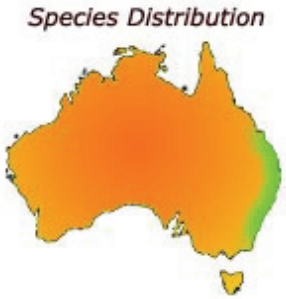
1. AUSTRALIAN BASS (AB)



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).

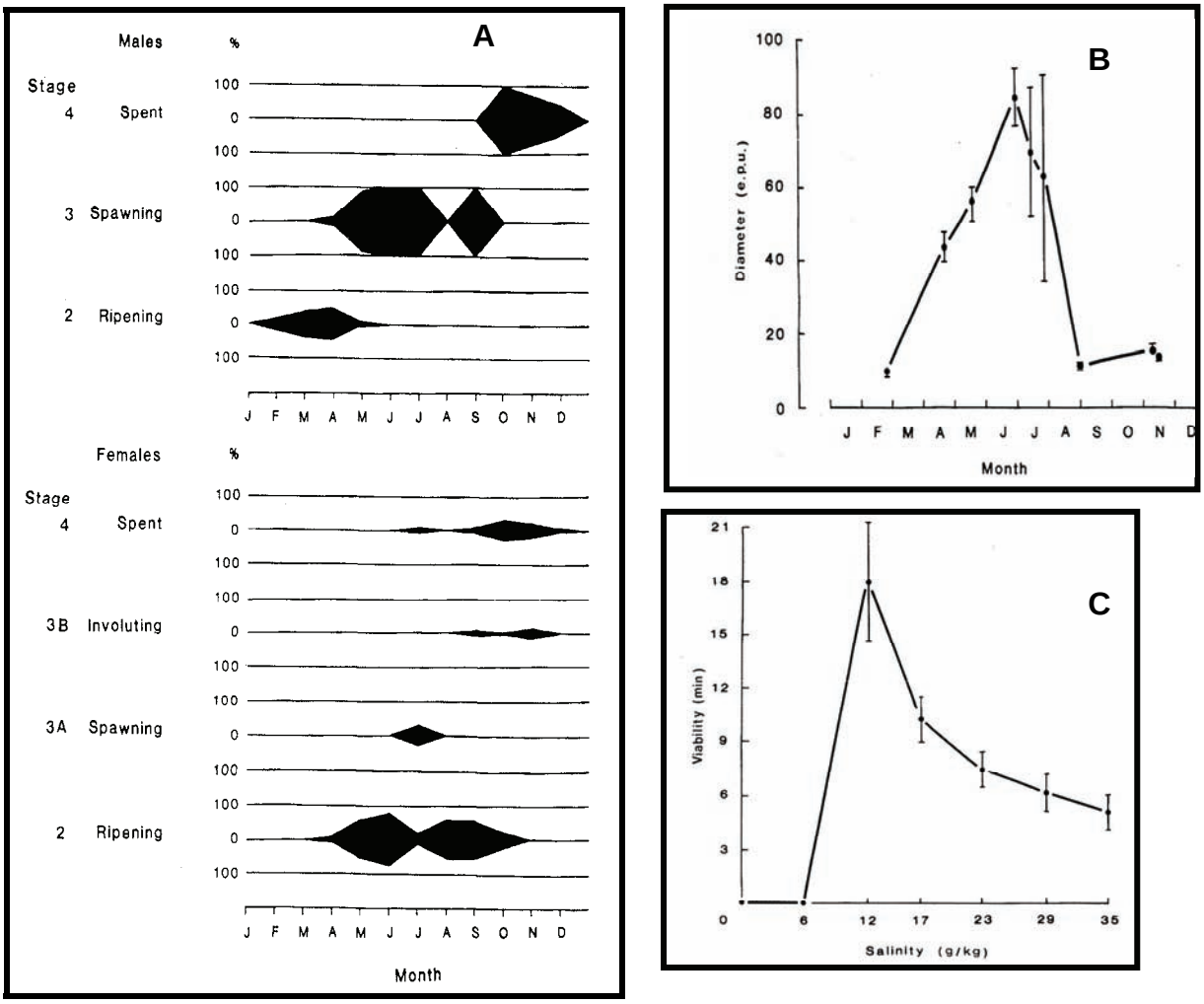


FIGURE 1: **A** Above left: Seasonal gonadal cycle of male and female AB shown by % frequency of macroscopically determined maturity stages **B** Top right: Seasonal changes in mean egg diameter **C** Bottom right: Effect of salinity on AB sperm viability (Source: Harris, 1986)

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.

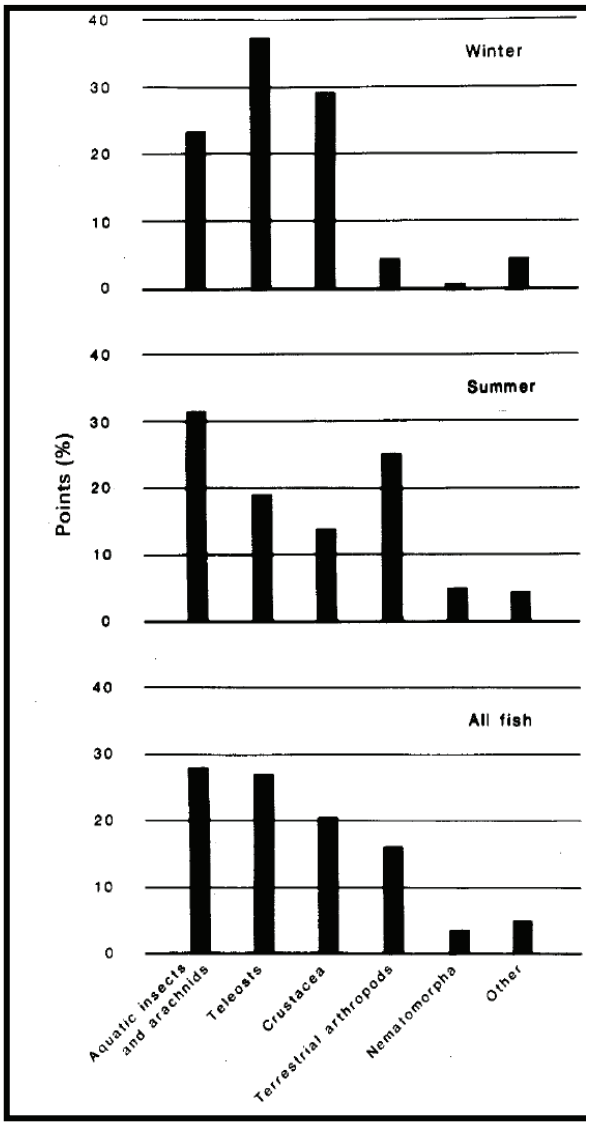


FIGURE 2: Percentage contributions of major prey groups in the diet of AB, in winter and summer (Source: Harris, 1985)

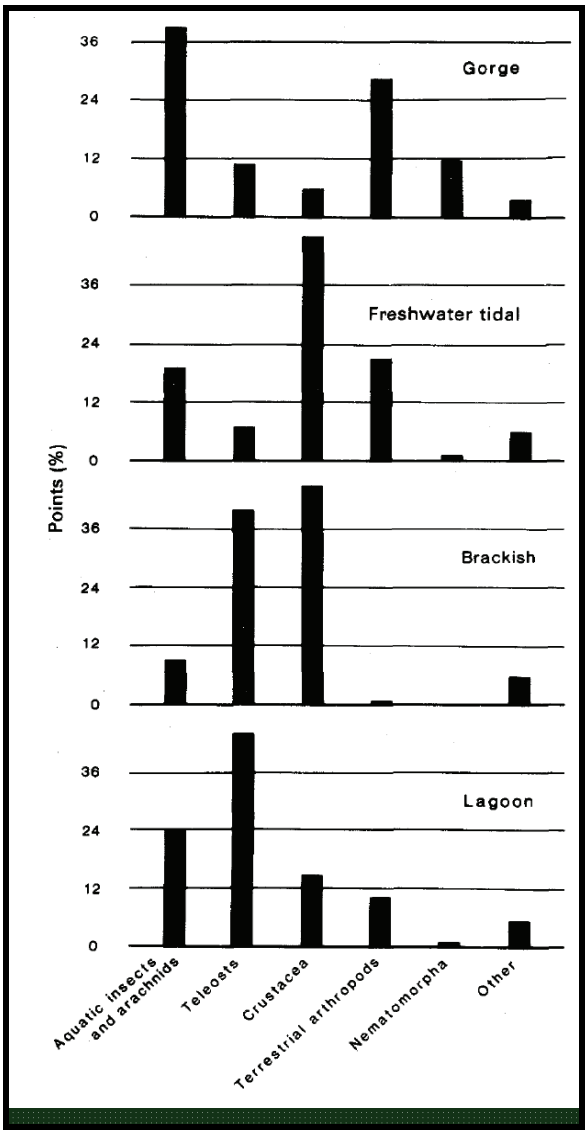


FIGURE 3: Percentage contributions of major prey groups in the diet of AB from 4 main habitat types (Source: Harris, 1985)

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).

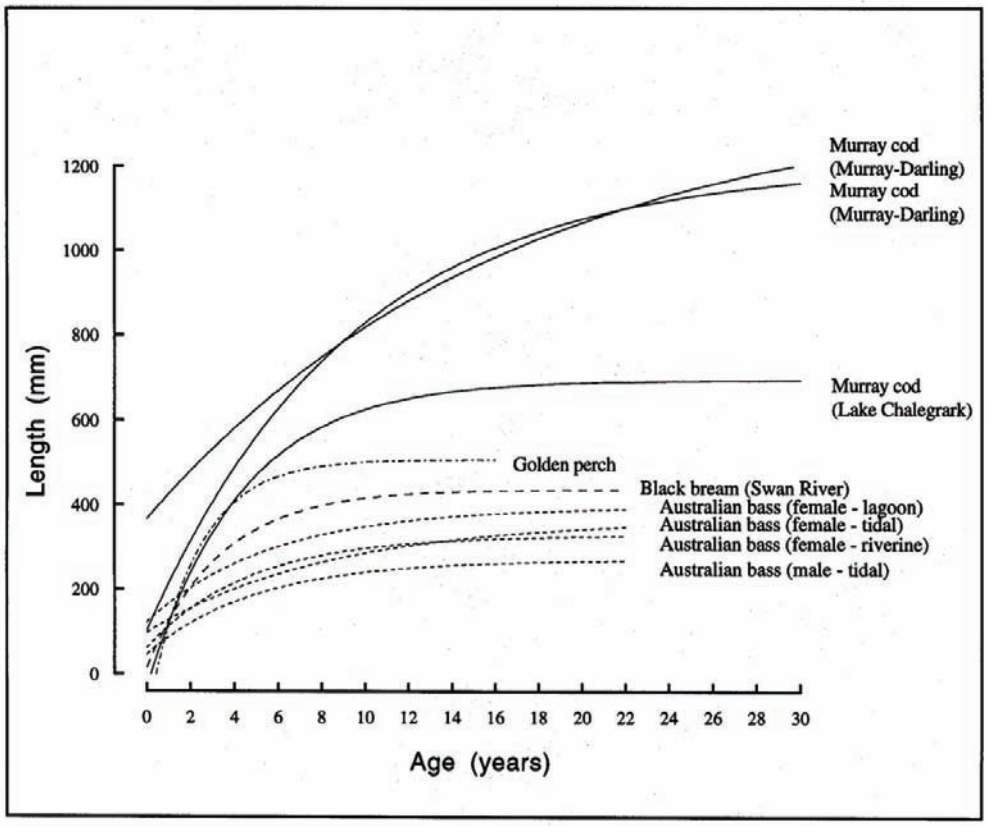


FIGURE 4: Von Bertalanffy growth curves for Murray cod, golden perch, AB and black bream. Taken by Anon (2004) from: Harris (1987); Anderson et al. (1992a,b); Gooley (1992; Rowland (1998) and Sarre and Potter (2000).

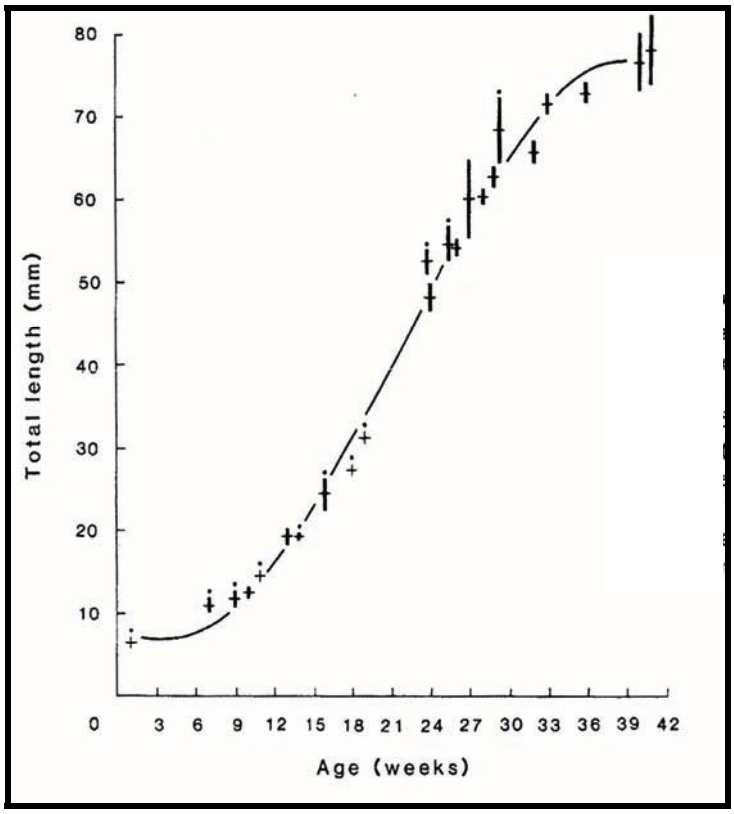


FIGURE 5: Early juvenile growth of AB in the Sydney Basin (Source: Harris, 1986)

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.

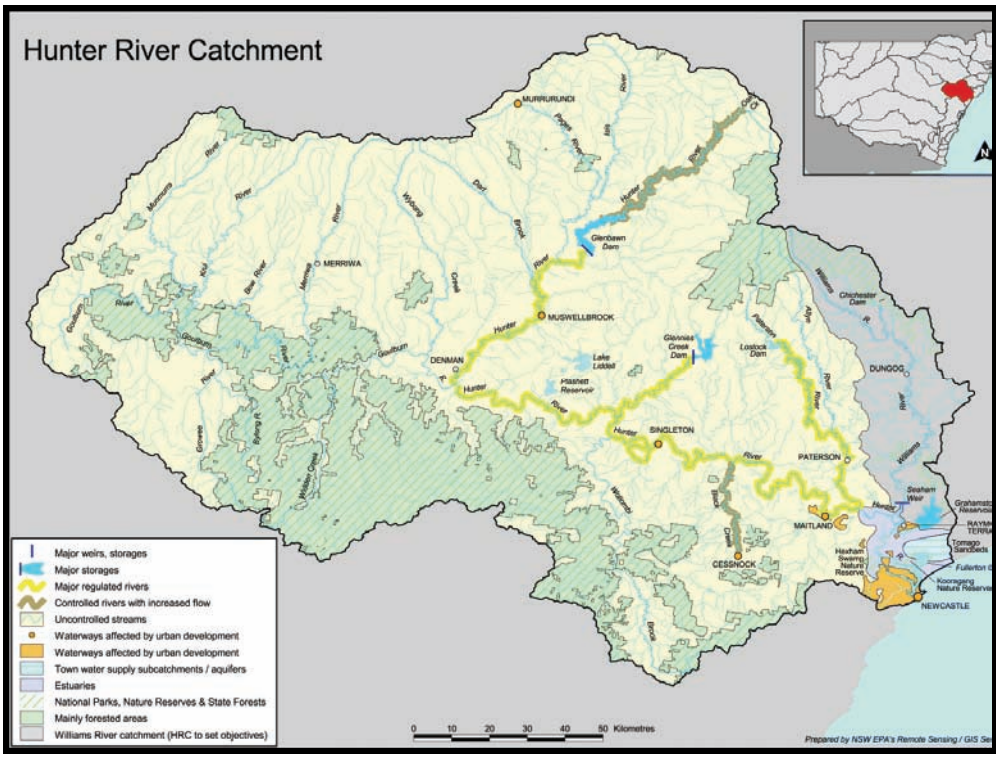


FIGURE 6: Australian Bass Broodstock collection sites local to the PSFI. Image from the NSW Department of Environment and Climate Change Website.

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.

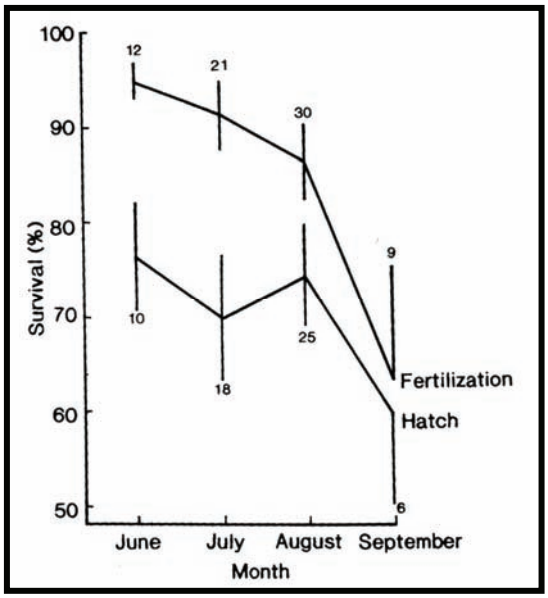


FIGURE 9: Mean monthly fertilization and hatch rates (1984-1986) for captive AB injected with hCG; bars are standard errors. (Source: Battaglene and Selosse, 1996)

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.

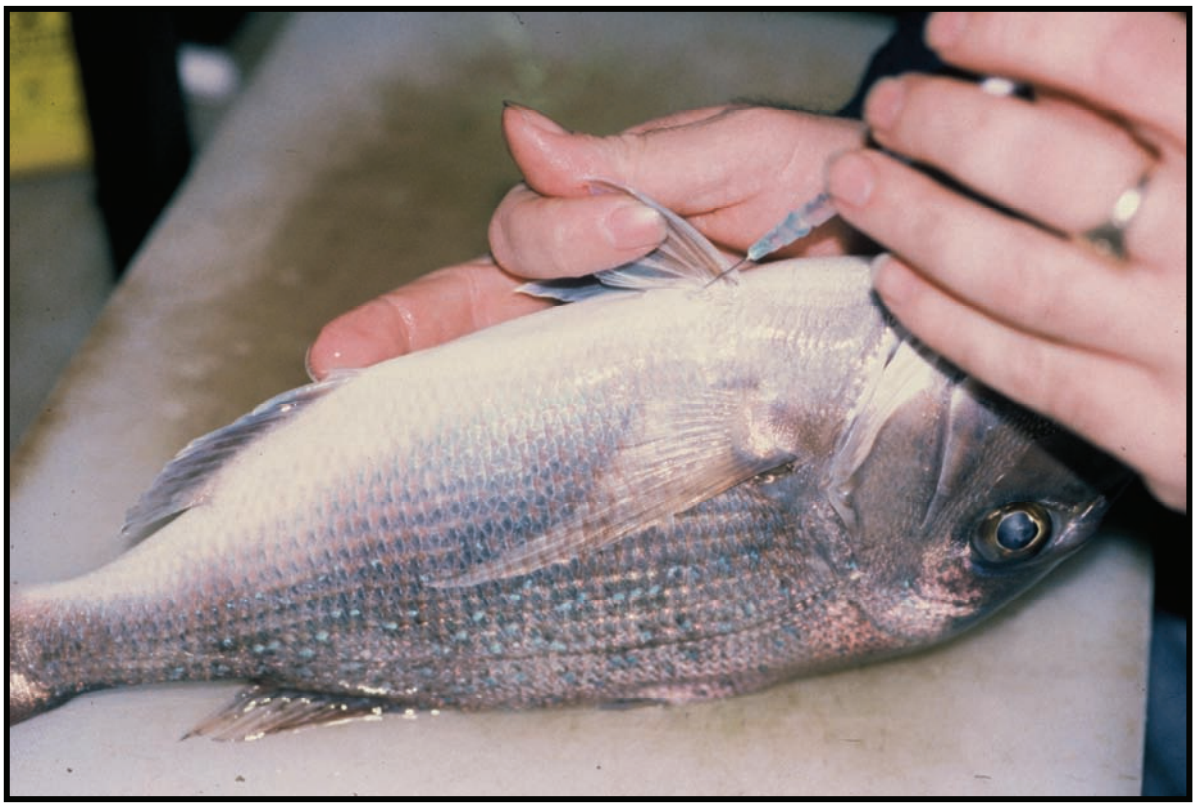


FIGURE 10: Injecting hCG at the base of the pelvic fin of a mature female snapper (*Pagrus auratus*).

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).

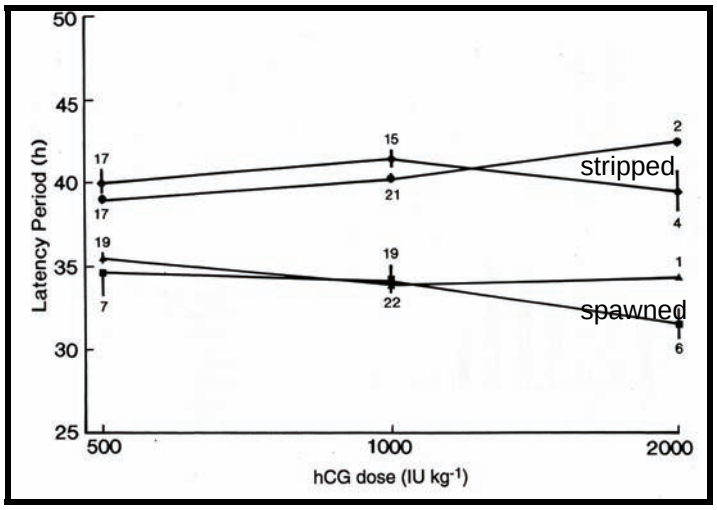


FIGURE 12: Mean latency period at 18 ±1º C for captive and wild AB injected with 500, 1000 or 2000 IU hCG./kg. Bars are standard errors, n. number of fish; (♦) stripped captive fish; (■) captive fish which spawned; (●) stripped wild fish; (▲) wild fish which spawned. (Source: Battaglene and Selosse,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

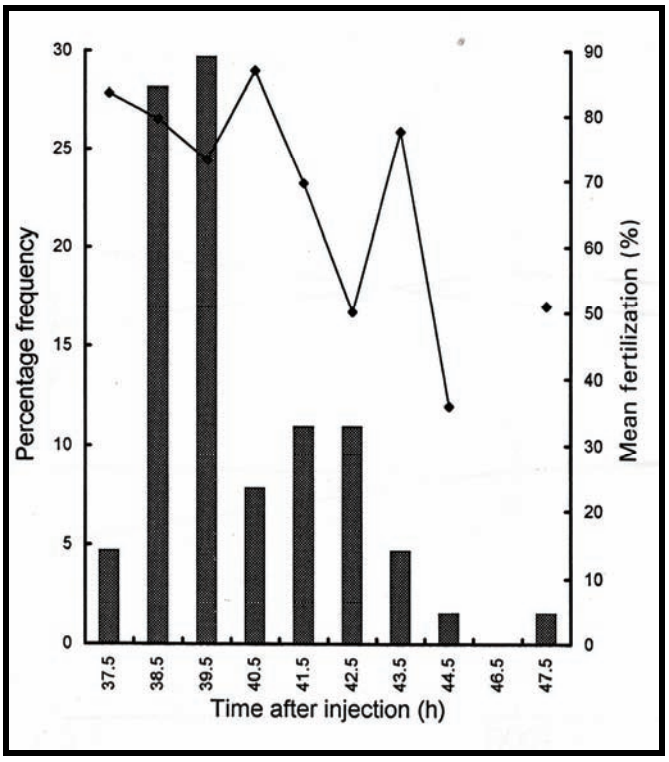


FIGURE 13: Frequency histogram of the period between injection and successful stripping at 18 ±1º C showing the mean percentage fertilization for each time interval. Data are for wild and captive fish injected with 500 or 1000 IU kg-' hCG (n = 64). (Source: Battaglene and Selosse, 1996**)**

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).

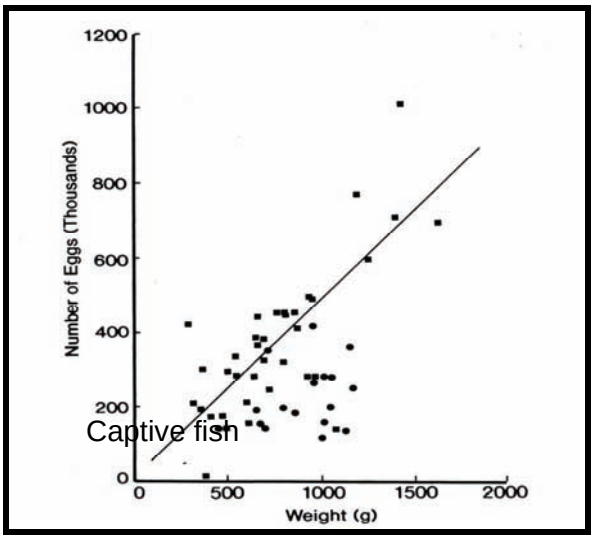


FIGURE 14: Relationship between weight and number of eggs spawned (■) or stripped (●) from wild AB injected with 500 or 1000 IU kg hCG following capture in May,June or July (Source: Battaglene and Selosse, 1996).

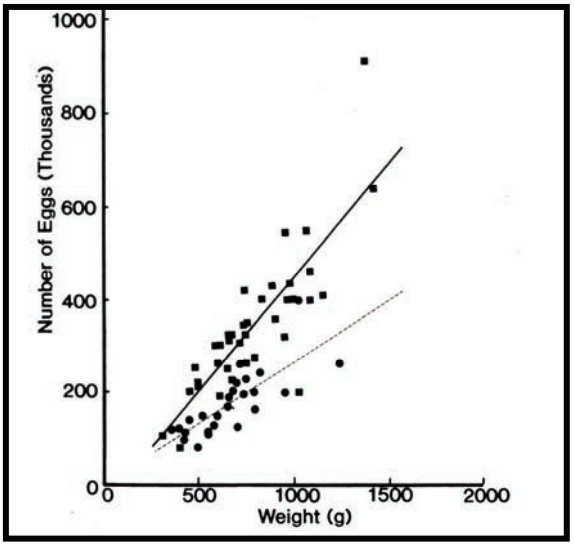


FIGURE 15: Relationship between the weight and number of eggs from spawned (■) and stripped (●) captive AB injected with 500 or 1000 IU kg hCG. (Source: Battaglene and Selosse, 1996).

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.

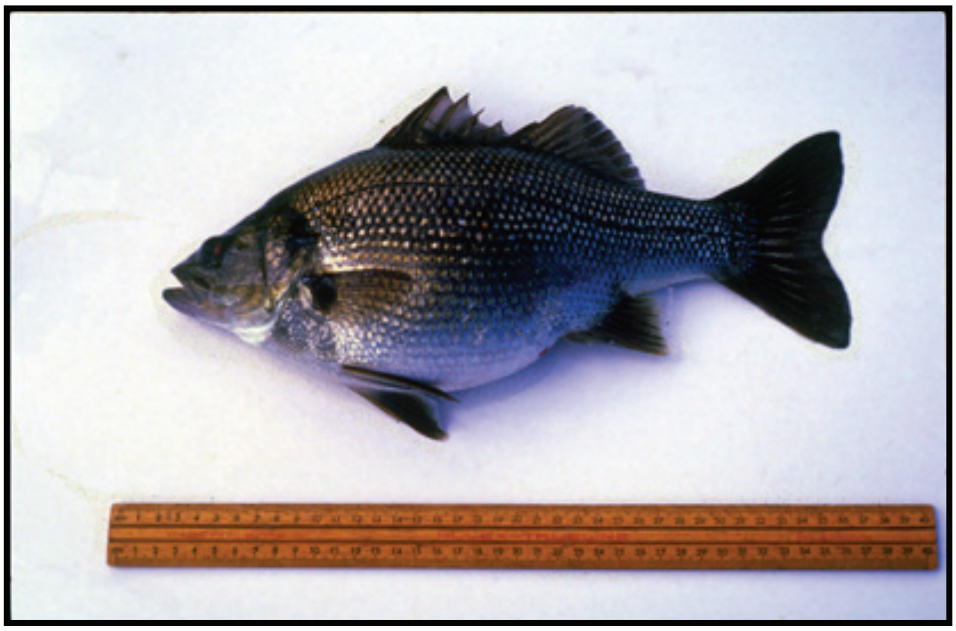


FIGURE 16: Female bass with distended abdomen after ovulation and egg hydration.

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.

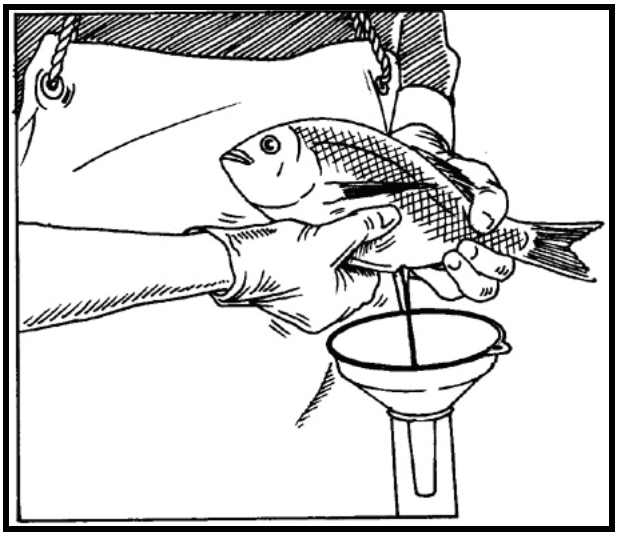


FIGURE 17: Stripping ovulated oocytes from a fish. (Source: Partridge et al., 2003).

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.

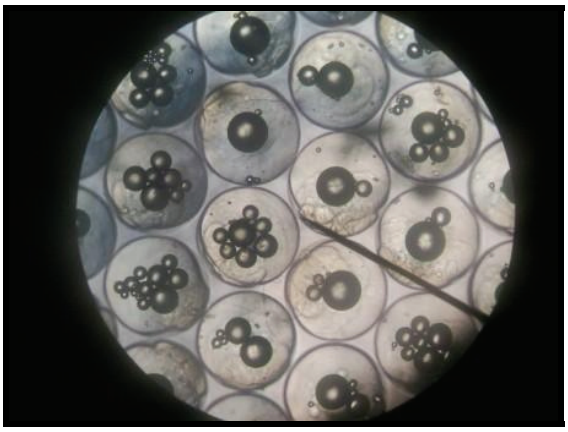


FIGURE 18: Microscopic observation of newly-fertilised AB oocytes. Note the normal, variable size and number of oil globules in oocytes.

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).

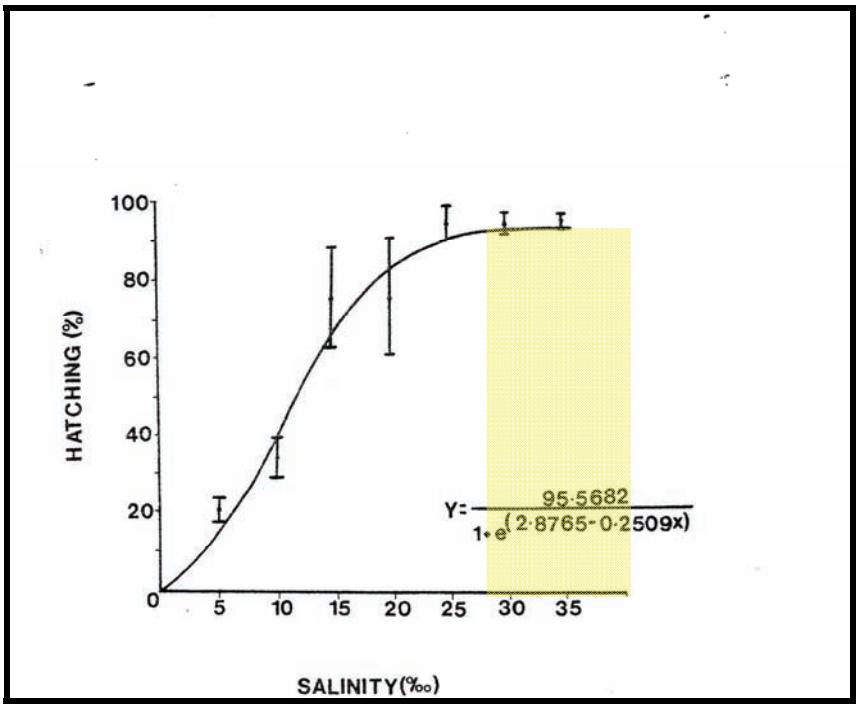


FIGURE 20: Effect of salinity on hatch rate of AB. Shaded area is recommended salinity band. (Source: Van der Wal 1985).

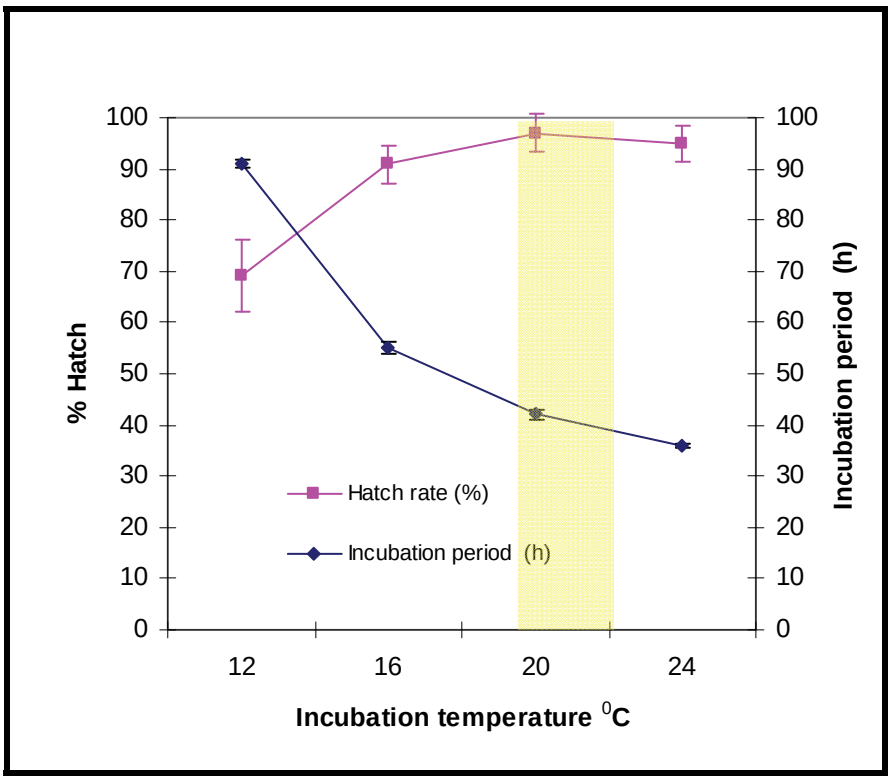


FIGURE 21: Effect of temperature on hatch rate and incubation time of AB eggs. Shaded area is recommended temperature band. (Based on data from Tables 1 and 2 of Van der Wal 1985).

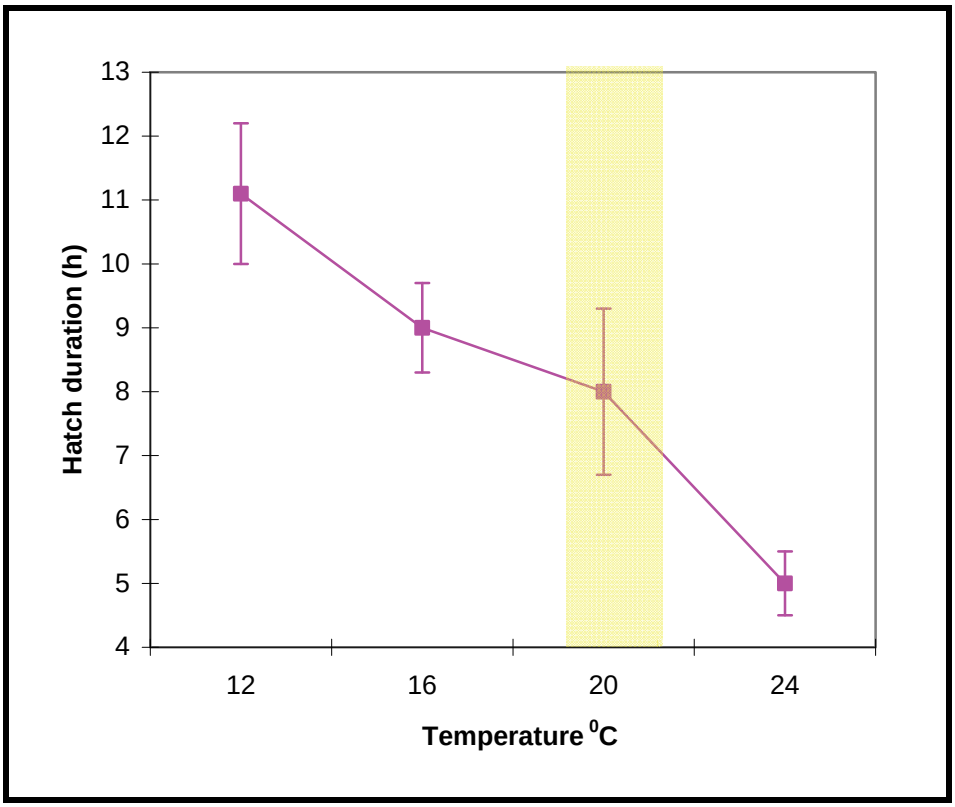


FIGURE 22: Effect of temperature on hatch duration of AB larvae (based on data from Tables 1 and 2 of Van der Wal, 1985). Shaded area is recommended temperature band.

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.

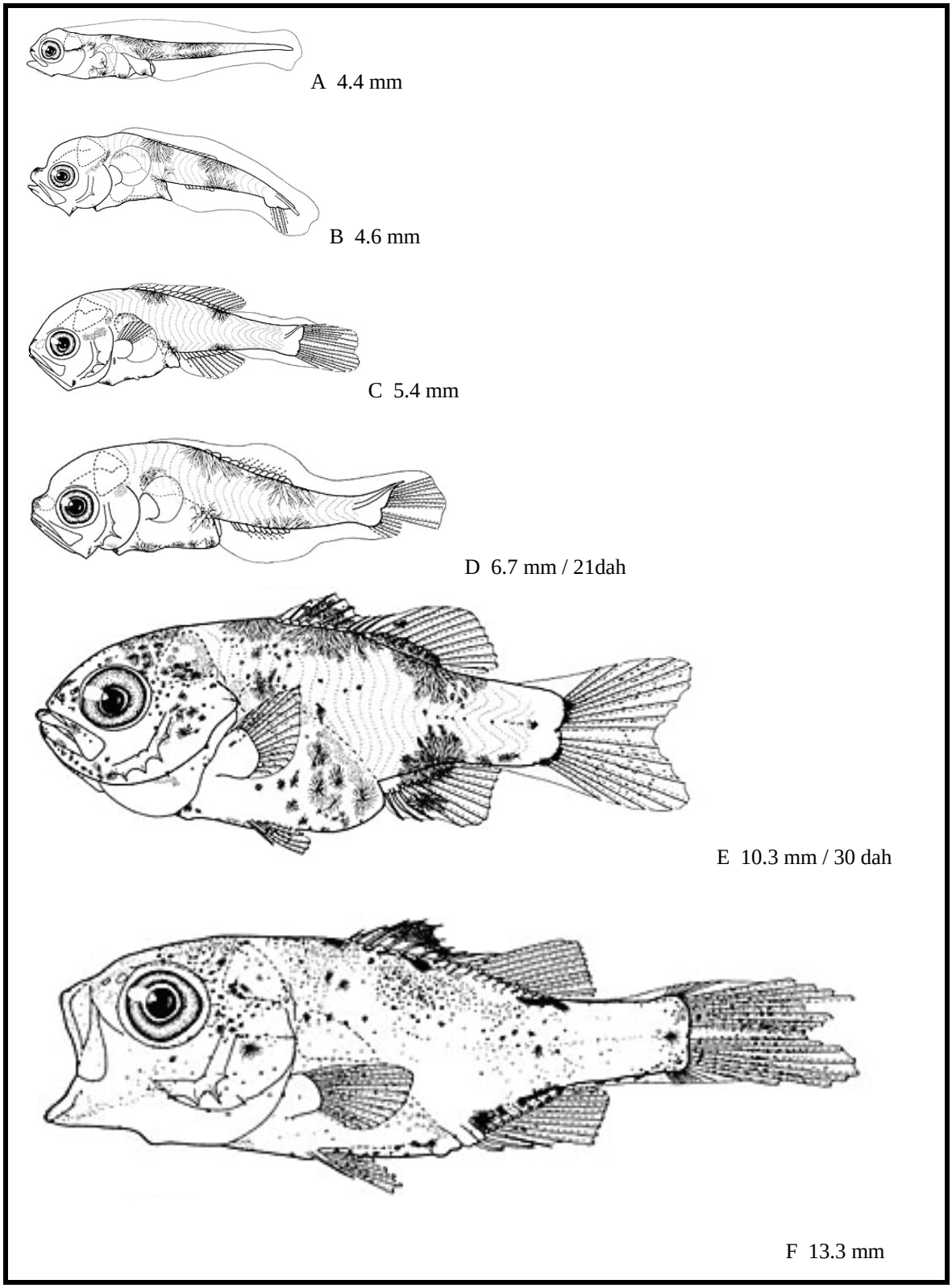


FIGURE 24: Life stages of AB. (Source: Trinski, T, Hay, A.C. & Fielder, D.S., 2005). Images down loaded from the Australian Museum Larval Fishes Website: http://amonline.net.au/larval_fishes/descriptions/macquaria-novemaculeata.html

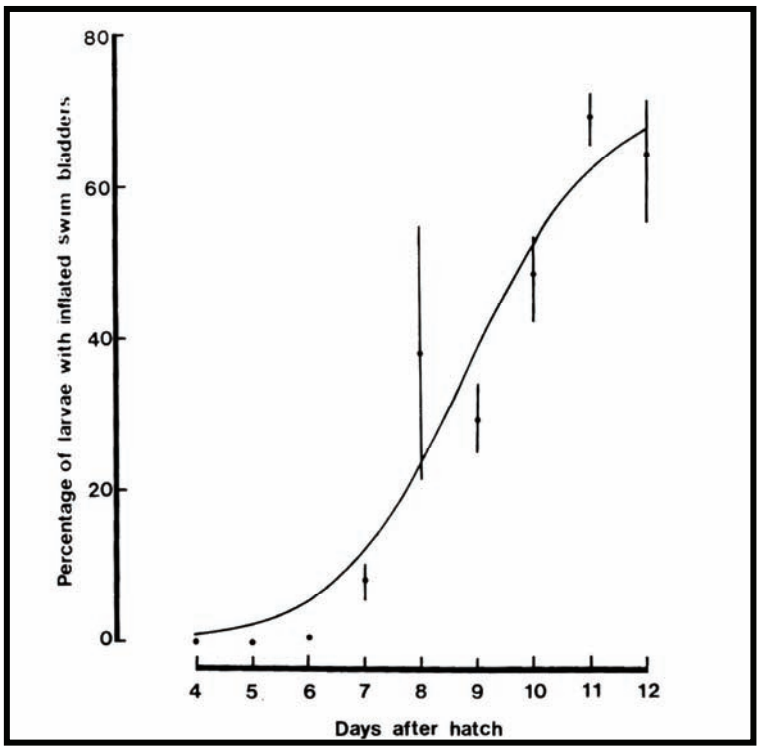


FIGURE 25: Percentages of AB larvae with inflated swim bladders from day 4 to day 12 after hatch. (means± se; n = 3) (Source: Battaglene and Talbot, 1990).

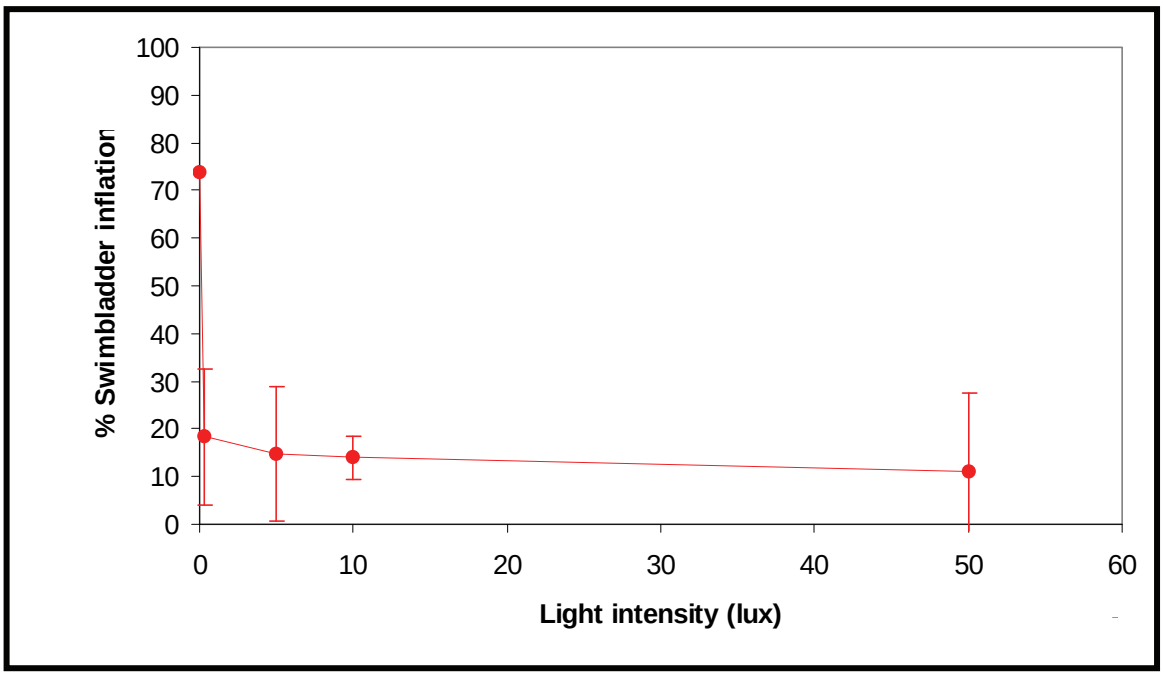


FIGURE 26: Effect of light intensity on initial swim bladder inflation (means ± sd) in larvae over the first 11 days after hatching when reared at optimum salinity (25g/kg), temperature (19 ±1 ºC), nil aeration and, with the exception of the continuous darkness (0 lux) treatment, a 12: 12-h light: dark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

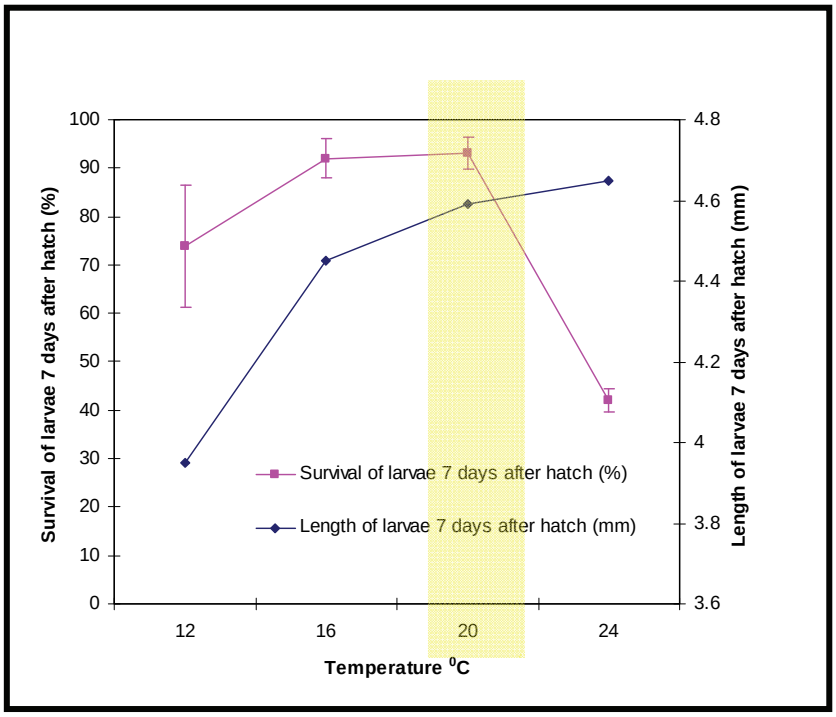


FIGURE 27: Effect of temperature on the growth and survival (mean ± s.d.) of AB larvae 7 days after hatch Shaded area is recommended temperature band. (Source: based on data from Table 1: Van der Wal, 1985).

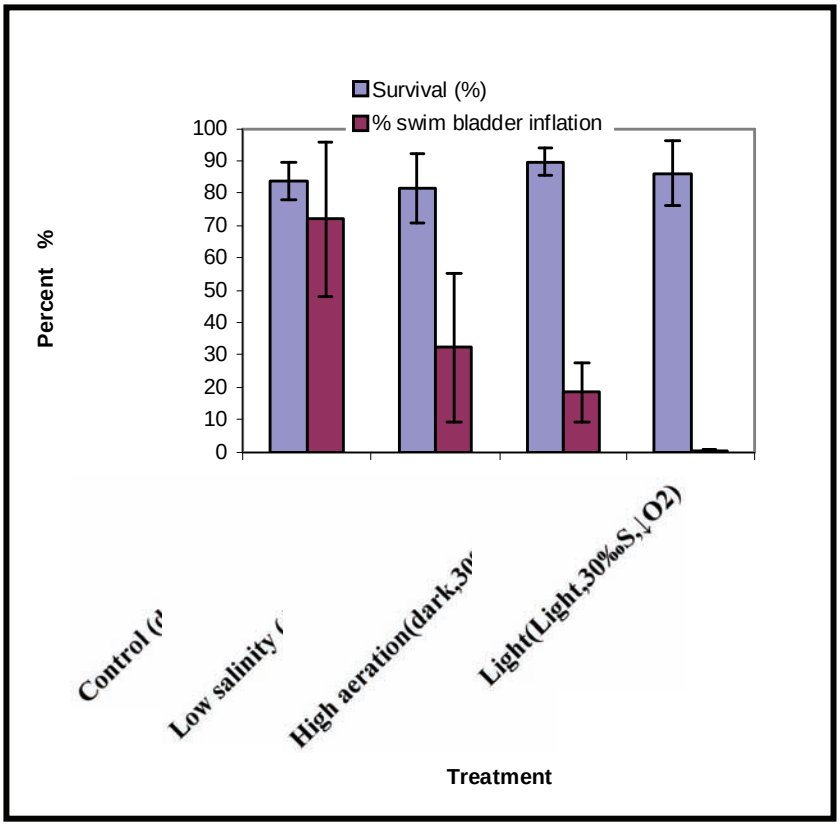


FIGURE 28: Separate effects of light, low salinity and high aeration on survival and swim bladder inflation in 11 day old AB larvae reared at 19±1 ºC. (Source: based on data provided in Table 1, Battaglene and Talbot, 1990).

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.*

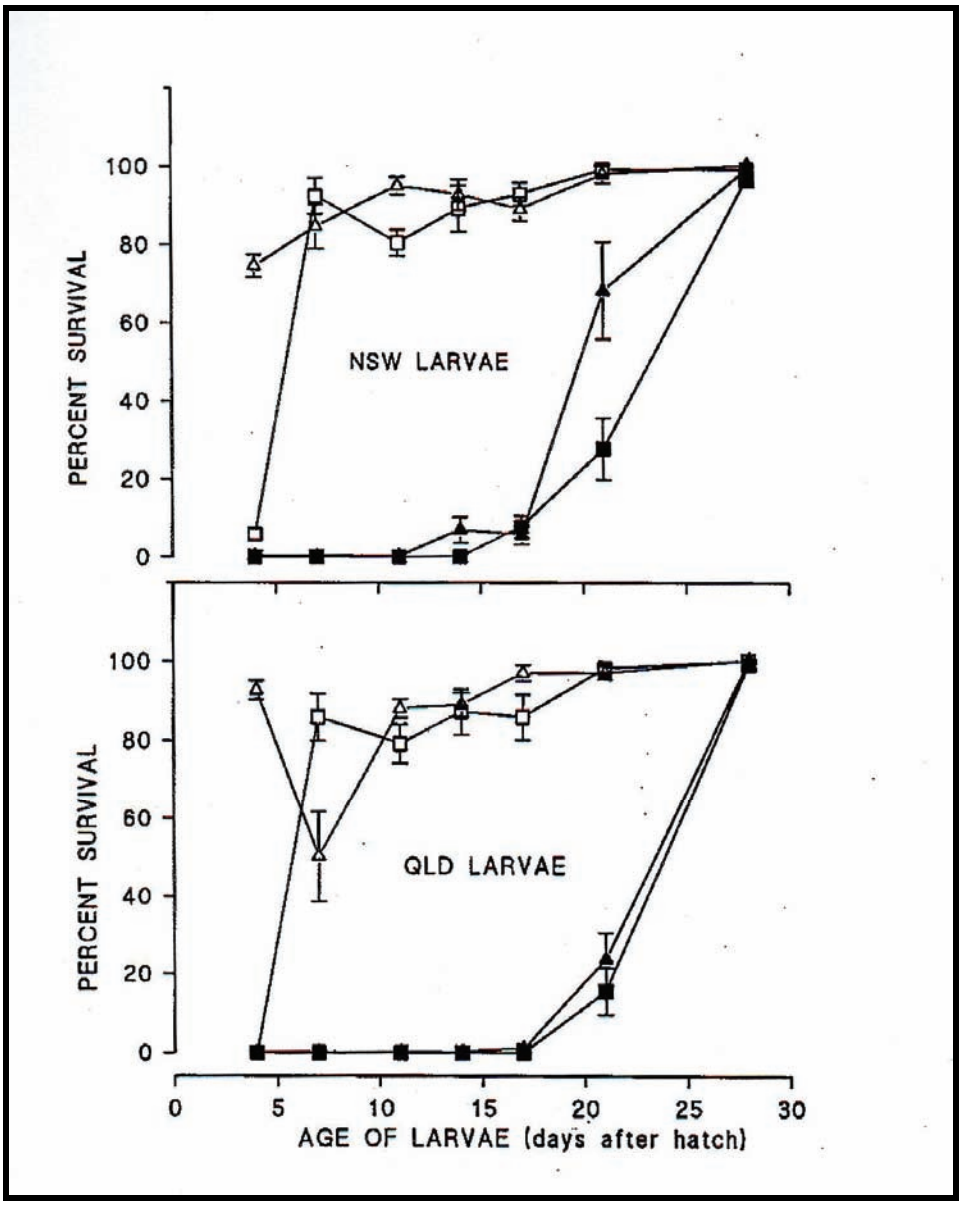


FIGURE 29: Percent survival at different ages of AB larvae from the Noosa River (Qld Larvae) and the Williams River, New South Wales (NSW larvae), in salinities of 0‰ and 2‰ following acclimation at salinities of 28‰ and 15‰ *Symbols*: ■ acclimated at 28‰ tested at 0‰ ; □ acclimated at 28‰ ,tested at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

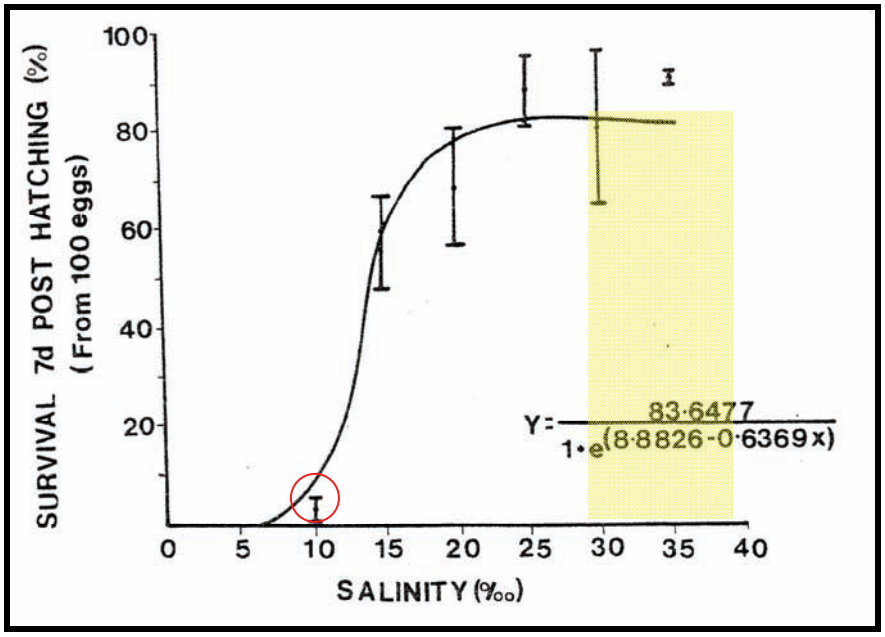


FIGURE 30: Effect of salinity on the survival rate (means ± sd) of 7dah AB larvae at 25-35‰ and 15°C. Shaded area is recommended salinity band. Encircled data point referred to in text. (Source: Van der Wal 1985).

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.

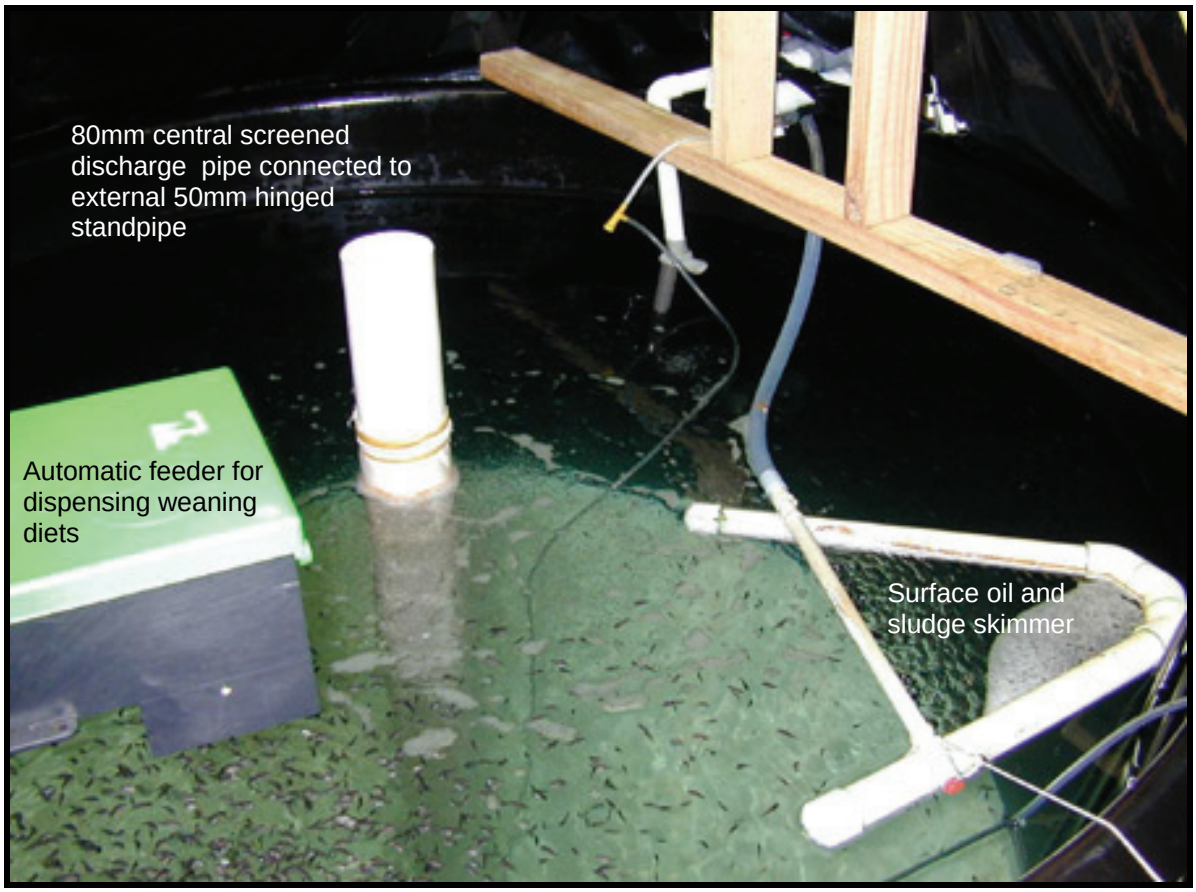


FIGURE 31: 2000 L intensive clear-water finfish hatchery rearing tank at PSFI. Note black sidewall and white conical bottom.

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.

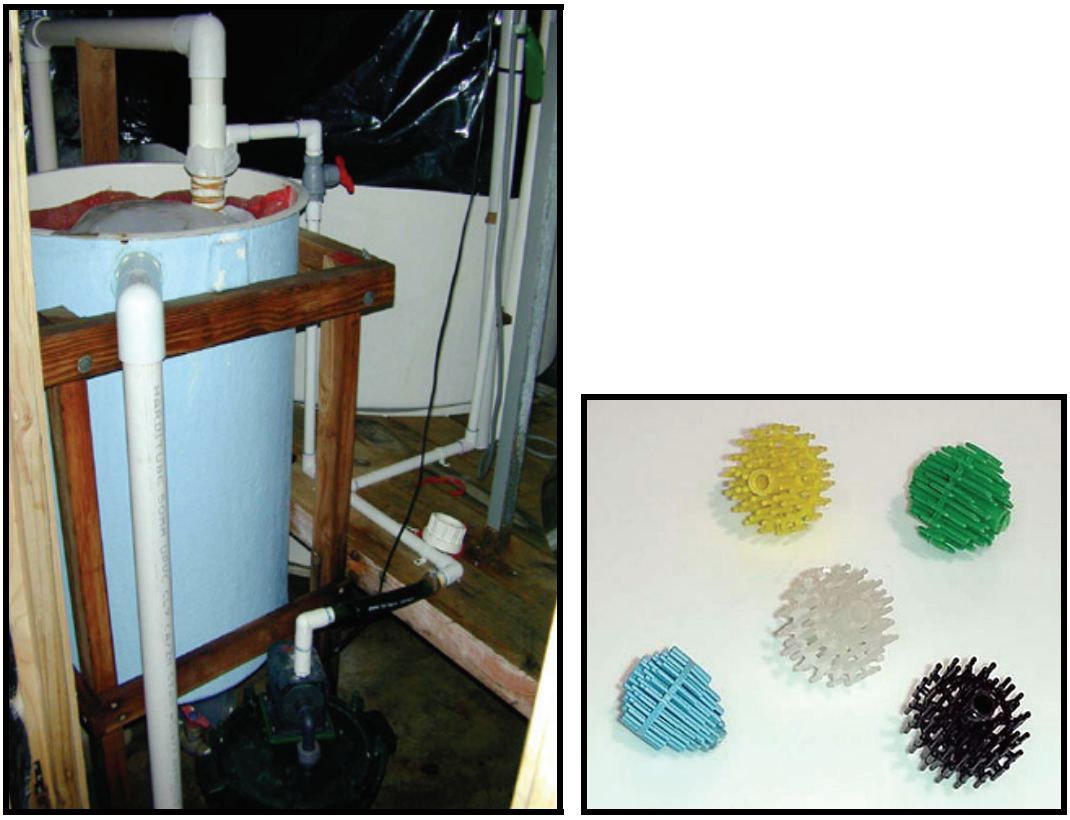


FIGURE 32: External bio-filter packed with onion bags filled with bio-balls.

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.

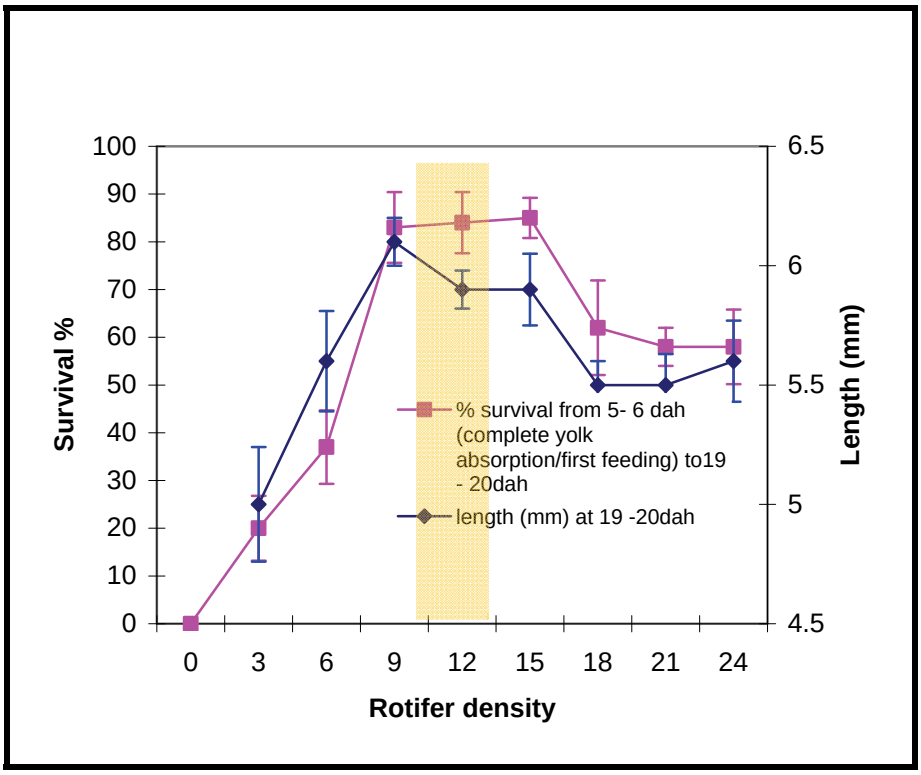


FIGURE 33: Effect of large strain rotifer (*Brachionis plicatilis*) concentration on growth and survival of AB larvae from first feeding/complete yolk absorption on 5-6 dah to day19-20 dah when reared at optimum salinity (28 -34g/kg) and temperature (20 ±1 ºC). Shaded area is recommended feeding concentration 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).

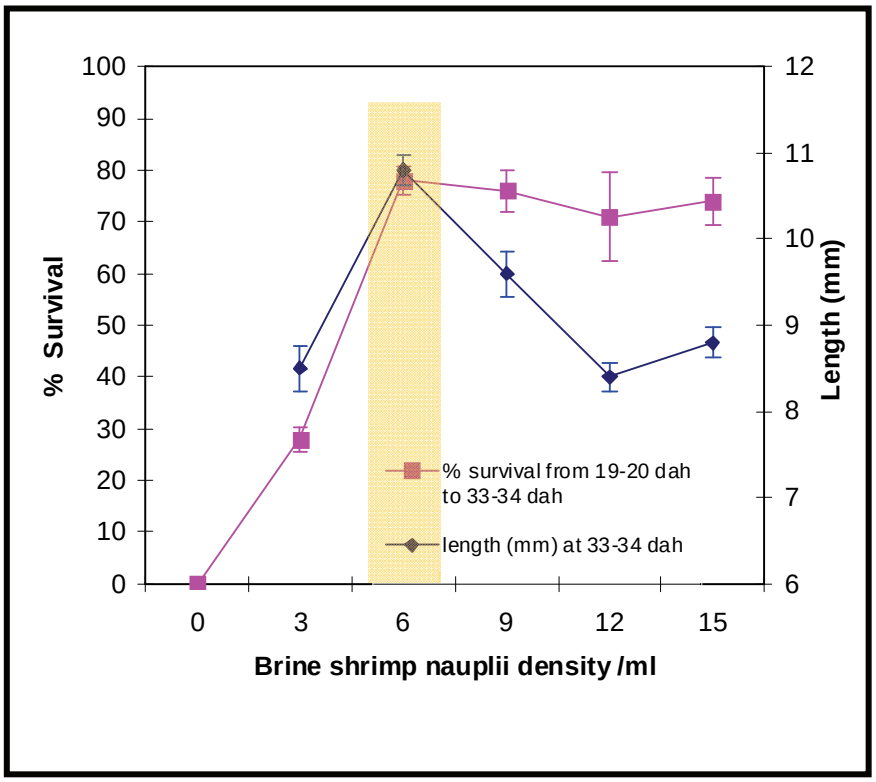


FIGURE 34: Effect of *Artemia* nauplii concentration on growth and survival of AB larvae over a 14 day period from 19 dah to day 33 dah when reared at optimum salinity (28 -34g/kg) and temperature (20 ±1 ºC). Shaded area is recommended feeding concentration of 6 *Artemia* nauplii /ml. (Source: derived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

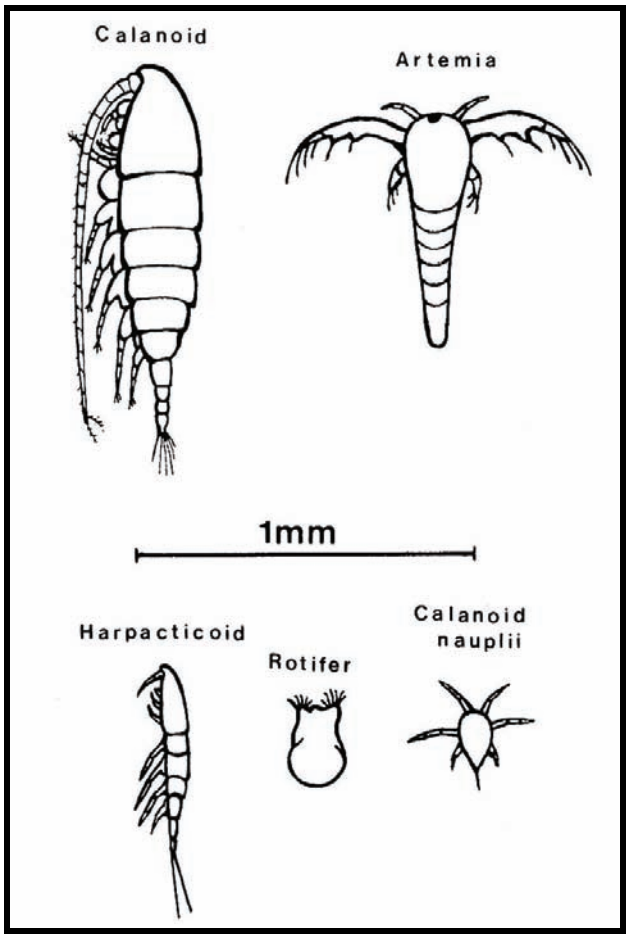


FIGURE 35: Left: Relative sizes and dominant categories of food items eaten by AB larvae in earthen prawn nursery ponds. (Source: Battaglene Talbot and Allan, 1992). Right: Photo of green- housed ponds at PSFI.

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.

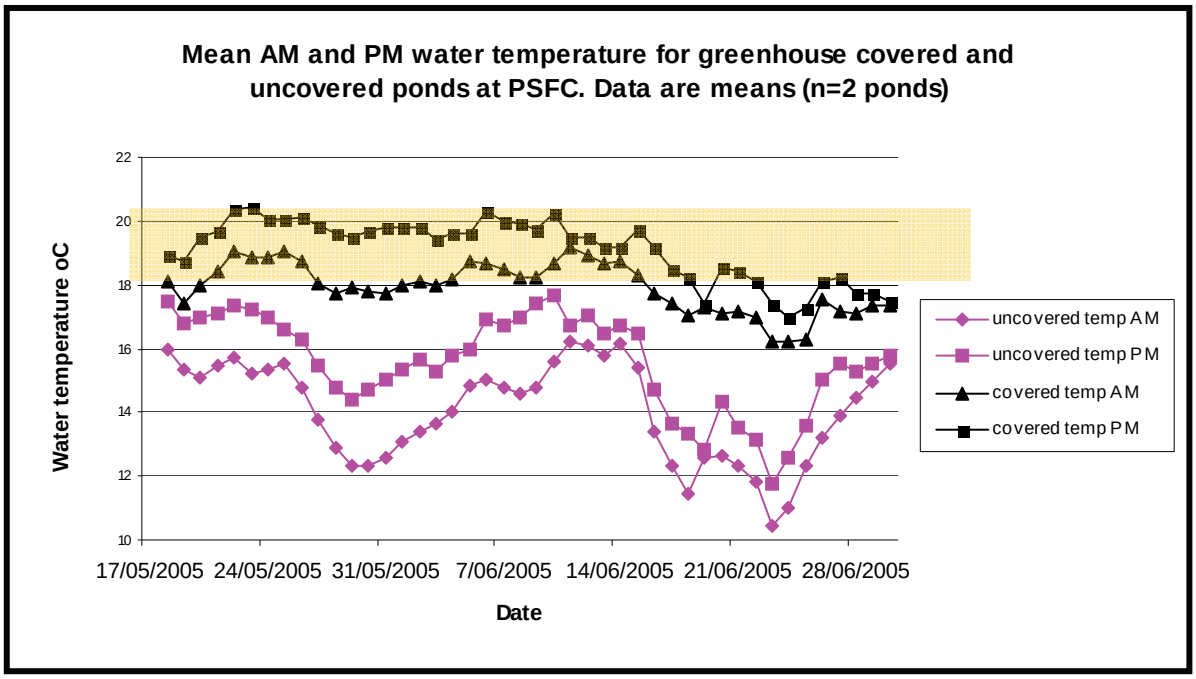


FIGURE 36: Typical effects of green-housing on 350 m^2 PSFI outdoor extensive fingerling production ponds. Shaded area is optimum rearing temperature for AB larvae. (Source: Fielder and Allan, 2008b)

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.

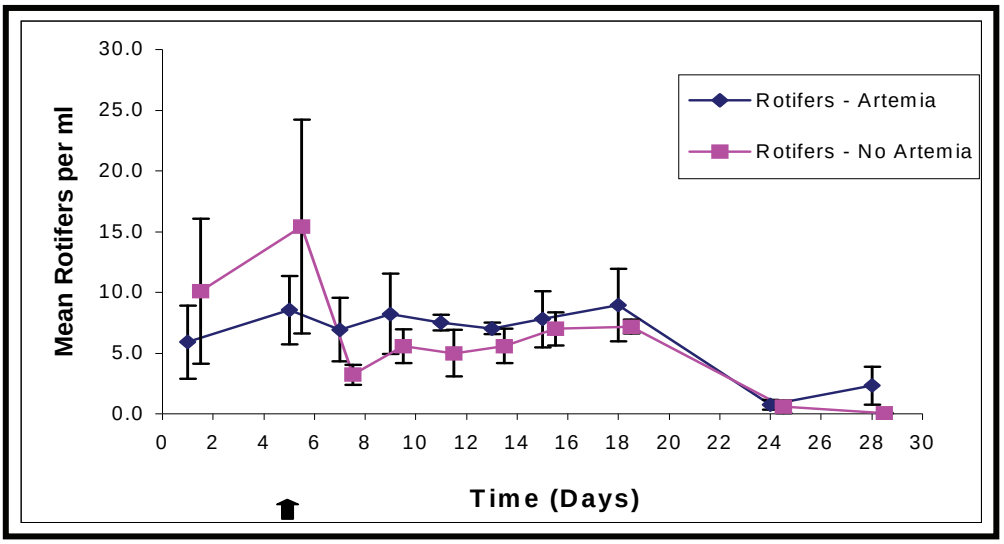


FIGURE 37: Mean number of rotifers in green housed ponds at the PSFI. Time is duration after filling. Data are means ± S.D (*n*=3 ponds). (Source: Fielder and Allan, 2008b).

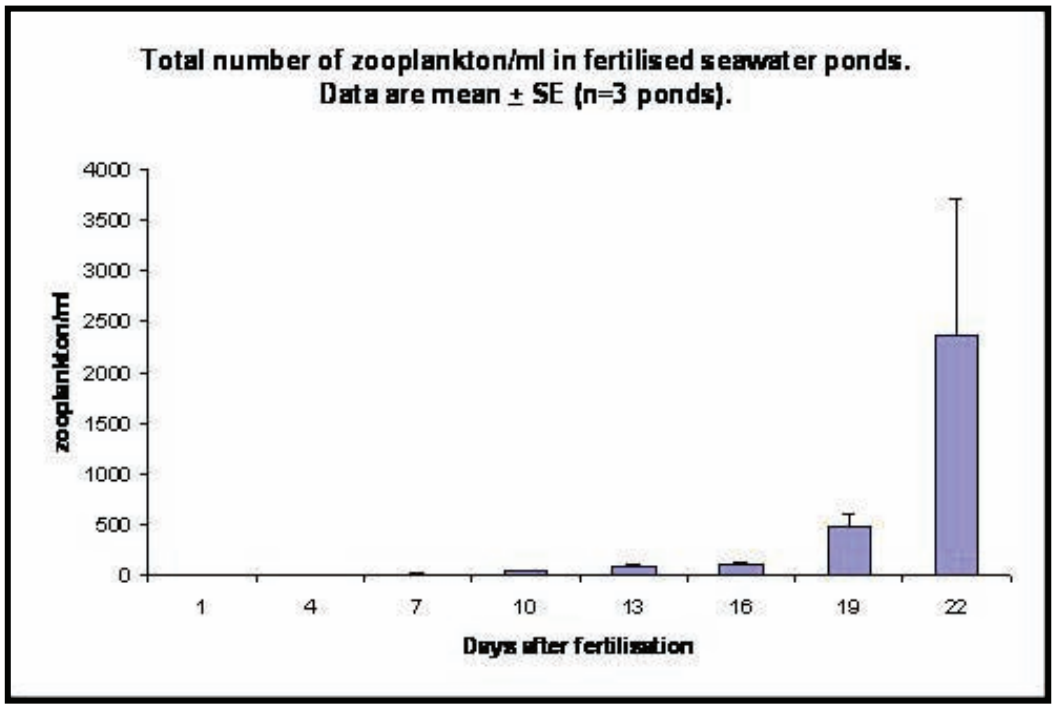


FIGURE 38: Total density of zooplankton density in plastic-lined ponds filled with seawater and fertilized. (Source: Fielder and Allan, 2008b).

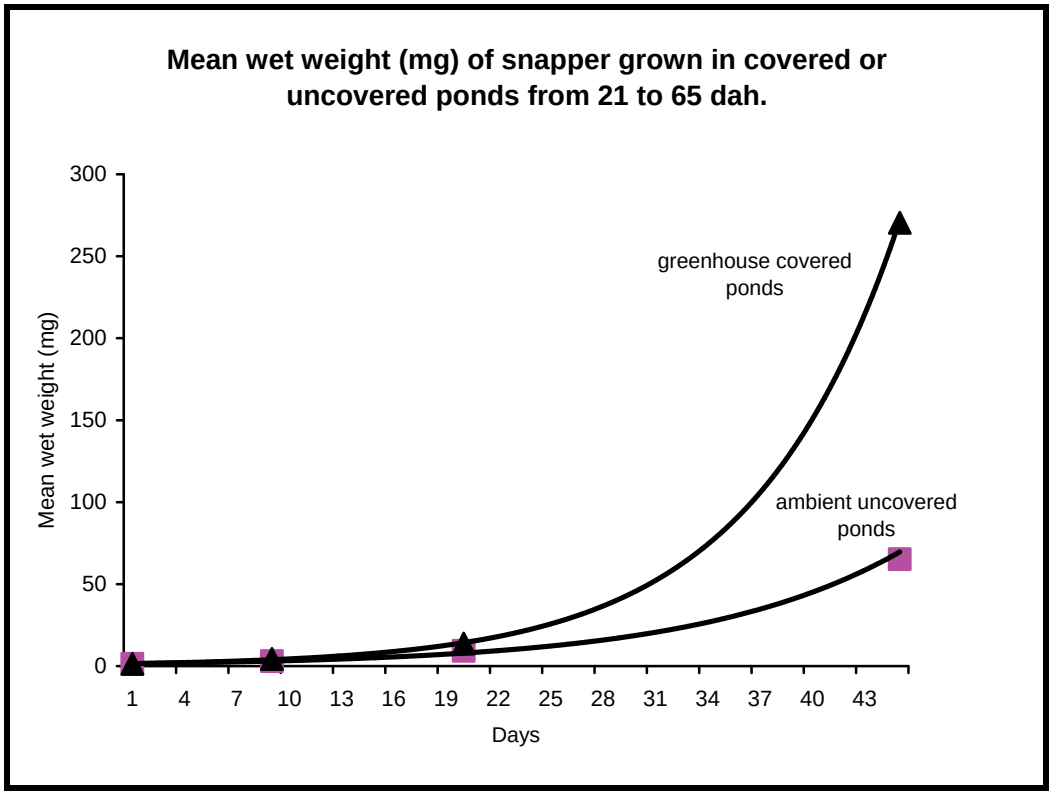


FIGURE 39: Mean wet weight (mg) of snapper grown in covered or uncovered ponds at the PSFI from 21 to 65 dah. (Source: Fielder and Allan, 2008b).

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