmed diagnosis of a notifiable diseases such as VNN, or of an important but unlisted emerging disease occurring for the first time at a particular facility at a particular site (i.e. at a quarantine facility), or within a state or region thereof previously believed to be free of that disease, are events wherein it may be advisable, if not required, to eradicate the disease by depopulating the affected facility and performing a thorough disinfection of all or part of the facility.
Fallowing of the affected facility for a defined period of time may be warranted in some situations.
Total eradication of an infective disease agent to prevent its possible spread into the wild
The following steps/actions may be used to achieve eradication of a disease through a total facility clean-up (TCU):
a. Depopulate all living stocks from the affected facility
b. Discontinue stocking of the facility.
c. Harvest and sell (if permitted) marketable stocks through normal market channels.
For unmarketable stocks the following are options for disposable after harvest:
i) Incineration: burn collected shrimp in a government approved (if required) incinerator. The limitations to this procedure are inherent to the disposal of fish. That is, fish contain large amounts of water and therefore this procedure may only be feasible for small quantities of fish or to larger quantities if the fish have been dried prior to incineration.

Disinfection of culture tanks and ponds – as for general disinfection described above.

Clean-up procedures for facility components other than culture areas

In order for a TCU to be effective, it may be necessary to disinfect the entire facility after all the fish have either been harvested or disposed of in some other manner. After depopulation of the facility, every possible animate and inanimate carrier of the disease agent must be identified and either removed from the facility or thoroughly disinfected. The movement of disease agents between live fish or dead numerous fish can be easily understood, while the same can not be said for their movement via inanimate components. Hence, all areas, units, subunits or components which are contaminated or potentially contaminated must go through a cleaning and disinfection process.

a) Buildings

The disinfection regime used should be building-specific and dependent upon the use-pattern of that particular building.

i) Office buildings: these buildings would most often be subject only to foot traffic from people who have been in contaminated buildings or culture areas. Because of this, the greatest focus of attention should be the floors and cold storage units in the building. Floors should be thoroughly cleaned (if they are non-porous) with standard detergents and cleaning solutions, followed by a thorough drying. If the floors are carpeted, they should be vacuumed and cleaned with a detergent appropriate for carpets, or 'steam' cleaned. All other areas within these buildings, such as walls, bathrooms, desks, refrigerators, freezers, etc. should be examined for possibly contaminated materials (i.e. frozen shrimp in freezers) and any such item found and its container should be cleaned and disinfected or disposed of in a sanitary manner.

ii) Culture buildings: it must be assumed that these buildings have had direct contact with the disease agents and will therefore be handled in a different manner from that of the office buildings. The disinfection regime for these buildings will consist of two steps. First, the building should be thoroughly swept and/or vacuumed (where appropriate) to remove as much large-sized organic and inorganic debris as possible. This should be followed with the second step, treatment with chlorine. Chlorine solution (~1600 ppm) should be applied (by spraying) to all surfaces which are not prone to the corrosive actions of chlorine. Those surfaces which should not be chlorinated, can first be sponged with a iodophor solution minimally providing 200 ppm of free iodine. These can then be covered with plastic or any other protective material. Floor surfaces can be soak-chlorinated to a depth of 5 cm with a 200 ppm chlorine solution. This should be allowed to set for a minimum of hours. If many of the sprayed surfaces are somewhat susceptible to corrosion by chlorine, those surfaces can be freshwater-rinsed after the 48-hour treatment.

In buildings where disinfection with chlorine is not practical, fumigation with formaldehyde gas should be considered. After a general cleaning, fumigation of a sealable building can be initiated. The entire process, from the time the building is first gassed until it can be occupied again, should take a minimum of 36-60& amp; nbsp;hours. The entire building must be totally sealed off during the actual fumigation; there should be no means by which the gas can escape once it is placed in the building. If possible, the electrical service for the building should be turned off. The required environment for formaldehyde gas disinfection is a minimum temperature of 18ºC with a high relative humidity (at saturation is best, i.e. floors should be wet, etc.). Generation of formaldehyde gas is accomplished by the addition of 17.5 g potassium permanganate to each 35 ml of 100% formalin (a 37-39% aqueous solution of formaldehyde gas) for each 2.83 m^3 (100 ft^3) of space. Ideally, each room in the structure should have its own source of formaldehyde gas to assure that all areas of the building are uniformly treated. The correct amount of each compound (potassium permanganate and formalin) should be weighed out into separate containers, the formalin should be placed in a non-plastic container that is at least 10 times the combined volume of both the formalin and the potassium permanganate.

(The person applying a formaldehyde gas fumigation should wear waterproof outer ware to protect their skin, an approved formaldehyde gas mask, and goggles or a face shield for eye protection.) The containers with the proper amounts of the two reagents should then be placed on the floor in the centre of the room, on a large disposable protective (plastic) mat. The formalin and potassium permanganate should not be mixed at this time. Once all rooms have the correct amounts of the two compounds, the building has been completely sealed and the environment modified as necessary, the actual fumigation can begin. The mixing of the two compounds must be done very rapidly and carefully as the reaction is

immediate and somewhat violent as formaldehyde gas is emitted. Starting with the room farthest from the exterior door, add the permanganate to the formalin and proceed to the next room. After all rooms have been completed, lock the exterior door and seal it from the outside with tape. The building should be allowed to set for a minimum of 12 hours. After this disinfection period the building should be flushed with clean air for 24-48 hours. There should be no detectable odour of formaldehyde when people are allowed to reoccupy the building.

An alternate method for the generation of formaldehyde gas is the sublimation of powdered paraformaldehyde. For each 2.83m^3 (100 ft^3) of space, approximately 28 g paraformaldehyde should be used. It can be sublimated by being placed in an electric fry pan, which has been set on high. This procedure is somewhat more dangerous, because formaldehyde is flammable and a spark from such a heating device could theoretically ignite the gas. The same procedures noted above for the formalin/permanganate mixture in regards to venting, etc. should also be followed for the use of paraformaldehyde.

iii) Processing buildings: these buildings are typically constructed to permit routine disinfection. For the most part, the procedures followed in the routine operation of such buildings are appropriate for a TCU, provided that the building, its cold rooms, and its freezers are also disinfected and thoroughly dried. If considered necessary, fumigation with formaldehyde gas may be done to insure destruction of the disease agent(s) of concern.

iv) Other buildings: buildings (feed storage, maintenance, tool rooms, etc.) should be treated somewhat like the office building. Care should be taken to remove all the large-sized debris, which would normally be found in relative abundance within these types of buildings. Potentially contaminated surfaces within such buildings should next be spray-chlorinated and allowed to set for 24-48 hours. This should be followed by a freshwater rinse. All equipment, which should not be exposed to the corrosive action of chlorine, should be removed before the spraying, and they should be disinfected by surface disinfection with 200 ppm of iodophor. Once the equipment has been disinfected, it can be brought back into the building. Fumigation with formaldehyde gas is another option for this type of building.

b) Culture support equipment and systems (not applicable to VNN until such time that infective pathways via algae and live feeds (rotifers and *Artemia*) have been demonstrated)

These are operational units of the culture facility which may be housed in a building.

i) *Artemia* systems: all *Artemia* decapsulation and cyst hatching units and tanks should be treated in the same manner as other tanks. They should be cleaned of all large debris, then filled to the top with clean water and calcium hypochlorite added to achieve a final concentration of 200 ppm (free Cl_2). Chlorination should be allowed to continue for 24-48 hours. The outside of such tanks may be spray-chlorinated (1600 ppm chlorine). Treated tanks can then be dechlorinated with sodium thiosulphate, drained, freshwater rinsed, and allowed to dry for a minimum of one week. Unopened containers of *Artemia* cysts at the facility can be retained. These should, however, be surface disinfected with chlorine (200 ppm) or iodophor (200 ppm).

ii) Algae systems: containers, tanks, incubators and rooms used to produce algae for feeding the larval stages of fish may be handled and disinfected in nearly the same way as other tanks systems. The only major difference being that special care must be taken to assure that all chlorine residues have been rinsed from the units before they are used again. In the case of the culture tubes, flasks, carboys, and flasks used to culture algae, a combination of acid (10% HCl) rinse or steam sterilisation can be used in lieu of disinfection with chlorine or idophor.

Disinfection of stock cultures of living algae is not possible. The use of disinfection is clearly out of the question; any compound which would kill the disease agent would likewise kill the algae. Hence, there are two basic methods of minimising the chance of a disease agent being present in the stock cultures.

Dilution: all stock cultures can be cloned from the existing stocks. Each culture should be diluted either by means of serial dilutions (for broth cultures) or streaked for single colonies (agar cultures). All dilutions must be performed using strict aseptic techniques with all media being properly autoclaved. Passages from the stock cultures should not occur until the algae culture room has itself been disinfected as per the above building procedures. Once a culture has been diluted and cloned by either of these methods, to the point where there remains only one cell of the original culture, the risk is negligible that a fish disease agent may be present.

New Stock Cultures: If existing stock culture are discarded in a TCU, new stocks should be purchased from algae supply laboratories, or obtained from other sources where contamination with (shrimp) disease agents is unlikely, such as isolating desired species from wild populations of algae. New stock cultures should not be obtained from any facility that also cultures fish and may be contaminated with fish disease agents of concern.

iii) Farm equipment: nets, seines, porous air-line tubing, etc. which are relatively inexpensive and easily obtainable should be discarded and removed from the facility during a TCU rather than being disinfected as they are not readily disinfected and chlorine is likely to damage them and shorten their useful life.

Non-expendable equipment such as large size flexible plastic tubing, pumps and pipes, transfer tanks, cages, harvest cages, harvest tables, Secchi disks, laboratory glassware, etc. should be soak-chlorinated in 200 ppm solutions for 24-48 hours. This is most easily accomplished by placing these objects in the tanks that are filled with 200 ppm solutions of chlorine. Care should be taken to have all items completely submerged (use heavy items to weigh-down more buoyant objects). A good guide is to place everything (except those that are to be thrown away) that is loose or can be unsecured from its point of attachment, into the 200 ppm chlorine solution in their respective tanks.

In the case of those similar type items which are associated with ponds, they should be placed in a special series of tanks set up near their respective ponds. These tanks should be filled with 200 ppm chlorine solutions. Following soak-chlorination, these items should be allowed to dry and be exposed to natural UV (sunlight) sterilisation. They should be turned at least once to expose all areas of the items to direct sunlight.

Tools and machinery, such as tractors, trucks, portable and stationary power tools, etc., should be thoroughly cleaned with standard cleaning solutions. All traces of mud, feed, etc. must be removed from these items. Following this, disinfection of surfaces likely to have been contaminated in normal use should be rinsed off with an iodophor solution (at a concentration of 200 & amp; nbsp;ppm) or cleaned with steam.

Small tools and instruments such as, scales and balances, test instruments, small power tools, etc., should be gently sponged off with 200 ppm of chlorine solutions if they are inert plastic or 200 ppm of iodophor if they are otherwise. These should then be placed back in their respective buildings during the formaldehyde fumigation. High precision electronic test equipment should not be subjected to the fumigation, especially if there has been little chance that it was ever contaminated.

iv) 'New-Water' Plumbing: all new-water plumbing which is contained within buildings, especially those which have blind ends or terminate in manifolds, should be filled with a minimum 200 ppm chlorine solution. The chlorine solution should be held in the lines for 24-48 hours minimum, followed by clean water rinsing. Pipes may also be disinfected by recirculating hot water (>60ºC) through them for several hours.

v) Uniforms, boots, etc.: all items worn or used by employees should be either disposed of or thoroughly washed and disinfected. In the case of clothing, such as coveralls, normal washing which incorporates a chlorine bleach is sufficient, especially if accompanied by sun drying. Other items, such as boots, gloves and other non-cloth items can be safely soak-chlorinated in a 200 ppm chlorine solution. This should be followed by a freshwater rinse. These items should also be contained within their respective buildings during formaldehyde fumigation.

vi) Feed items: all feed items, such as prepared feeds, fresh feeds should be removed from the facility and replaced with new feeds from sources known to be free of contamination by disease-causing agents.

Re-stocking of disinfected farms

Following a TCU, restocking of the disinfected facilities or farms should be accomplished only with stocks known to be free of VNN and other notifiable or other emerging or significant fish diseases of concern.

5. REFERENCES

Anderson, A.J., Arthington, A.H. & Anderson, A. (1990). Lipid classes and fatty-acid composition of the eggs of some Australian fish. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 96:267-270.

Anderson, J.R., Morrison, A.K. & Ray, D.J, (1992a). Validation of the use of thin-sectioned otoliths for determining the age and growth of golden perch, *Macquaria ambigua* (Perciformes: Percichthyidae), in the lower Murray-Darling basin, Australia. *Australian Journal of Marine and Freshwater Research* 43:1103-1128.

Anderson, J.R., Morrison, A.K. & Ray, D.J, (1992b). Age and growth of Murray cod, *Maccullochella peeli* (Perciformes: Percichthyidae), in the lower Murray-Darling basin, Australia, from thin-sectioned otoliths. *Australian Journal of Marine and Freshwater Research* 43:983-1013.

Anon, (2004). Eastern Freshwater Cod (*Maccullochella ikei*) Recovery Plan. NSW Fisheries, Nelson Bay.

Ballagh, D., Fielder, D.S. & Pankhurst, T. (2010). In: Fielder, D.S., A. Roberts-Thomson, M.A. Booth, G.L. Allan and R. Adlard, 2008. Aquafin CRC project 1B3: Increasing the profitability of snapper farming by improving hatchery practices and diets Volume 3: Fingerling production and health. NSW Department of Primary Industries, Aquafin CRC and Fisheries Research and Development Corporation.

Bardsley, W.L., Fielder, D.S. & Allan, G.L. (1998). Evaluation of small strain rotifers *Brahionus rotundiformis* for use as first-feeds in Australian marine fish hatcheries. Proceedings of Australian Marine Science Association Conference, Glenelg, Adelaide, South Australia.

Battaglene, S.C. (1995). Induced ovulation and larval rearing of Australian marine fish. PhDThesis, University of Tasmania, Launceston, Tasmania.

Battaglene, S.C. & Allan, G.L. (1990). Australian bass - a potential winter crop for the prawn farming industry in NSW and southern Queensland. *Australian Fisheries* 49:31-32.

Battaglene, S.C. & Talbot, R.B. (1990). Initial swimbladder inflation in intensively reared Australian bass larvae, *Macquaria novemaculeata* (Steindachner) Perciformes: Percichthyidae. *Aquaculture* 86:431-442.

Battaglene, S.C. &Talbot, R. B. (1994). Hormone induction and larval rearing of mulloway, *Argyrosomus hololepidotus* (Pisces: Sciaenidae). *Aquaculture* 126:73-81.

Battaglene, S.C., Talbot, R.B. & Beevers, P.J. (1989a). Australian bass culture – recent advances. *Australian Fisheries* 49(1):28039.

Battaglene, S.C., Beevers, P.J. & Talbot, R.B. (1989b). A review of research into the artificial propagation of Australian bass (*Macquaria novemaculeata*) at the Brackish Water Fish Culture Research Station, Salamander Bay,1979 to 1986, NSW Agriculture & Fisheries, Sydney, Australia. Fisheries Bulletin No. 3, 1. PP Bureau of Rural Resources Proceedings No. 15.

Battaglene, S.C. & Morehead, D.T. (2006). Tolerance of striped trumpeter *Latris lineate* embryos to ozonated seawater. *Aquaculture International* 14(5):421-429.

Battaglene, S.C. & Selosse, P.M, (1996). Hormone-induced ovulation and spawning of captive and wild broodfish of the catadromous Australian bass, *Macquaria novemaculeata* (Steindachner), (Percichthyidae). *Aquaculture Research* 27:191-204.

Battaglene, S.C., Talbot, R.B. & Allan, G.L. (1992). Supplementary feeding with brine shrimp, *Artemia salina* in the extensive brackish water culture of Australian bass *Macquaria novemaculeata* (Steindachner). In: Allan, G.L. & Dall, W. (Eds.), Proceedings of Aquaculture Nutrition Workshop, Salamander Bay, 15-17 April 1991. NSW Fisheries.

Benetti, D.D., Matera, J.A., Stevens, O.M., Alarcon, J.F., Feeley, M.W., Rotman, F.J., Minemoto, Y., Banner-Stevens, G., Fanke, J., Zimmerman, S. & Eldridge, L. (2002). *Journal of the World Aquaculture Society*: 33:349-357.

Booth, M.A., Pirozzi, I., Allan, G.L. & Fielder, D.S. (2010). Aquafin CRC Project 1B5: Feed technology for temperature fish species Volume 2: Diet development. Industry and Investment NSW and Fisheries Research and Development Corporation. 376 pp.

Burke, J.B. (1994). Larvae of Australian bass, *Macquaria novemaculeata* (Steindachner 1866), can survive low salinities within 7 days of hatching. *Aquaculture Research* 25:579–584.

Carton, A.G. (2005). The impact of light intensity and algal-induced turbidity on first-feeding *Seriola lalandi* larvae. *Aquaculture Research* 36:1588-1594.

Chenoweth, S. F. and J.M. Hughes, 1997. Genetic population structure of the catadromous Perciform: *Macquaria novemaculeata* (Percichthyidae). *Journal of Fish Biology* 50:721-733.

Fielder, D.S. & Allan, G.L. (2008a). Effect of polyhouse covers and addition of *Artemia* on larval rearing of snapper in fertilized ponds. In: Fielder, D.S., Roberts-Thomson, A., Booth, M.A., Allan, G.L. & Adlard, R. (Eds.), Aquafin CRC Project 1B3: Increasing the profitability of snapper farming by improving hatchery practices and diets Volume 3: Fingerling production and health, pp 32-41. NSW Department of Primary Industries, Aquafin CRC and Fisheries Research and Development Corporation.

Fielder, D.C. & Allan, G.L. (2008b). Evaluation of seawater zooplankton production in plastic-lined ponds for extensive and intensive culture of snapper larvae. In: Fielder, D.S., Roberts-Thomson, A., Booth, M.A., Allan, G.L. & Adlard, R. (Eds.), Aquafin CRC Project 1B3: Increasing the profitability of snapper farming by improving hatchery practices and diets Volume 3: Fingerling production and health, pp 47-50. NSW Department of Primary Industries, Aquafin CRC and Fisheries Research and Development Corporation.

Fielder, D.S. & Bardsley, W. (1999). 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* 30(3):380-387.

Fielder, D.S., Bardsley, W.J. & Allan, G. L. (1999). Enhancement of Mulloway (*Argyrosomus japonicus*) in intermittently opening lagoons. NSW Fisheries Final Report Series No 14.

Fielder, D.S., Purser, G.J. & Battaglene (2000). Effect of rapid changes in temperature and salinity on availability of the rotifers *Brachionus rotundiformis* and *Brachionus plicatilis*. *Aquaculture*189:85-99.

Fielder, D.S., Roberts-Thomson, A., Booth, M.A., Allan, G.L. & Adlard, R. (2008). Aquafin CRC project 1B3: Increasing the profitability of snapper farming by improving hatchery practices and diets Volume 3: Fingerling production and health. NSW Department of Primary Industries, Aquafin CRC and Fisheries Research and Development Corporation.

Fielder, D.S., Ballagh, D., Booth, M.A., Pankhurst, P.M., Becker, C.E. & Allan, G.L. (2010). Aquafin CRC project 1B5: Feed technology for temperate fish species, Vol 1: Feeding strategies. Industry and Investment NSW, Aquafin CRC and Fisheries Research and Development Corporation.

Gillanders, B.M., Ferrell, J.F. & Andrew, N. L. (1999). Size at maturity and seasonal changes in gonad activity of yellowtail kingfish (*Seriola lalandi*; Carangidae) in New South Wales, Australia. *New Zealand Journal of Marine and Freshwater Research* 33:457-468.

Gillanders, B. M, J. F. Ferrell, and N. L. Andrew, 2001. Estimates of movement and life-history parameters of yellowtail kingfish (*Seriola lalandi*): how useful are data from a cooperative tagging program? *Marine and Freshwater Research* 52:179-92.

Glazebrook, J.S., Heasman, M.P. & de Beer, s.W. (1990). Picornalike viral particles associated with mass mortalities in larval barramundi, *Lates calcarifer* (Bloch). *Journal of Fish Diseases* 13:245-249.

Gooley, G.J. (1992). Validation of the use of otoliths to determine the age and growth of Murray cod, *Maccullochella-Peelii* (Mitchell) Percichthyidae), in Lake Charlegrark, Western Victoria. *Australian Journal of Marine and Freshwater Research* 43:1091-1102.

Hall, D. (1984). The Coorong: Biology of the major fish species and fluctuations in catch rates 1976-1983. *SAFIC* 8:3-15.

Harris, J.H. (1983). The Australian bass (*Macquaria novemaculeata*). PhD Thesis. University of NSW.

Harris, J.H. (1985). Age of Australian bass, *Macquaria novemaculeata* (Perciformes, Percichthyidae), in the Sydney Basin. *Australian Journal of Marine and Freshwater Research* 36:235-246.

Harris, J.H. (1986). Reproduction of the Australian bass, *Macquaria novemaculeata* (Perciformes, Percichthyidae) in the Sydney Basin. *Australian Journal of Marine & Freshwater Research* 37:209-235.

Harris, J.H. (1987). Growth of Australian bass (*Macquaria novemaculeata*) (Perciformes, Percichthyidae) in the Sydney Basin. *Australian Journal of Marine and Freshwater Research* 38:351-361.

Harris, J.H. (1988). Demography of Australian bass (*Macquaria novemaculeata*) (Perciformes, Percichthyidae) in the Sydney Basin. *Australian Journal of Marine & Freshwater Research* 39:355-369.

Harris, J.H. (1985a.). Diet of Australian bass, (*Macquaria novemaculeata*) (Perciformes, Percichthyidae) in the Sydney Basin. *Australian Journal of Marine & Freshwater Research* 36:219-234.

Harris, J.H. (1985b). Age of Australian Bass, (*Macquaria novemaculeata* (Perciformes: Percichthyidae), in the Sydney Basin. *Australian Journal of Marine & Freshwater Research* 36 :235-246.

Kolkovski, S., (2005). Evaluation of Yellowtail Kingfish (*Seriola lalandi*) grow out in and-based systems and broodstock management. Final Report to the Aquaculture Development Council, Western Australia. Department of Fisheries – Research Division, Western Australian Fisheries and Marine Research Laboratories, Western Australia 6920. ISBN 1 877098 74 4.

Kolkovski, S. & Sakakura, Y. (2004). Yellowtail kingfish from larvae to mature fish – problems and opportunities. In Cruz Suárez, L.E., Ricque Marie, D., Nieto López, M.G., Villarreal, D., Scholz, U. & González, M. Advances del VII Symposium Internacional de Nutrición Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mėxico.

Lom, J. & Dykova, I. (1992). Protozoan parasites of fishes (Developments in Aquaculture and Fisheries Science 26). Elsevier, Amsterdam.

Moran, D., Smith, C.K., Gara, B.G.& Poortenaar, C.W. (2007). Reproductive behaviour and egg development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). *Aquaculture* 262: 95–104.

Moretti, A., Pedini Fernandez-Criado, M., Cittolin, G. And Guidastri, R., 1999. Manual on hatchery Production of Seabass and Gilthead Seabream. Volume 1. Rome, FAO, 194 p.

Papadakis, I.E., Chatzifotis, S., Divanach, P. & Kentouri, M. (2008). Weaning of greater amberjack (*Seriola dumerilii* Risso 1810) juveniles from moist to dry pellet. *Aquaculture International* 18(1):13-25.

Partridge, G., Jenkins, G. & Frankish, K. (2003). Hatchery Manual for the Production of Snapper *(Pagrus auratus)* and Black Bream *(Acanthopagrus butcheri)***.** WestOne Publishing, Perth.152 pp.

PIRSA (2002). Yellowtail Kingfish Aquaculture in SA. Fact Sheet. Primary Industries and Resources South Australia. ISSN 132-0409. (www.pir.sa.gov.au).

Poortenaar, C.W., Hooker, S.H. & Sharp, N. (2001). Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. *Aquaculture* 201:271-286.

Rimmer, M.A. & Franklin, B. (1997). Development of Live Fish Transport Techniques. FRDC Projects 93/184 and 93/185. DPI (Department of Primary Industries), Cairns, Queensland, Australia. 151 pp.

Rimmer, M.A. & Reed, A. (1989). Effects of nutritional enhancement of live food organisms on growth and survival of barramundi/ seabass *Lates calcarifer* (Bloch) larvae. In: Advances in Tropical Aquaculture, 20 February - 4 March, 1989, Tahiti. *AQUACOP IFREMER Actes de Colloque* 9:611-623.

Roberts-Thomson, A. (2008). Investigating cryopreservation of *Amyloodinium ocellatum*. In: Aquafin CRC Project 1B3: Increasing the profitability of snapper farming by improving hatchery practices and diets Volume 3: Fingerling production and Health (Eds. Fielder, D.S., Roberts-Thomson, A., Booth, M.A., Allan, G.L. & Adlard, R.). Final Report to Fisheries Research and Development Corporation Project No. 2001/208. NSW Department of Primary Industries, Aquafin CRC and Fisheries Research and Development Corporation, 88-92.

Rowland, S.J. (1983). The hormone-induced ovulation and spawning of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson) (Percichthyidae). *Aquaculture* 35:221-238.

Rowland. S.J. (1986). The hormone-induced spawning and larval rearing of Australian freshwater fish, with particular emphasis on golden perch (*Macquaria ambigua*). In: Proceedings of the first Freshwater Aquaculture Workshop, Narrandera.1983, (Ed. L.F.Reynolds) pp.23-32. (NSW Agriculture and Fisheries, Sydney).

Rowland, S.J. (1998). Age and growth of the Australian freshwater fish Murray cod, *Maccullochella peelii peelii. Proceedings of the Linnean Society of New South Wales* 120:168-179.

Rowland, S.J., Landos, M.L., Callinan, R.B., Allan, G.L., Read, P., Mifsud, C. Nixon, M., Boyd, P. and Tully, P. (2007). Development of a health management strategy for the silver perch aquaculture industry. *NSW Department of Primary Industries – Fisheries Final Report Series* No. 93, ISSN 1449-9967.

Rutledge, W.P. (1989). The Texas marine hatchery program – it works! *CalCOFI Report* 30:49-52.

Rutledge, W., Rimmer, M., Russell, J., Garrett, R. & Barlow, C. (1990). Cost Benefit of Hatchery-reared Barramundi, *Lates calcarifer* (Bloch), in Queensland. *Aquaculture and Fisheries Management* 21:443-448.

Rutledge, W.P. & Rimmer, M.A. (1991). Culture of larval sea bass, *Lates calcarifer* (Bloch), in Queensland. *Aquaculture and Fisheries Management* 21:443-448.

Sarre, G.A. & Potter, I.C, (2000). The age comparisons and growth rates of *Acanthopagrus butcheri* (Sparidae) vary among estuaries: some possible contributing factors. *FisheryBulletin* 98:785-799.

Schnierer, S.B. (1982). The biology of the Australian bass (*Macquaria novemaculeata*) (F. Steindachner) in the Richmond River, New South Wales, MSc. Thesis, University of Queensland.

Silberschneider,V. & Gray, C. (2005). Arresting the decline of the commercial and recreational fisheries for mulloway (*Argyrosomus japonicus*). *NSW DPI Fisheries Final Report Series* No. 82, NSW Fisheries: Cronulla. 71 pp.

Stewart, J., Ferrell, D.J. & van der Walt, B. (2004). Sizes and ages in commercial landings with estimates of growth, mortality, and yield per recruit of yellowtail kingfish (*Seriola lalandi*) from New South Wales, Australia. *Marine and Freshwater Research* 55:489-497.

Trinski, T., Hay, A.C.& Fielder, D.S. (2005). Larval development of estuary perch *(Macquaria colonorum*) and Australian bass (*M. novemaculeata*) (Perciformes:Percichthyidae), and comments on their life history**.** *Fishery Bulletin*103:183-194.

van der Wal, E. (1985). Effects of temperature and salinity on the hatch rate and survival of Australian bass *(Macquaria novemaculeata)* eggs and yolk-sac larvae. *Aquaculture* 47:239-244.

van der Wal, E.J. (1989). Australian bass. Proceedings of the Fourth Annual Conference of the Australian mariculture association, Brisbane, Queensland.

van der Wal, E.J. & Nell, J.A. (1986). Effect of food concentration on the survival and growth of Australian bass (*Macquaria novemaculeata*) larvae. *The Progressive Fish-Culturist* 48: 202-204.

Vergani, M., Acha, E.M., Astarloa Diaz, J.M. & Giberto, D. (2008). Food of the yellowtail amberjack *Seriola lalandi* from the south-west Atlantic. *Journal of the Marine Biological Association of the United Kingdom* 88 (4):851-852.

Wilde, G. R., & Sawynok, W. (2005). Use of relative risk to assess the effect of hook removal on recapture rates of angler-caught fishes in Australia. Fourth World Recreational Fishing Conference. Trondheim, Norway. June 2005.

Williams, N.J. (1970). A comparison of the two species of the genus Percalates Ramsey and Ogilby (Percomorphi: Macquariidae) and their taxonomy. *NSW State Fisheries Research Bulletin*, 11:60.

6. APPENDICES

6.1 Harvesting, Counting and Transportation and Stocking of AB Fingerlings (Adapted from **Appendix 11 of Partridge et al., 2003**)

Mass weighing is a relatively quick and accurate method of counting large numbers of juvenile fish. Equipment needed for the weighing includes: 10-L plastic buckets with handles (the number dependent on how many fish are to be weighed); 5-L buckets; air supply with numerous outlets (oxygen preferred); anaesthetic (AQUI-S is used at the ADU and PSFI); wide mouthed, small mesh scoop nets to gather fish; and electronic bench scales capable of weighing up to 3-5 kg to 0.01 g accuracy.

The nursery tank holding the fish to be weighed is drained to a depth which minimises the use of anaesthetic and makes the anaesthetised fish easy to scoop. In the case of intensive indoor hatchery tanks this constitutes about 100L. Fish are concentrated within an internal harvest sump incorporated into extensive ponds. Multiple air-stones are added to provide sufficient aeration during anaesthesia. The bench scales are set up close by in a cool low light intensity area with the hatchery or for outdoor ponds in a shaded wind and rain sheltered area.

Seawater is added to the 5L buckets and the buckets are then tarred whilst sitting on the scales. Sufficient quantity of anaesthetic is added to the tank to mildly anaesthetise the fish in the tank. Seawater is added to the 10 L buckets and aeration is provided to each of these buckets. Four people are required for smooth operation (one scooping out the anaesthetised fish, one person setting up the buckets and recording weights, one person weighing and one for counting fish in the sub-samples).

Once the fish have been mildly anaesthetised, smoothly and quickly scoop a random sample of fish (100-500). Hold the net containing the fish over the tank for a short period until water drips have almost stopped (approximately 15-20 seconds). Pour the fish from the net into one of the tarred 5 L buckets. Re-weigh the bucket containing the fish sample on the bench scales then accurately count the fish. Three samples are taken following this procedure to provide mean fish weight.

Whilst this sampling is occurring another person is scooping the remaining fish out of the tank, waiting until dripping from the net is almost stopped and placing approximately 0.5-1 kg into further 5 L tarred buckets. If the fish are being transported immediately after counting, they can be revived in the transport tank.

Once the three sample batches of fish are counted the average fish weight for each batch is determined by dividing the mass weight of the fish by the number of fish in the sample. To ensure that reasonably accurate estimate is obtained, the range of these three weights should be within 5%. The average of the three weights is then used to calculate the total number of fish in the tank. This is determined by dividing the total mass weight of all the fish by the average weight as recorded from the sampling process. If this procedure is being used to count fish prior to sale, the maximum fish weight should be used to ensure the client receives no less than they have purchased.

6.2 Transportation (adapted from pages 69, **Chapter 7 Partridge et al., 2003**)

Juvenile AB may be transported either in a tank open to the atmosphere (open system) or in a sealed plastic bag (closed system). The choice of method depends on the mode of transport available, the number of fish to be transported and economics. Sealed plastic bags are the only legal choice for air transport and often the only practical choice for producers not set up for tank transport. Transport by tank is more specialised than bag transport and usually involves having a truck or trailer with a life support system capable of supplying both air from a blower (usually driven by a portable generator) and/or oxygen from pressurised cylinders.

6.2.1 Closed system

A plastic bag containing fish in oxygenated water under a sealed atmosphere of oxygen is called a closed system. Aquarium fish are transported around the world using this method and, providing the water quality and oxygen requirements of the species being transported are known, it is a reliable and efficient method. As AB are not as tolerant of poor water conditions as freshwater aquarium fish, bags must be stocked with much lower numbers of fish, resulting in increased transport costs relative to freshwater aquarium species.

A commonly used method for transportation of marine fish juveniles involves placing 4.5 L of water (the salinity of which is dependent on the water to which the fish have been acclimatised) into a 40 L clear plastic bag of 100 µm thickness (the dimensions of the standard bag are 800 x 450 mm). The fish are not fed on the day they are to be transported. For 20-25 mm AB fingerlings, no more than 100 should be stocked into each bag. Once the fish are bagged, an oxygen line is inserted into it and the air squeezed out while the top is twisted closed. Approximately 4.5 L of medical grade oxygen is then added, the oxygen line removed and the bag sealed by twisting, doubling the twist over on itself and securing with a sturdy rubber band. A second bag is then placed over the first and again secured with a rubber band. Three double bags thus prepared are placed into a standard size, air-freight foam esky (560mm long, 380 mm wide and 260 mm high) with a 500 mL ice brick. For air transport the foam box must also have a plastic liner and an absorbent pad placed between each double bag.

This method is dependable for transport times of up to 12 hours duration provided the water and AB fingerlings are held at constant temperature in the tolerable range 10 to 20 ºC.

6.2.2 Open system

Transporter tanks should thoroughly cleaned and disinfected and rinsed with transporting water prior to filling with 5 micron filtered water. As with the closed system described above the physio-chemical profile of transportation water should closely match that of water from which they are being harvested or in which they have been stockpiled. The internal surfaces should be smooth and insulated against rapid and/or large changes of temperature and well baffled to minimise turbulence and slop. Drainage lines and valves must be large enough to avoid any risk of congestion during harvesting but flexible to enable static head adjusted control over the rate drainage. The tanks should also be fitted with a screened overflow to enable blending of transportation water with water into which the fish are to be stocked and hence an opportunity for the fish to acclimate as described below. Other important criteria to be adhered to are as follows:

- Stocking rate should not exceed 5 kg/m^3 of water volume
- Ensure the vehicle (utility, truck, trailer) is in good working order and rated for the intended load
- Ensure the life support system is reliable;
- Do not feed the juveniles for 24 hours prior to transportation to minimise stress and contamination of the water during transport;
- Transport fish during the cooler hours of the day or at night in summer.

6.2.3 Acclimation on delivery

Acclimation of AB fingerlings to a new water body can be safely accomplished relatively quickly provided that the temperature and salinity of the receiving waters are comparable to those of the transport water. If this is not the case then the procedure described below should be followed. Safe acclimation of 20-25mm AB fingerlings to a new water body, even with a different salinity, is relatively fast; however, as the fish grow, they become less tolerant of rapid acclimatisation and should be treated more gradually and with great care.

The most important water quality parameters in respect to acclimating fish are temperature, salinity and pH; other factors that may affect fish survival and growth include oxygen, ionic composition and, in the case of black bream being stocked into fresh water, hardness and alkalinity. On arrival at the destination, the temperatures (and salinities if necessary) of the transport water and water body are measured. Half of the water is drained from the bag, or tank, and then topped up from the water body to the original volume. After a period of 15 minutes this process is repeated with the container topped up again from the water body. After a further 15-20 minutes the fish are released into the water body. Air or oxygen is supplied to the container during the acclimatisation period.

6.3 Manufacture and Implantation of Slow-Release LHRH - a Cholesterol Pellet for Spawning Induction of Broodstock Snapper.

Disclaimer: The importation, purchase and use of chemicals to treat animals (including fish) in Australia is controlled through the National Registration Authority for Agriculture and Veterinary Chemicals. Information on chemicals and dosage rates are provided in this document, based on published data, for specific life stages of individual species under laboratory conditions. The provision of the chemical and dose rate information in this document does not infer that the chemicals may be legally used for aquaculture in Australia. Some of the stated products in this text are unregistered, or not registered for the particular use. This should not be interpreted as a recommendation for use and the authors of this publication take no responsibility for losses should these chemicals or dosages be used in aquaculture. It is an offence to import and supply unregistered chemicals and the supply must be authorised by either a veterinary prescription or permit. Farmers should check the current registration status of chemicals with the National Registration Authority (02) 6272 5158, or http://www.nra.gov.au.

(Contributed by Stewart Fielder, Port Stephens Fisheries Institute)

Analogues of Luteinising Hormone Releasing Hormone (LHRH-a) are commonly used to induce final-stage oocyte maturation, ovulation and spawning of many fish species including snapper at PSFI. The techniques described below for manufacture and implantation of LHRH-a cholesterol pellets have been adapted from Lee et al. (1986) to suit the size of snapper held and available materials at PSFI. The concentration of LHRH-a in pellets can be modified by adding more or less LHRH-a or cholesterol to the mix to suit fish size and final target concentration of LHRH-a/kg fish. The LHRH analogue mostly used at PSFC is: (des-Gly10, [D-Trp6], pro9 – Ethylamide) LHRH analogue (Sigma Aldrich, Cat. No. L5386).

The pellets we manufacture are designed for implanting into fish with a needle and syringe designed to administer Passive Internal Transponder (PIT) tags. These PIT tags are commonly used by veterinarians to identify domestic animals and are readily available from veterinarian supply companies.

1. The Mixture

- To a pre-weighed 5 mg vial of LHRH-a add 0.5 ml of 50% punctilious ethanol and dissolve contents.
- Weigh 380 mg of cholesterol (Sigma Aldrich, Cat. No. C3292) and add the LHRH-a/ethanol solution. Mix thoroughly and dry for about 1 hr at 37°C.
- Mix approximately 20 mg (2 molten drops) of cocoa butter into the hormone/cholesterol mixture until it appears ‘flakey'.
- Accurately weigh (to at least 2 decimal places) the whole mixture to determine the concentration of the LHRH-a.

2. The Mould

- The mould used at PSFC is made from 5 mm thick plastic sheet with dimensions of approximately 10 cm x 10 cm.
- Six holes of 2.4 mm diameter are drilled at equal intervals along the centre line of the plate.
- Each hole is countersunk to allow easy packing of cholesterol mixture.

3. Making the pellet

- Place the mould onto a clean, hard surface and clamp down to prevent the mould from lifting off the surface.
- Carefully pack a hole with cholesterol mixture and hammer the mixture into the mould with a flat-ended rod or sawn-off nail.
- When the mould is completely full with hard-packed mixture unclamp the mould from the bench top and hammer the pellet out of the mould.
- Accurately weigh each pellet, and calculate the amount of LHRH-a in each pellet based on original whole mixture concentration.
- Store the pellet in a small sample jar in the freezer until needed.
- As well as the amount of hormone present in each pellet, we also record on the sample jar the mass of fish each pellet will be suitable for. For example if we require a dose of 100 µg LHRH-a/kg fish a 2 kg fish will need a pellet with 200 µg LHRH-a. This is often useful during induction when fish are anaesthetised and rapid selection of a pellet with the correct concentration of LHRH-a is necessary.

4. Implantation

- Anaesthetise the target fish, determine its weight and lay flat on a bench top.
- Insert a cholesterol pellet, of the appropriate dose, into the needle of a PIT tag applicator.
- We usually implant the pellets into the dorsal musculature of the fish.
- Place the needle tip under a scale at approximately 45° angle and insert the needle into the muscle for 1-2 cm.
- Inject the pellet and withdraw the needle.
- Check the needle to ensure that the pellet was ejected.

6.4 Broodstock and Hatchery Data Sheets used at PSFI.

BROODSTOCK INDUCTION DATA

DATE: **SPECIES:** **REGION:**

Hormone:

FEMALES

No.	Pit-tag	Fork L (mm)	Weight (g)	Oocyte dia. (µm)	x (µm)	Dose /kg	Dose ius/ug	Time	Spawning Tank
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

MALES

No.	Pit-tag	Fork L (mm)	Weight (g)	Description eg. Running milt	x (µm)	Dose /kg	Dose ius/ug	Time	Spawning Tank
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									

Comments:

PSFC - Australian Bass Production - Spawning/Incubation Tank Data

Date: **Season/Year:** **Region:**

Spawning tank	1	2	3	4	5	6	7	8
Brood ID M								
F								
Water tempºC								
Spawning time								
Viable eggs								
Total eggs								
Fert %								
Est.total larvae								
Hatch rate								
Volume transferred (L)								
No. larvae transferred								
Transferred to								
Volume transferred (L)								
No. larvae transferred								
Transferred to								
Volume transferred (L)								
No. larvae transferred								
Transferred to								
Volume transferred (L)								
No. larvae transferred								
Transferred to								

BROODSTOCK DATA SHEET

YEAR: TANK: SPECIES: GENERATION/REGION:

DATE	WATER QUALITY					Buffer(g)	Food		Contacts	Comment
	pH	DO	Temp	Sal	NH+4		Type	Amount(g)	Treatment	

YEAR:

MARINE FISH BREEDING: LARVAL REARING AND FINGERLING GROWOUT DATA

SPECIES: DATE STOCKED: DATE HARVESTED:

FAM/GEN: NO STOCKED: NO HARVESTED:

TANK NO: ORIGIN: DESTINATION:

Date	Dah	Water quality					Residual Food		Food Added		Larvae Sample			Contact/Comment
		pH	DO	Temp	Sal	NH+4	Rotes	Arts	Rotes	Arts	TL	SB	Food	

Aquafin CRC

NSW GOVERNMENT Industry & Investment

Australian Government
Fisheries Research and Development Corporation