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HATCHERY MANAGEMENT OF TIGER GROUPER (*Epinephelus fuscoguttatus*):

a best-practice manual

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Foreword

Aquaculture of high-value finfish species, such as groupers, is an industry of increasing importance throughout the Asia–Pacific region and one that provides a livelihood for small-scale farmers throughout Asia. In the past, a major constraint to the expansion of this industry has been the limited supply of 'seed stock'—small fish that are subsequently grown out in sea cages or ponds before being sold to market.

Research undertaken by scientists in Australia, Indonesia and the Philippines has been instrumental in improving the technology to produce marine finfish seed stock in hatcheries. The Australian Centre for International Agricultural Research (ACIAR) has also contributed significantly to this outcome by funding collaborative research between institutions in the Asia–Pacific region.

These research findings have now been adopted by commercial hatcheries, particularly in Indonesia, but also Australia and many other countries. Millions of tiger grouper seeds produced by Indonesian hatcheries have been marketed not only to the domestic market but also exported to neighbouring countries including Singapore, Malaysia, Vietnam, Thailand, Taiwan, Hong Kong and China. This developing industry makes an important contribution to farmers' incomes, job opportunities and export earnings.

This manual provides guidelines for the production of tiger grouper fingerlings. It outlines best-practice methods for broodstock maintenance, spawning, egg incubation and rearing of larvae through to 2–3 cm, fully metamorphosed juveniles. The guidelines have been developed from the outcomes of ACIAR-funded research, as well as from the experience of Indonesian, Philippine and Australian scientists and commercial hatchery operators, and published information. The hatchery manual provides a valuable aid for improving the availability of grouper seed stock to support sustainable small-scale aquaculture in the Asia–Pacific region.

Nick Austin
Chief Executive Officer, ACIAR

Contents

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Abbreviations

ACIAR	Australian Centre for International Agricultural Research
DAH	days after hatching
DHA	docosahexaenoic acid (22:6n-3)
ppm	parts per million
ppt	parts per thousand
RIM	Research Institute for Mariculture (Gondol, Bali, Indonesia)
S	'small' (type rotifer—*Brachionus rotundiformis*)
SS	'super-small' (type rotifer—*B. rotundiformis*)
TL	total length
VNN	viral nervous necrosis

Introduction

Groupers belong to the subfamily Epinephelinae, family Serranidae, and are commercially important fish, particularly for live seafood markets in Asia in countries such as Hong Kong, China, Taiwan, Singapore and Malaysia (Johnston and Yeeting 2006). Species that are commonly found in the seafood markets are usually representatives of three genera: *Epinephelus*, *Cromileptes* and *Plectropomus*. Because of the high prices that groupers bring in these markets, there is considerable interest in commercial aquaculture production of a range of grouper species (Rimmer et al. 2004).

Groupers are widespread throughout the Indo-Pacific region, from southern Japan to Palau, Guam, New Caledonia, southern Queensland, Australia, and the eastern Indian Ocean from the Andaman and Nicobar Islands to Broome, Western Australia. In Indonesia, groupers are found in coastal and marine waters throughout the archipelago. They are carnivores, feeding on small fish and crustaceans, and are protogynous hermaphrodites, maturing first as females then changing into males as they grow older.

Tiger grouper (*Epinephelus fuscoguttatus*) is a large (up to 120 cm total length; TL) grouper widely distributed in the Indo-Pacific region. In the last decade it has become a popular candidate for aquaculture due to its rapid growth, hardy nature in culture and good market price. This manual provides a guide to hatchery management for the production of tiger grouper, based on research results and the experience of the authors in both experimental and small-scale commercial hatchery production.

Tiger grouper

Although the correct international marketing name for *E. fuscoguttatus* is 'brown-marbled grouper', it is commonly known throughout Asia as 'tiger grouper'. Some other common names are listed in Table 1. *Epinephelus fuscoguttatus* is brownish-yellow to light brown in colour with large, irregular-shaped, dark brown blotches on the head, back and sides (Figure 1). The head, body and fins have small dark spots and a black saddle spot on the caudal peduncle. It has 11 dorsal spines, 14–15 dorsal soft rays, 3 anal spines and 8 anal soft rays.

This species is often confused with the similar camouflage grouper, *Epinephelus polyphekadion*, because of likeness in colour pattern. *Epinephelus fuscoguttatus* has an indentation in the head profile above the eye and a more deeply incised dorsal fin membrane. *Epinephelus polyphekadion* has fewer pectoral-fin rays (16 or 17, compared with 18–20 for *E. fuscoguttatus*), usually fewer lower gill rakers (16–18, compared with 17–21 for *E. fuscoguttatus*), a smoothly convex dorsal head profile, and interspinous dorsal-fin membranes less deeply incised (Heemstra and Randall 1993). For detailed descriptions of both species, refer to FishBase (<www.fishbase.org>). *Epinephelus microdon*, which is occasionally mentioned in the aquaculture literature, is a synonym of *E. polyphekadion*. Although *E. polyphekadion* is regularly marketed along with other live reef-fish species, there is little demand for fingerlings of *E. polyphekadion* because it exhibits much slower growth than *E. fuscoguttatus* (James et al. 1998).

Tiger grouper are distributed widely in the Indo-Pacific region: from the Red Sea and eastern Africa, as far east as Samoa and the Phoenix Islands, north to Japan and south to Australia (Figure 2). In the wild, tiger grouper are found associated with coral reefs, at depths ranging from 1 to 60 m. They are reported to reach 120 cm TL. Like other groupers, the tiger grouper is a carnivore, and reported stomach contents include fishes, crabs and cephalopods (Heemstra and Randall 1993).

Table 1 List of common names for *Epinephelus fuscoguttatus* (various sources)

Common name	Country/region
Tiger grouper	English name—common usage
Brown-marbled grouper	English name—marketing
Flowery cod	Australia
Lo fu pan	Hong Kong
Kala cobra	India (Andaman Islands)
Kerapu macan	Indonesia
Kerapu kodok	Aceh, Indonesia
Kerapu hitam	Malaysia
Lapu-lapu	Philippines
Kerapu hitam, lao hu ban	Singapore
Pla karang-lai-hin-on	Thailand
Ca song hoa nau	Vietnam

Figure 1 A tiger grouper on the Great Barrier Reef, Australia, where it is locally known as 'flowery cod' (Photo: Great Barrier Reef Marine Park Authority)

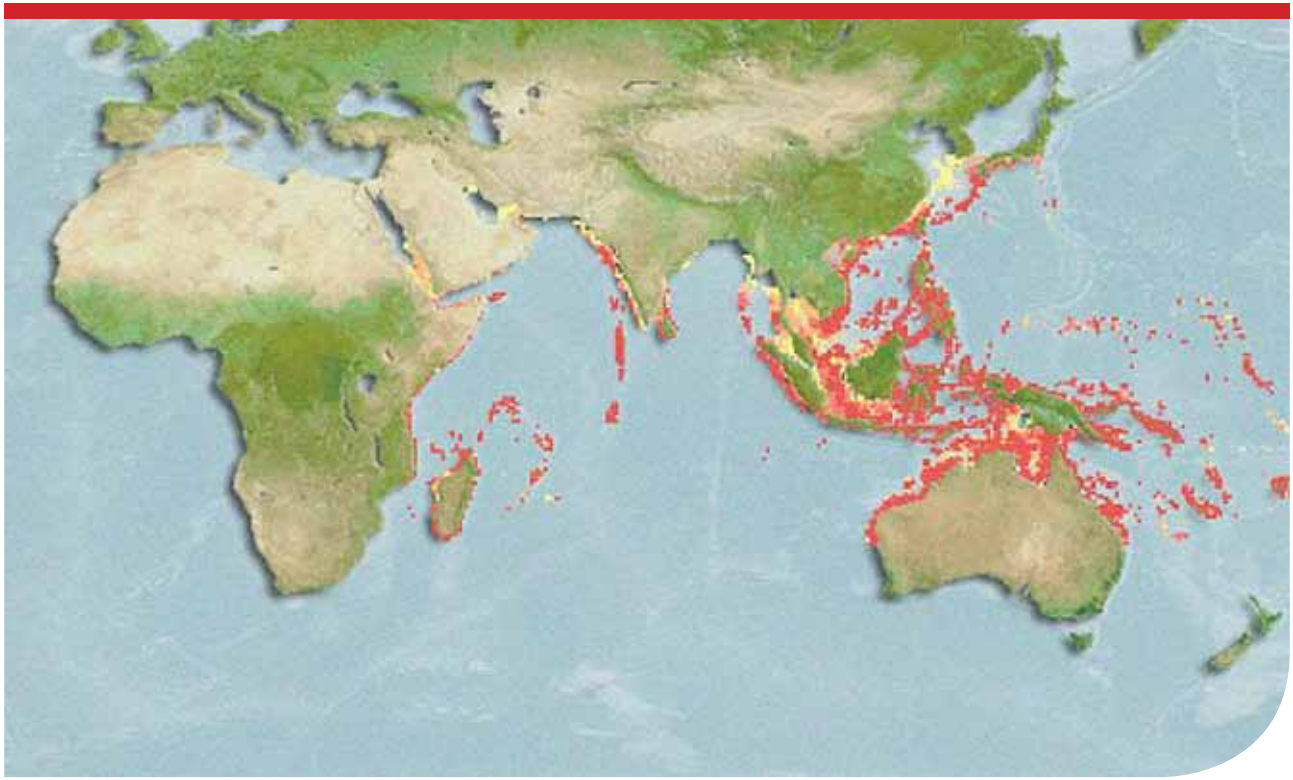


Figure 2 Map showing distribution of *Epinephelus fuscoguttatus*—reported captures are shown as red dots (Source: AquaMaps 2010)

Hatchery technology for tiger grouper

Over the past decade, considerable research effort has been directed to developing technology for artificial propagation and for larval rearing of grouper. Grouper hatchery technology has been pioneered by the Research Institute for Mariculture (RIM) at Gondol, Bali, Indonesia, and since 1998 continual improvements in grouper hatchery techniques, and the extension of the technology to industry, have provided a substantial boost to development of the Indonesian marine finfish aquaculture industry (Sugama et al. 2001, 2002).

Subsequently, RIM Gondol, supported by the Australian Centre for International Agricultural Research (ACIAR), has improved the technology for hatchery production of grouper fingerlings by undertaking research activities in collaboration with scientists from Australia, the Philippines and other Indonesian institutions. Since 2000, RIM Gondol has successfully been producing tiger grouper fingerlings (Figure 3), and the seed production techniques developed by RIM Gondol have been widely adopted by farmers in northern Bali, East Java (Situbondo) and South Sumatra (Lampung).

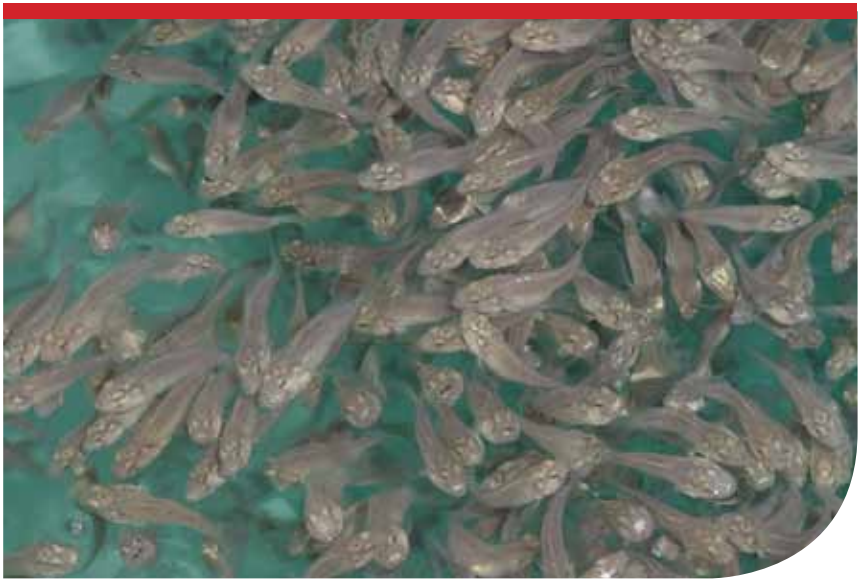


Figure 3 Hatchery-reared tiger grouper fingerlings (Photo: Sih Yang Sim)

Technology developments at RIM Gondol have provided a strong stimulus for commercial hatchery development in the surrounding area. In northern Bali's Buleleng regency there has been a steady increase in the number of hatchery tanks being used to produce grouper fingerlings (Figure 4). Although these hatcheries produce small numbers of mouse grouper (*Cromileptes altivelis*) and coral trout (*Plectropomus leopardus*), the main production is of tiger grouper, for which there is strong demand from farmers throughout Indonesia and overseas (Figure 5).

Millions of tiger grouper fingerlings have been marketed, not only to the domestic market but also exported to neighbouring countries, including Singapore, Malaysia, Vietnam, Thailand, Taiwan, Hong Kong and China. The hatchery techniques developed by RIM Gondol have now been transferred to the private sector, reportedly contributing to farmers' income and job opportunities as well as export earnings (Heerin 2002; Siar et al. 2002).

Hatchery technologies developed through ACIAR research and development projects have also been adopted by government and commercial hatcheries in Australia (Rimmer and McBride 2008) as well as in many other countries. These technologies have been incorporated in the Grouper Hatchery Production Training Course that is run annually through the Asia–Pacific Marine Finfish Network. By 2008 this course had trained over 100 people from 21 countries in grouper hatchery technology.

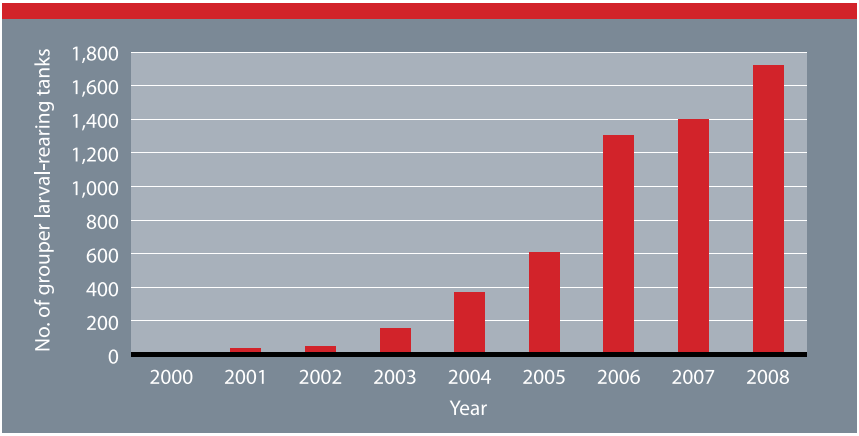


Figure 4 Total numbers of larval-rearing tanks producing grouper fingerlings in hatcheries in Buleleng regency, northern Bali

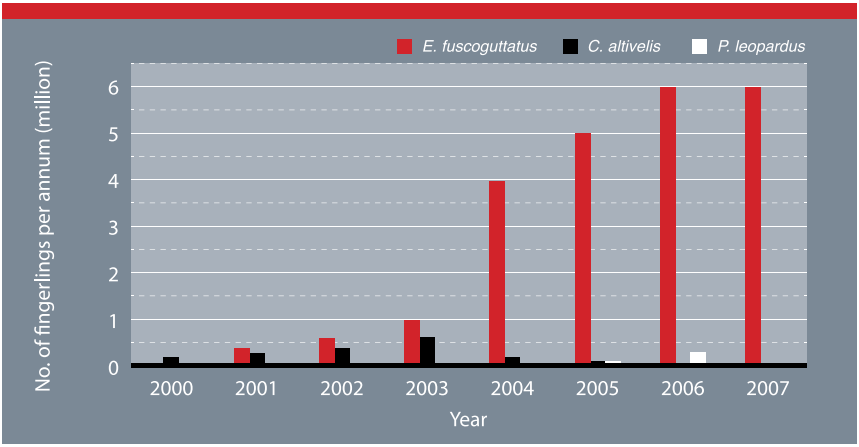
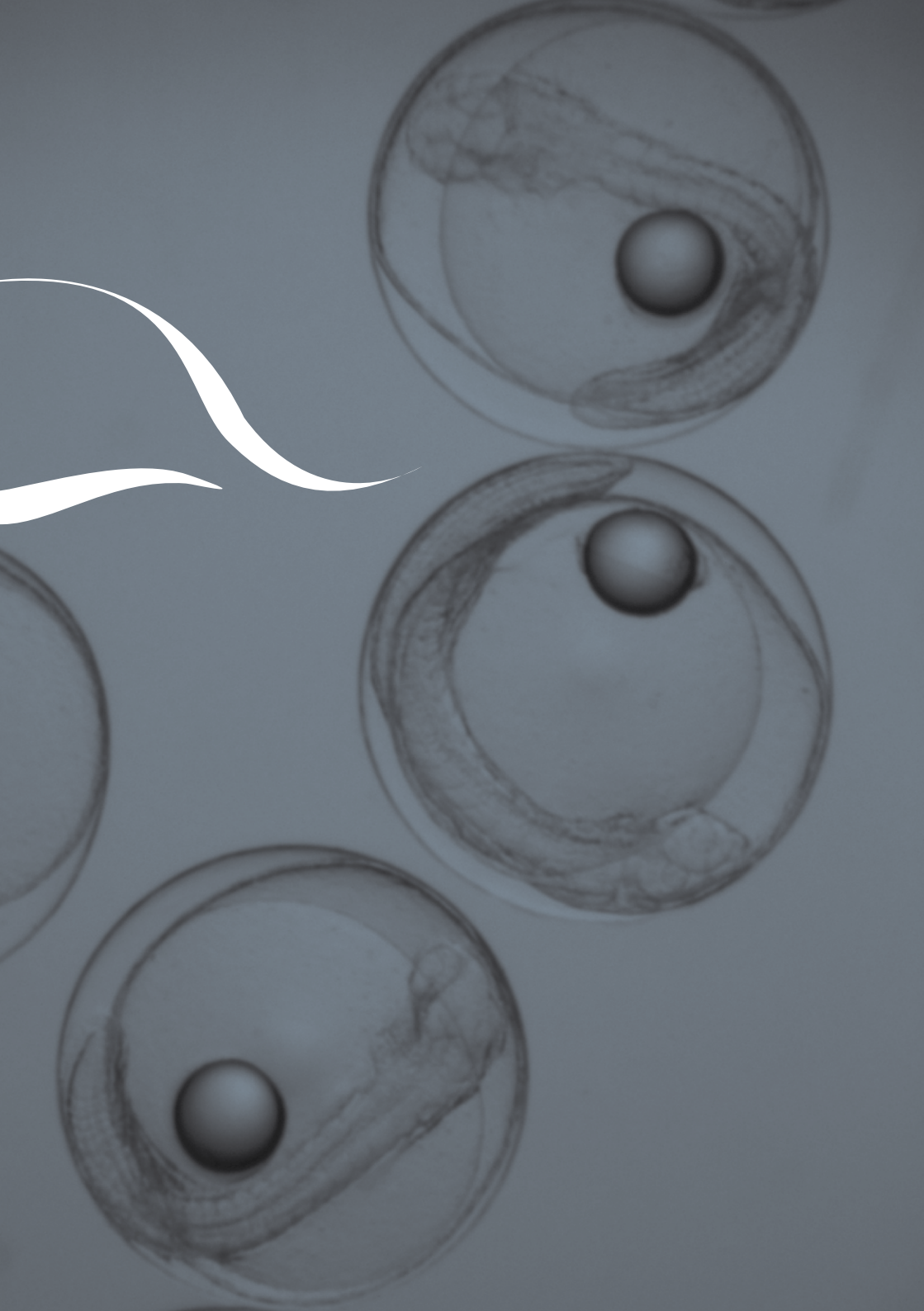


Figure 5 Total numbers of fingerlings of tiger grouper (*Epinephelus fuscoguttatus*), mouse grouper (*Cromileptes altivelis*) and coral trout (*Plectropomus leopardus*) produced annually by hatcheries in Buleleng regency, northern Bali



Hatchery design and operation

Design of small-scale hatcheries for marine finfish fingerling production, including grouper, is covered in the publication 'A guide to small-scale marine finfish hatchery technology' (Sim et al. 2005). However, a key component in the design and operation of hatcheries, regardless of scale, is the implementation of biosecurity to reduce the incidence of disease, particularly viral nervous necrosis (VNN). Biosecurity design and practice will not be covered in detail in this publication, but key features of biosecurity best practice are summarised in Box 1.

To further support biosecurity and reduce the incidence of disease, hatcheries should be sited away from other aquaculture facilities, particularly the effluent from other hatchery, nursery and grow-out operations.

BOX 1

Key features of biosecurity for hatcheries

- Separation of various functional areas (broodstock, live food production, larval rearing etc.) with footbaths and hand washes at access points (Figure 6)
- Access to hatchery limited to essential personnel only
- Disinfection and thorough rinsing of all equipment, including water-quality monitoring equipment, nets, basins etc. before use and when moving between areas
- Quarantine of new fish (broodstock, larvae or fingerlings)
- 'Batch' production of larvae, with disinfection and dry-out of hatchery between batches
- Training of staff in biosecurity and health management
- Strict isolation of batches of fish showing disease
- Routine monitoring for pathogens and disease, and prompt diagnosis of any disease events
- Optimisation of water quality and nutrition to improve the overall health and resistance of the larvae.

Figure 6 To ensure biosecurity, hatcheries should be fitted with lockable doors and each entrance should have a footbath. Signage should include instructions to disinfect hands and footwear on entry (inset) and note restriction of unauthorised visitors (not shown). (Photo: M. Rimmer)

Broodstock and spawning

Broodstock

Acquisition

Systematic selection of broodstock and keeping of records of fish being brought into the hatchery and used for production are important. Initially, tiger grouper broodstock (Figure 7) can be acquired through collecting or purchasing wild fish. Since mature male and female broodstock are externally indistinguishable, it is necessary to obtain fish in a wide range of sizes.

Figure 7 Tiger grouper broodstock maintained in fibreglass broodstock tanks at the Northern Fisheries Centre, Cairns, Queensland, Australia. The material in the background is artificial habitat to partly mimic the tiger grouper preference for coral reef habitat. (Photo: Queensland Department of Primary Industries and Fisheries)

Tiger grouper, like other members of the Epinephelinae, are protogynous hermaphrodites; that is, they mature initially as females, then change sex to male at a later age (Pears et al. 2007). At RIM Gondol the smallest recorded size of mature tiger grouper captured from the wild is 3.7 kg (female) and 8.2 kg (male). In the Philippines, the smallest recorded size of mature tiger grouper grown in captivity and fed on dry pellets is 2.2 kg (female) and 3.5 kg (male).

Another method of acquiring broodstock is to grow fish produced in the hatchery. Cage, pond or tank-reared fish are already accustomed to culture conditions and consequently easier to develop into suitable broodstock. However, it can take 4 years to grow juvenile tiger grouper up to broodstock size. Moretti et al. (1999) list the characteristics to look for when selecting broodstock of European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*), and this approach applies to other marine finfish species, including grouper:

- normal body shape and colour
- absence of skeletal deformities
- overall healthy status, i.e. absence of large wounds, haemorrhages, infections and parasites
- normal behaviour, such as a good response to food distribution, controlled buoyancy to maintain position in the water column
- the best growth and food conversion rate within its age group.

Transport

Broodstock fish, including grouper, should be transported in dark-coloured, covered tanks containing aerated or oxygenated water, to reduce stress. Dissolved oxygen levels should be maintained at >75% saturation at all times. Mild sedation, using approved sedatives for fish, can be used to reduce stress and make handling the fish easier and safer. Fish to be transported should not be fed for at least 24 hours beforehand to prevent faeces and regurgitated feed from fouling the transport water.

Treatment before stocking

Before the fish are stocked into broodstock tanks, it is advisable to quarantine them to reduce the opportunity for new fish to transmit parasites or diseases to the established fish. This process usually takes

between 1 and 4 weeks, and can be carried out in small (0.5–2 m^3) tanks to facilitate water exchange and fish handling. During the quarantine period, broodstock management focuses on reducing the parasite load of the fish by regularly placing them in a freshwater bath for 5 minutes to help eliminate common parasites such as skin flukes (*Benedenia* spp. and *Neobenedenia* spp.), protozoans (e.g. *Cryptocaryon irritans*) and parasitic copepods (e.g. *Caligus* spp.) (Koesharyani et al. 2005). Note that a single freshwater bath will not completely eliminate protozoan parasites such as *C. irritans*. While the visible theront stage can be eliminated using freshwater baths, the trophont stage is encysted in the epithelium and is not affected by freshwater exposure, hence the need for quarantine and repeated freshwater treatments before the newly acquired fish are stocked in the broodstock tanks.

If water quality (particularly temperature and salinity) in the broodstock tanks is markedly different from the previous holding environment, the fish should be acclimatised for up to 1 hour before being released into the tank. To acclimatise the fish, place them in a tank filled with the original water, and slowly add the new tank water until conditions in the transfer tank and the new tank are similar.

Broodstock tanks

Broodstock tanks are used not only for culture and maintenance, but also for spawning. Because of the size of tiger grouper broodstock (usually >10 kg), larger tanks in the range 50–100 m^3 are preferred (Figures 8 and 9). Tanks should be round, or square or rectangular with rounded corners. Medium-range blue, green or grey is preferred as the tank colour; not very light or very dark shades. There is general agreement that tanks should be at least 2.0 m deep and preferably >2.5 m to allow sufficient room for spawning behaviour, which involves pairs or groups of fish swimming upward from the tank bottom while releasing eggs and sperm (Okumura et al. 2003; Sudaryanto et al. 2004). Each tank has an overflow pipe with an egg collection tank with nets installed for egg collection (upper left in Figure 8; see also Figure 12). It is advisable that broodstock tanks are roofed in order to reduce the growth of algae on the tank walls, which makes egg collection difficult and increases the risk of parasite infestation. Moreover, dirty tanks need to be cleaned frequently which may stress the broodstock and cause spawning failure or lower the quality of spawned eggs.

Broodstock tanks are continuously supplied with fresh sea water at a daily exchange rate of 200–300%. Sea water used for broodstock tanks should be filtered and clear, with stable salinity (33.0–35.0 parts per thousand (ppt)) and water temperature (27.0–30.5 °C).

Tanks located outdoors (Figure 8) are subject to the natural photoperiod, while indoor tanks may be provided with artificial lighting (Figure 9) to simulate different photoperiod regimes. In general, photoperiod and temperature manipulation seems to have little impact on grouper spawning periodicity or success.

Figure 8 Concrete tanks used for holding tiger grouper broodstock at the Brackishwater Aquaculture Development Centre, Ujung Batee, Aceh, Indonesia. Each of these tanks is about 50 m^3 in volume. (Photo: M. Rimmer)

Figure 9 Fibreglass tanks used for holding tiger grouper broodstock at the Northern Fisheries Centre, Cairns, Queensland, Australia. The tanks in the foreground are about 20 m^3 in volume and each is fitted with a recirculation system comprising a biological filter (white elevated tanks) and ozone system to maintain water quality, and a heat exchanger to maintain water temperature. Some tanks are covered (background) and fitted with computer-controlled lighting systems to control day length as well as water temperature. (Photo: Queensland Department of Primary Industries and Fisheries)

Broodstock management

Feeding

At RIM Gondol broodstock are fed to satiation six times each week, four times with fish (Figure 10) and twice with squid. This feeding schedule may vary between hatcheries, depending on the availability of fish and squid. At RIM Gondol the fish used are mainly members of the families Clupeidae (herrings) and Scombridae (mackerels). The feed is supplemented with a vitamin mix included at 1% of feed. Commercial or custom-formulated vitamin mixes can be used; the components of a formulation (originally developed for barramundi (*Lates calcarifer*) broodstock) are listed in Table 2.

Figure 10 Wet fish (often called 'trash' fish) used for feeding grouper broodstock (Photo: M. Rimmer)

Table 2 Vitamin premix formulation originally developed for use in soluble form for barramundi broodstock, but which can also be used for grouper broodstock. Allowance is based on a mixing rate of 100 g premix in 1 L of water and injected at a rate of 1 mL/50 g feed fish; with broodstock fed fresh fish at a rate of 2% body weight/day. Formulation developed by Queensland Department of Primary Industries and Fisheries.

Ingredient	Amount/kg premix	Allowance/kg broodfish/day
A	2×10^6 IU	80 IU
D3	0.8×10^6 IU	32 IU
E (DL-α-tocopherol)	40 g	1.6 mg
K3	2 g	0.08 mg
Ascorbic acid	40 g	1.6 mg
Thiamine	4 g	0.16 mg
Riboflavin	4 g	0.16 mg
Pyridoxine	4 g	0.16 mg
Panthothenic acid	10 g	0.4 mg
Biotin	100 mg	4.0 µg
Nicotinic acid	30 g	1.2 mg
Folic acid	1 g	0.04 mg
B12	4 mg	0.16 µg
Choline chloride	200 g	8.0 mg
Inositol	50 g	2.0 mg
PABA	20 g	0.8 mg
Ethoxyquin	30 g	1.2 mg
Dextrose	(to 1.0 kg)	–

IU = international units

PABA = para-aminobenzoic acid

Another method to incorporate vitamins and minerals in the broodstock feed is to use a locally made 'sausage' mixture of fish and squid using the enzyme transglutaminase as a binder. The mixture mainly comprises fish, squid, shrimp or other fishery ingredients, some rice (or other) powder, vitamin mix and transglutaminase (Table 3). The method for preparing the feed is:

1. Weigh the required amount of dry ingredients (Figure 11a).
2. Weigh the required amount of fish etc. and mince using a meat mincer (Figure 11b).
3. Add dry ingredients to minced fish and mix well, either by hand or using a Hobart mixer (Figure 11c).
4. Using a sausage machine or similar equipment, extrude the mixture into a mould of the required shape, such as a length of polyvinylchloride (PVC) pipe cut in half (Figure 11d).
5. Place the mould with the feed in the freezer to harden (Figure 11e). The feed should be used within 1 week.
6. Lengths of the frozen feed can be cut and fed directly to the broodstock (Figure 11f).

Table 3 Composition of transglutaminase-based 'sausage' feed for marine finfish broodstock

Ingredient	Amount
Minced fish, squid, shrimp etc.	793 g
Rice flour or other finely ground starch product	195 g
Transglutaminase B	10 g
Vitamin mix	1–2 g (depending on recommended inclusion rate)
Total	**1 kg**

Figure 11 Making transglutaminase-based wet feed for marine finfish broodstock (see text for method): (a) measuring dry ingredients; (b) mincing fish; (c) mixing; (d) extruding; (e) freezing; and (f) feeding (Photos: M. Rimmer)

Tank cleaning

Faeces and excess feed that accumulate on the tank bottom are siphoned out regularly to prevent water-quality degradation. It is advisable to clean broodstock tanks after spawning is complete to remove excess or dead eggs which decay and pollute the water. To reduce the incidence of parasite infestations, broodstock should be bathed in fresh water for 5–7 minutes during tank cleaning.

Gender identification

Grouper broodstock are held at low densities in tanks, usually <1 kg/m^3. Sex ratios are usually around 1 males to 5 females, but can vary depending on the availability of fish and on operator preferences. As noted earlier, tiger grouper are protogynous, so females will change sex to male. However, in broodstock tanks this change may be socially mediated, and the presence of male fish may repress sex change by females. The sex of individual fish can be confirmed only by physical examination. To confirm sex, the abdomen of an anaesthetised fish is gently massaged in a head-to-tail direction. A sexually ripe male spawner will extrude copious milt from its urinogenital pore. If no milt is expressed, the fish is immature, a male not in spawning condition, or a female. Cannulation of the genital pore of females is necessary to obtain egg samples to assess the development stage of the ovaries. However, cannulation of female tiger grouper is often difficult if the fish are not in spawning condition because the genital orifice is either completely closed or difficult to access.

The cannula is a 40–50 cm length of clear flexible plastic tubing (3 mm outside diameter, 1.2 mm inside diameter), which is inserted into the urinogenital orifice of males and the oviduct of females. Fish to be cannulated are anaesthetised and a wet cloth or towel is placed over the eyes to assist in calming the fish. The cannula is guided into the fish for a distance of 6–7 cm and suction is applied to the other end of the cannula as it is withdrawn. After withdrawal, the sample within the cannula is expelled onto a microscope slide for immediate examination or into a vial containing 1% neutral buffered formalin for later measurement of egg diameter. Generally, females in spawning condition will have oocytes over 400–500 µm diameter.

Spawning

Tiger grouper broodstock are allowed to spawn naturally in the tanks. Spawning generally occurs at night (9 pm – 3 am) for three to six nights each month during the new moon phase. At RIM Gondol grouper broodstock generally spawn throughout the year (Sugama et al. 2002). During the spawning period, tiger grouper may spawn between 0.8 and 6.0 million eggs each night. In Bali in July and August, cooler southerly winds cause water temperatures to drop to around 25 °C. During this period, tiger grouper broodstock usually cease spawning. If they do spawn during this period, only a small number of poor-quality eggs are produced and these cannot be used for hatchery production.

Egg-handling procedures

Collection

When spawning occurs, eggs are collected from the overflow egg collector tank using a fine (400 µm mesh) net (Figure 12). The fertilised eggs of grouper are non-adhesive and pelagic, and range from 0.8 to 0.9 mm in diameter.

Grouper eggs are sensitive to handling during the early developmental stages, and should only be removed from the collection nets once the embryo has developed optic vesicles, i.e. at the eyed stage (see Figure 15) (Caberoy and Quinitio 1998). Handling eggs before this stage will result in increased mortality and a higher incidence of deformities (Caberoy and Quinitio 1998).

Figure 12 Egg collector fitted to an overflow tank adjacent to the broodstock tank. Water in the broodstock tank flows through the egg collector and the eggs are collected in the fine mesh net. (Photo: M. Rimmer)

Disinfection

To minimise the chances of vertical transmission of VNN, fertilised eggs of many marine finfish species are treated with ozone (Battaglene and Morehead 2006; Buchan et al. 2006) (see Box 2), and this treatment is also recommended for grouper eggs (Liao et al. 2001). Grouper eggs should be subjected to a concentration × exposure time (CT) score of around 1.0 (Su et al. 2001)—this means they should be treated with ozone at a concentration of 1 mg/L for 1 minute or equivalent (e.g. 0.8 mg/L for 1.25 minutes).

Incubation

After washing in ozone-treated water, the eggs are rinsed with clean, disinfected (using ozone) sea water (Figure 13) and, for incubation, transferred into 0.5–1.0 m^3 tanks with aerated sea water. Only floating eggs are used for larval rearing because these are more likely to be fertilised than sinking eggs, which are unfertilised or dead. Unfertilised eggs settle to the bottom of the broodstock tank and are removed by siphoning. If any unfertilised eggs are transferred to the incubation tanks, they should be siphoned out and discarded to prevent water-quality degradation. Recommended values for conditions in the incubation tank are listed in Table 4.

BOX 2

Safety note: use of ozone

Ozone (O_3) is used to oxidise organic matter and kill bacteria and other pathogens in water. Ozone is highly toxic to fish and extremely hazardous to human health. The treatment tanks should be outside any enclosed rooms (although under cover) in a well-ventilated area. Staff should be trained in the use of ozone, and should wear gloves and respirator masks when using the ozone system. Ozone degrades quickly; its half-life is about 15 minutes.

Table 4 Recommended values for physico-chemical parameters for incubation of tiger grouper eggs. Note that there is very little information available on the tolerances of grouper larvae to various environmental parameters.

	Recommended	Reference
Temperature	28–30 °C	–
Salinity	32–34 ppt	–
Stocking density*	400 eggs/L	Toledo et al. (2004)
Aeration*	100 mL/min	Toledo et al. (2004)

* Refers to other *Epinephelus* species, but in the absence of more specific information, provides a guide to the requirements for tiger grouper

Figure 13 Ozone generator (on top of chest freezer) and seawater tanks set up to wash grouper eggs. The eggs are washed in one tank (foreground), then rinsed in a second tank (out of view). (Photo: M. Rimmer)

Qualitative evaluation of the eggs

Egg quality in marine finfish is generally evaluated using both qualitative and quantitative methods.

Fertilised eggs (Figure 14) are examined under a microscope (10× or 20× magnification is sufficient) for the following:

- eggs should be regular in shape
- during the early stages of embryonic development, the individual cells should be regular in size
- eggs and embryos should be completely transparent, with no dark areas
- chorions (eggshells) should be free of any parasites or fouling organisms.

If there is only a low proportion of eggs that are irregularly shaped, dark or with aberrant embryonic development, the eggs can be used in the hatchery because it is likely that the poor-quality larvae will simply die during the larval-rearing procedure. If there is a high proportion of eggs exhibiting abnormal characteristics (>10%), the batch should be discarded. If the eggs have parasites or fouling organisms, the batch should be discarded because of the probability that they will transfer pathogens to the hatchery. Following disposal, all tanks and equipment used should be cleaned and disinfected (see Appendix 1 for a list of disinfection procedures).

Figure 14 Fertilised eggs of grouper have a well-developed larva curled around the yolk (e.g. centre and upper right). Unfertilised eggs have no visible larva (e.g. centre left and upper left). (Photo: R. Knuckey)

Quantitative estimation of fertilisation and hatching rates

Fertilisation and hatching rates are also used as indicators of egg quality. For grouper, both fertilisation and hatching rates should be higher than 50%, and preferably higher than 80%. Fish larvae from batches of eggs with poor fertilisation and hatching rates (<30%) are regarded as being of poor quality, and generally exhibit low survival and a high incidence of deformities and other health problems. Such batches are usually discarded. Records of fertilisation and hatching rates should be kept to allow an evaluation of broodstock performance and spawning success, particularly on an annual basis.

To estimate fertilisation and hatching rates, 10 samples, each containing about 100 eggs and/or larvae, are usually enough to provide an accurate estimate. However, samples need to be taken with a high degree of consistency to ensure reliable results.

Fertilisation rate

Estimation of the fertilisation rate (Box 3) should be undertaken several hours after fertilisation has taken place, and well before hatching, so that embryonic development makes fertilised eggs easier to discriminate. Tiger grouper eggs hatch between 18 and 22 hours after fertilisation at 27–29 °C.

BOX 3

Calculating fertilisation rate

To calculate the fertilisation rate, inspect representative samples of eggs under a microscope. Count the number of fertilised eggs (N_F) and the number of unfertilised eggs (N_{Uf}).

$N_{Uf} + N_F$ = total number of eggs in the sample (N_T)

The fertilisation rate (%) is $N_F/N_T \times 100$

Example:

Examination of 10 samples of eggs indicates that 1,215 are fertilised and 103 are unfertilised.

N_F = 1,215 and N_T = 1,318

Fertilisation rate = 92%

To successfully measure the fertilisation rate, it is necessary to be able to distinguish fertilised from unfertilised eggs. This requires a good microscope, and some experience, to discriminate fertilised eggs during the early developmental stages. Figure 14 provides some examples of fertilised and unfertilised tiger grouper eggs.

Hatching rate

Because dead eggs, live eggs and hatched larvae distribute differently in the tank, before sampling eggs and larvae to calculate hatching rate (Box 4), it is necessary to mix the eggs and larvae in the tank by swirling the tank by hand (but see Box 5). Swirling should be adequate to fully mix eggs and larvae, but should not be violent enough to damage newly hatched larvae. If using equipment such as beakers or small containers, ensure that they have been disinfected before use (see Appendix 1). Because grouper eggs are stocked directly to the larval-rearing tanks before hatching, hatching rates are usually done on a separate sample of larvae, not those intended for larval rearing.

Estimation of the hatching rate should be undertaken when hatching is completed. The time to hatching and the duration of hatching depends on temperature: larvae will take longer to hatch, and the duration of hatching will be extended, at lower temperatures.

BOX 4

Calculating hatching rate

To calculate the hatching rate, count the number of hatched larvae (N_H) and the number of unhatched eggs with developed larvae (N_{Uh}).

$N_{Uh} + N_H$ = total number of eggs in the sample (N_T)

The hatching rate (%) is $N_H/N_T \times 100$

Example:

Examination after hatching has finished of 10 samples of eggs and larvae indicates that 988 larvae have hatched, 122 eggs contain advanced embryos that have not hatched and 15 eggs are unfertilised or undeveloped. (Note that the 15 unfertilised eggs have been accounted for in the estimation of fertilisation rate, and thus are not included in the estimation of hatching rate.)

N_H = 988 and N_T = 1,110

Hatching rate = 89%

BOX 5

Cautionary note: chemical contamination of tanks

A wide range of chemicals, and even soap, will kill fish eggs and larvae. Ensure that there are no chemicals, including sunscreen or insect repellent, on your skin before stirring the tank.

Ensure that any items of equipment used to sample eggs or larvae from the tanks are disinfected before use, and disinfected between use in different tanks, to reduce the chances of disease transfer.

Stocking larval tanks

Generally, grouper eggs are stocked at the eyed stage before hatching (Figure 15) because at this stage they are more robust than the newly hatched larvae. Newly hatched larvae (Figure 16) are very sensitive to physical shock or changes in water quality, and moving them to the larval-rearing tanks may result in high levels of mortality. Because the hatching rate is not known before the eggs are stocked in the larval-rearing tanks, the number of eggs to be stocked needs to be estimated using historical hatching rates for that hatchery. Accurate estimates of the number of larvae stocked can be back-calculated using data from the actual batch stocked, as described above. If hatching rates are low, the larvae in the larval-rearing tanks should be discarded, and the tanks cleaned and disinfected (Appendix 1).

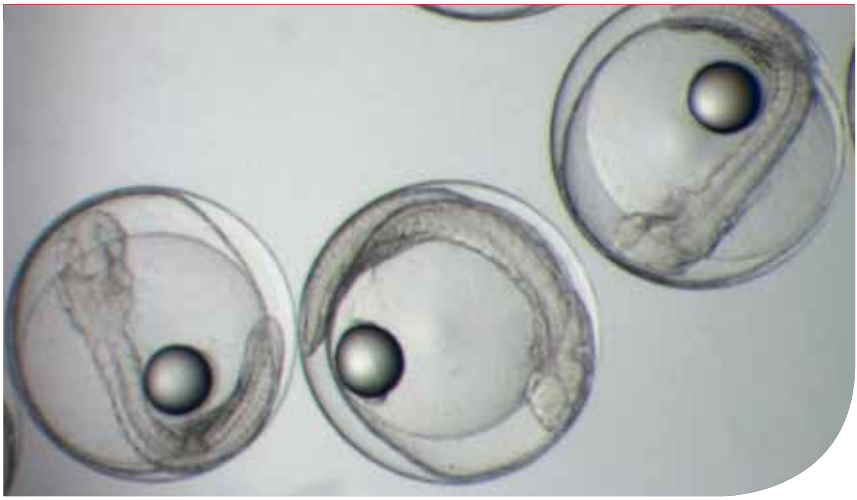


Figure 15 Late-stage tiger grouper eggs immediately before hatching. At this stage the larvae will be visibly 'twitching' within the chorion. (Photo: R. Knuckey)

Figure 16 Newly hatched tiger grouper larva (Photo: R. Knuckey)

Larval-rearing procedures

Larval-rearing tanks

At RIM Gondol, both round and rectangular tanks are used for larval rearing. For rectangular tanks, the corners of the tank should be rounded to avoid larval aggregation in the tank corners. The preferred size of larval-rearing tanks is about 10 m^3 in volume with a depth of 1.2 m. Based on experience in rearing grouper larvae of several species at RIM Gondol, the preferred colour for larval-rearing tanks is bright yellow or pale blue (Figure 17). These colours allow the grouper larvae to discriminate prey (such as rotifers and brine shrimp) more easily, and make tank management, particularly cleaning, easier.

Aeration should be provided in a 'grid' pattern to ensure even mixing of the water in the tanks and to ensure dissolved oxygen levels are maintained throughout the tank. Airstones should be placed in each corner of the tank to prevent stagnation. Aeration should be light during the early stages of larval rearing, to avoid physically damaging the larvae. It can be increased during the larval-rearing cycle, as the larvae become more robust.

Water to the larval-rearing tanks should, as a minimum requirement, be filtered through a sand filter (Figure 18). More complex filtration and water treatment systems, such as ultraviolet or ozone disinfection and cartridge filters, will help maintain biosecurity in the hatchery. Larval-rearing tanks should at least be roofed to avoid direct sunlight and rain. Better still is to enclose the larval-rearing tanks in a building—this will help maintain optimal water temperature, reduce diurnal fluctuations in water temperature and facilitate biosecurity.

The larval-rearing tanks should be maintained as a separate quarantined area within the hatchery, with entry only to authorised persons, hand and foot washes on entry and exit, and disinfection of all equipment before use. Following each production cycle, all tanks and equipment (such as nets, feeding buckets, airstones and airlines etc.) should be cleaned and disinfected. Details of disinfection procedures are provided in Appendix 1.

Figure 17 Larval-rearing tanks in a small-scale commercial hatchery in Bali. Preferred tank colour for larval rearing of groupers is yellow or pale blue. (Photo: M. Rimmer)

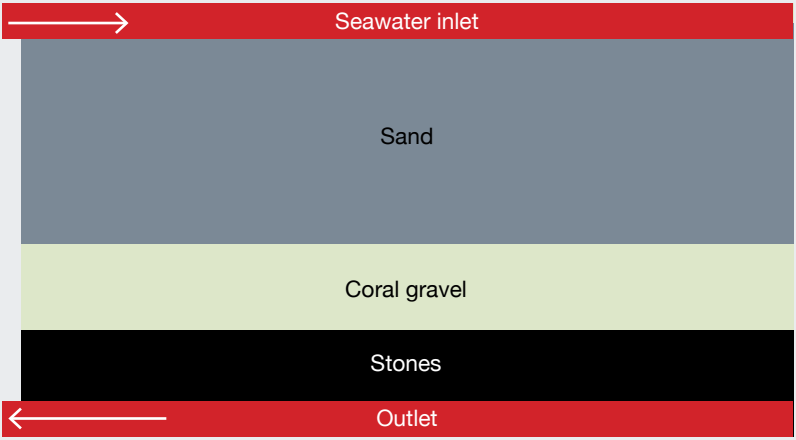


Figure 18 Cross-sectional diagram of a gravity sand filter showing arrangement of graded substrates and inlet and outlet configuration

Larval development

The newly hatched larvae of tiger grouper measure 1.4–1.7 mm TL. The larval development of tiger grouper is shown in Figure 19. The mouth opens 2–3 days after hatching (DAH) and the yolk is completely absorbed by 4–5 DAH. By 10–30 DAH, most tiger grouper larvae have the elongated dorsal and pelvic spines typical of larval serranids. When the larvae are reared at high density, they often become entangled with each other via these spines. This can lead to high mortality between 10 and 30 DAH. Tiger grouper larvae show drastic changes in their shape as they grow from the newly hatched larva to the juvenile stage (Figure 19). A detailed description of the larval morphological development of tiger grouper can be found in Kohno et al. (1993). Until the larvae have completed metamorphosis to the juvenile stage, they are very sensitive to environmental conditions and substantial mortality can occur due to apparently minor stresses. Tiger grouper metamorphose to juvenile at about 40–45 DAH (Figure 19), although this can be delayed due to low water temperatures or poor nutrition. Because of the sensitivity of larvae, careful management is required throughout the larval-rearing phase.

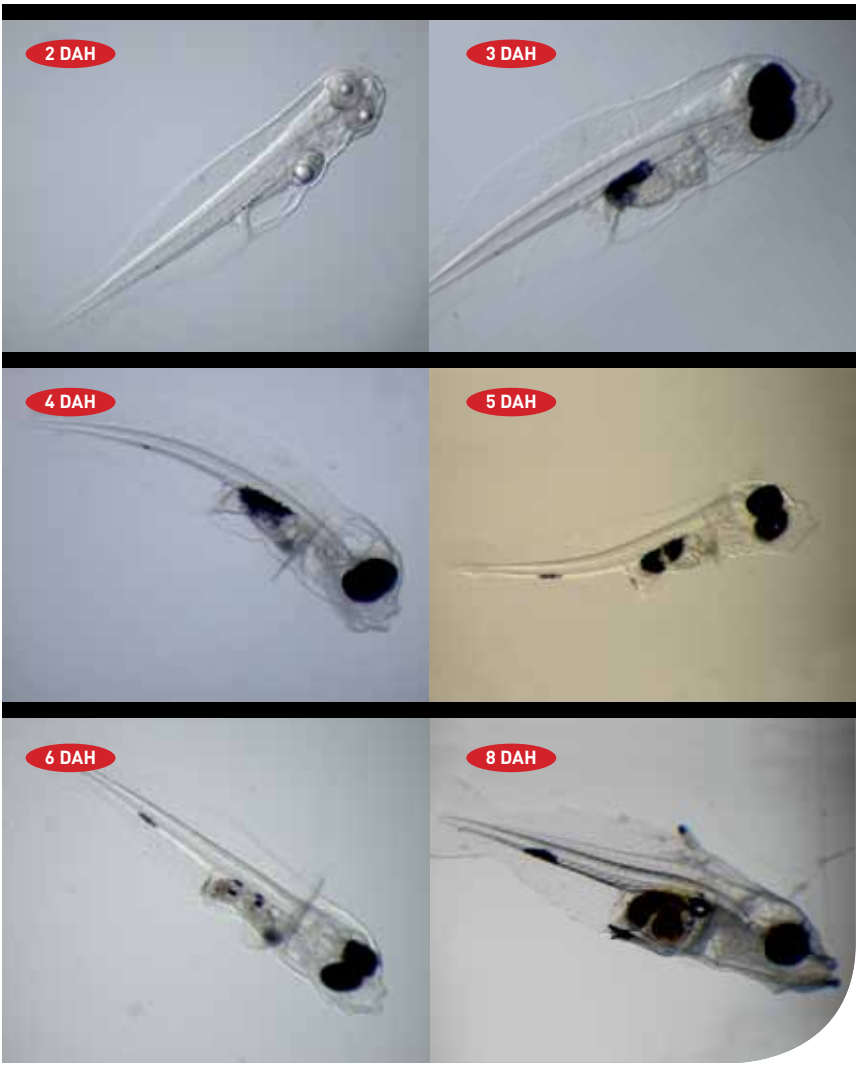


Figure 19 Larval development of grouper. There can be substantial variation in development rates between and within batches of tiger grouper larvae, so this schedule should be used only as a guide. At 2 days after hatching (DAH) the larvae have not yet begun to feed and the yolk and oil globule, which provide endogenous nutrition at this stage, are visible. At 3 DAH the mouth has opened and the larvae begin feeding, the gut has formed and the stomach and eyes are pigmented. From 4 to 6 DAH there are no major morphological changes, but pigmentation around the stomach increases. At 8 DAH the buds of the dorsal and pectoral spines appear.

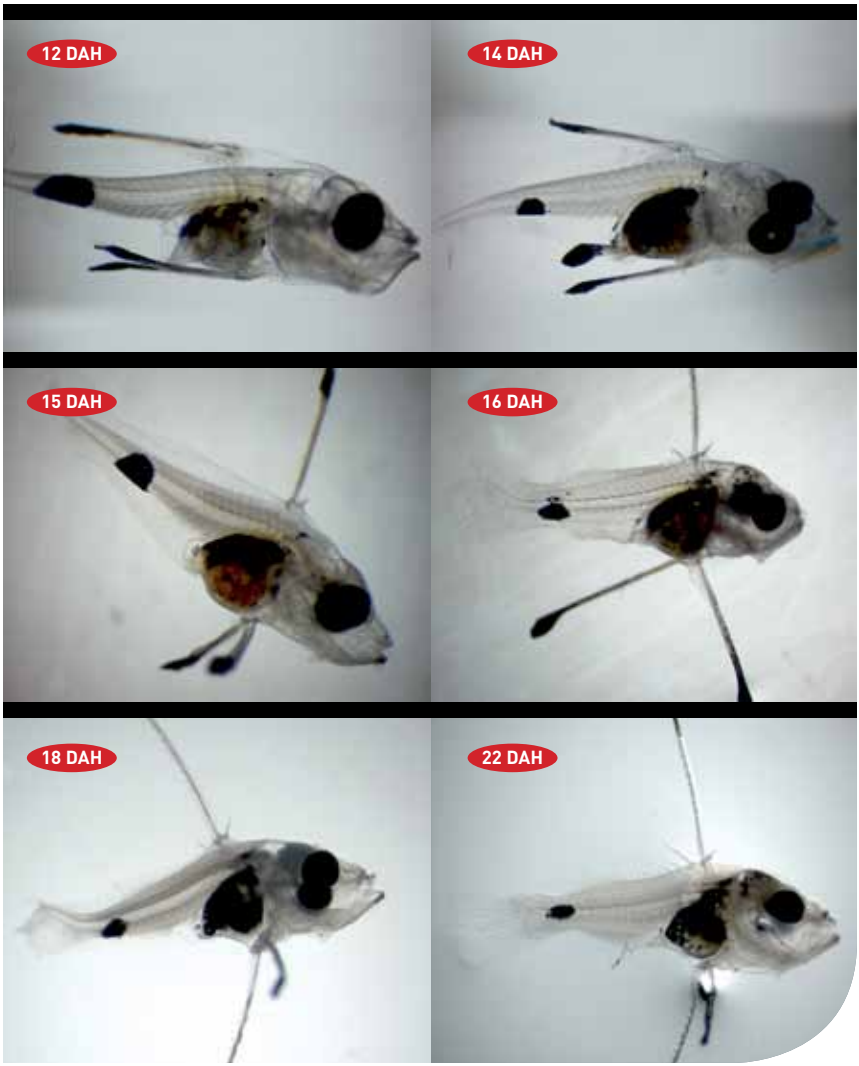


Figure 19 **(continued)** Throughout the second week (8–14 DAH) the gut continues to develop, the dorsal and pectoral spines elongate, and the head and body develop. In the third week (15–20 DAH), the larvae continue to grow and develop and appear less transparent.

Figure 19 **(continued)** Growth during the fourth week (21–27 DAH) may be rapid as the larvae feed on brine shrimp (*Artemia*). Body pigmentation increases and the larvae appear darker in colour. The dorsal and pectoral spines begin to recede. Most of the larvae will metamorphose by the end of week 5, although some will not metamorphose until week 6. At this time cannibalism will begin, with metamorphosed fish attacking smaller fish. (Photos: R. Knuckey)

Rearing the larvae

Important points to remember when rearing larvae are listed in Box 6. The sea water used in larval-rearing tanks should be pre-treated using a sand filter to remove particulates, and then sterilised using ozone (see above) or chlorine (see Appendix 1) to reduce the potential for pathogen introduction in the water supply. Recommended initial stocking density for tiger grouper is 10 larvae/L.

As discussed further below, oil can be added to form a thin film on the water surface (around 0.2 mL/m^2) at 1–5 DAH to prevent surface aggregation mortality in early-stage grouper larvae.

BOX 6

Best practice for grouper larval rearing

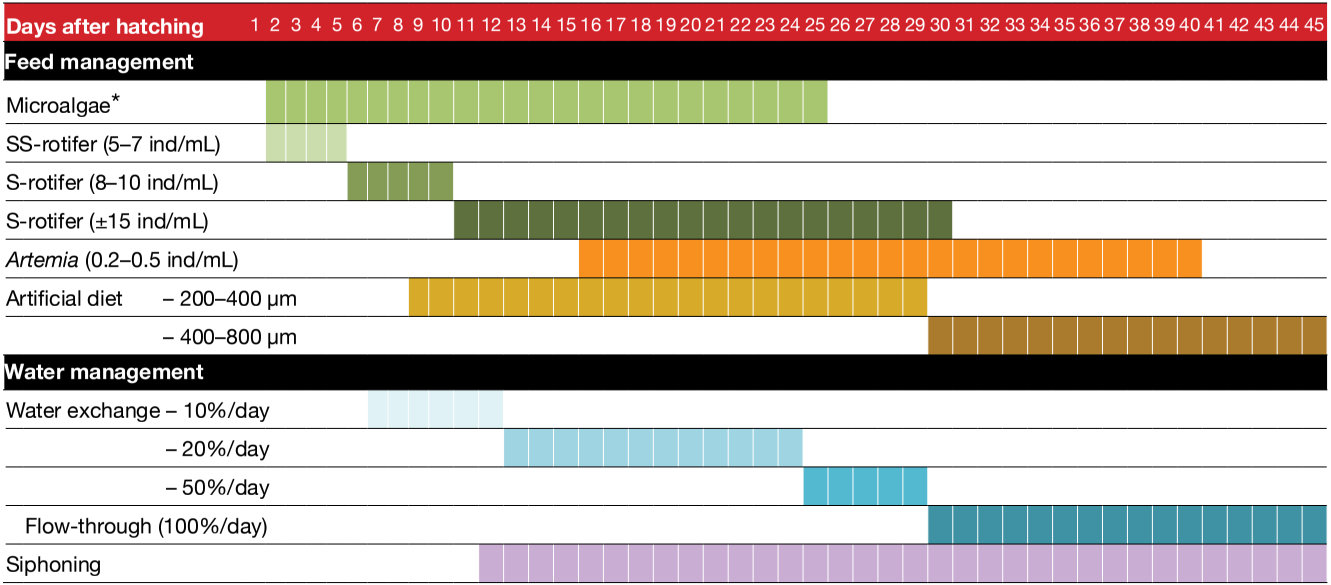
- Maintain low densities of larvae; stock at 10 larvae/L
- Supplement live food organisms with an enrichment product high in docosahexaenoic acid (DHA)
- Maintain optimal water quality
- Regularly check and maintain food densities in the tanks
- Regularly examine larvae under the microscope for full stomachs and signs of disease
- Store artificial diets and enrichment products in a refrigerator or coldroom
- Keep good records of feeding, water quality and other aspects of hatchery management. Some example data sheets are included in Appendix 2.

Live foods used for larval rearing comprise microalgae (*Nannochloropsis* sp.), super-small (SS-type, 60–100 µm) and small (S-type, 120–180 µm) rotifers (*Brachionus rotundiformis*) and brine shrimp (*Artemia*) nauplii. Artificial diets are introduced before feeding *Artemia* nauplii. The larval-rearing protocol is summarised in Figure 20. Note that the protocol described here and summarised in Figure 20 is a guide only, and specific hatchery protocols will depend on a wide range of factors including operator preferences and experience. Production of live food is not covered in this publication—we recommend 'Manual on the production and use of live food for aquaculture' by Lavens and Sorgeloos (1996) for a thorough guide to this topic.

Microalgae (usually *Nannochloropsis*) are introduced to the larval-rearing tanks 2 DAH, i.e. 2 days after stocking the larvae. The algal cell density is maintained at 300–500 × 10^3 cells/mL. SS-type rotifers are introduced at 2 DAH (afternoon) when the larvae have partly absorbed their yolk. The SS-type rotifer density in the larval-rearing tanks should be maintained at 5–7 individuals/mL during 2–5 DAH. Following the period of feeding with SS-type rotifers, small (S-type) rotifers are fed at a density of 8–10 individuals/mL from 6 to 10 DAH, increasing to around 15 individuals/mL from 11 to 30 DAH. Rotifer density gradually decreases as the rate of rotifer consumption by the larvae increases and eventually rotifers disappear by around 30 DAH.

The use of calanoid copepods as live feed during the early larval rearing of groupers has been shown to improve larval growth and survival (Doi et al. 1997; Toledo et al. 1997, 1999) and larval grouper will actively select copepod nauplii in preference to rotifers (Toledo et al. 1997), suggesting that copepods are a more acceptable and nutritional prey than rotifers. However, copepods are not widely used in commercial hatcheries and, while their application to larval rearing holds much promise, there is still considerable research and development required before they can be reliably produced and used in hatcheries (McKinnon et al. 2003).

From 9 DAH, small-size commercially formulated diet with a particle size of 200–400 µm is used. The formulated feed is sprinkled onto the surface of the water in small amounts frequently (as often as hourly) throughout the day. Only small amounts of feed are added such that the feed is consumed within 5 or 10 minutes; excess feed should not be allowed to accumulate on the bottom of the tank where it will decompose and degrade water quality. The feed size is increased to 400–800 µm from 30–45 DAH. Only high-quality



* At concentration of 300–500 × 10^3 cells/mL;

Note: S = small type; SS = super-small type; ind = individuals

Figure 20 Larval-rearing protocol for tiger grouper. Note this is a guide only—individual hatcheries may find substantial differences in growth rates which will require modification of this guideline.

microdiets specifically formulated for marine finfish should be used and these should be stored in a refrigerator or freezer to maintain their quality.

From 16 to around 40 DAH, *Artemia* are fed at a density of 0.2–0.5 individuals/mL. As noted below, the *Artemia* should be supplemented with a commercial enhancement product that will increase the levels of essential fatty acids.

Larval-rearing tanks are maintained statically until 7 DAH. Initially, water exchange is limited to only about 10%/day (7–12 DAH) to avoid sudden changes in water quality, increasing to 20%/day when both artificial diets and *Artemia* are being fed (13–24 DAH). From about 12 DAH, faeces, dead larvae and uneaten food accumulating on the bottom of the tank are siphoned out at least once daily to maintain water quality. Initially, only one-quarter of the tank bottom is siphoned each day and this is gradually increased until the whole tank is siphoned daily. Water exchange increases to around 50%/day from 25 DAH, then to a slow flow-through equivalent to around 100%/day from about 30 DAH.

Towards the end of the larval-rearing cycle, the metamorphosed juveniles should be fully weaned to pellets. This is particularly important if the fingerlings are destined for nurseries or farms using pellet feed, to reduce mortality associated with weaning fish from wet diets (e.g. 'trash' fish) to

Figure 21 Belt feeder used to wean newly metamorphosed grouper fingerlings (Photo: R. Knuckey)

pellets. This requires frequent feeding of small amounts of pellets during daylight hours. To reduce labour, belt feeder can be used to deliver the feed either as a constant stream or in small batches (Figure 21).

Recommended larval-rearing conditions for tiger grouper are listed in Table 5. It is important to regularly measure water quality in the larval-rearing tanks. If water quality degrades, it may be necessary to replace the water at rates higher than the rates recommended above. However, the water used should be of similar temperature and salinity to the water in the rearing tanks to avoid stressing the larvae. It is also important that records are kept of water quality, feeding and other management aspects of the hatchery. Some examples of data sheets to keep such records are given in Appendix 2.

Table 5 Recommended values for physico-chemical parameters for larval rearing of tiger grouper. Note that there is very little information available on the tolerances of grouper larvae to various environmental parameters.

	Recommended	Reference
Temperature	28–30 °C	
Salinity	32–34 ppt	
Light*	500–700 lux	Toledo et al. (2002)
Photoperiod	Natural	
Aeration*	0.62–1.25 mL/min/L	Toledo et al. (2002)
Dissolved oxygen	80–100% saturation	
Ammonia (NH_3-N)	<0.1 ppm	
Nitrite (NO_2-N)	<1.0 ppm	

* Refers to other *Epinephelus* species, but in the absence of more specific information, provides a guide to the requirements for tiger grouper

Nutritional enhancement of live foods

Larvae of the closely related green or greasy grouper (*E. coioides*) require high levels of the highly fatty unsaturated fatty acids eicosapentaenoic acid (EPA, or 20:5n-3), arachidonic acid (ARA, or 20:4n-6) and docosahexaenoic acid (DHA, or 22:6n-3) for proper development, and provision of these fatty acids in the diet, via incorporation in the live foods used for larval rearing, improves survival, growth and pigmentation of the larvae and fingerlings (Alava et al. 2004). Our research has demonstrated that tiger grouper have a similar requirement for highly unsaturated fatty acids in the diet during larval development. For this reason, the live foods used for larval rearing should be treated to increase the levels of these essential fatty acids.

Various commercial preparations have been developed for nutritional enhancement of rotifers and brine shrimp (Alava et al. 2004). Because groupers have a very high requirement for DHA, we recommend the use of products high in DHA, particularly for *Artemia* because *Artemia* retroconvert long-chain fatty acids to shorter-chain fatty acids, thus lowering the levels of the essential fatty acids such as DHA. These nutritional supplementation products are packaged as liquid or spray-dried products. Generally, preparation involves measuring the required quantity, blending either to hydrate (for spray-dried products) or emulsify (for liquid products) the material, then applying to the live-food culture tanks. The manufacturers provide technical information on the application of their products. Of particular importance is the need to maintain high dissolved oxygen levels in the culture tanks during the application period (usually <12 hours). This may require the use of pure oxygen, or oxygen-supplemented air, particularly if the live-food organisms are at high density.

Problems in larval rearing

There are several commonly encountered problems in the larval rearing of *Epinephelus* species, including *E. fuscoguttatus*.

Surface aggregation mortality

Grouper larvae are attracted to patches of sunlight in the tank, and where patchy sunlight is present may swim to the surface of the tank. This often results in (a) the larvae becoming 'stuck' on the water surface or (b) groups of larvae becoming entangled in each other's spines. Both problems cause significant mortality among early stage larvae.

To reduce this problem, only indirect light should fall evenly on the larval-rearing tanks. All direct light should be screened. To prevent surface tension death, oil can be added twice daily to the tank (at about 0.2 mL/m^2) to form a thin film at 1–5 DAH (Yamaoka et al. 2000).

Larval mortality at first feeding

Usually there is relatively high mortality among first-feeding larvae. Samples of larvae should be checked under a microscope around the time of initial feeding (3 DAH) to ensure that they are feeding on the rotifers provided. If the larvae are not feeding, check the size and density of the live-food organisms to ensure that there are enough food items of appropriate size for the larvae.

Viral nervous necrosis

VNN is a common disease problem in marine finfish hatcheries and affects most cultured marine finfish species, including groupers (Harikrishnan et al. 2011; Manin and Ransangan 2011). This infectious disease is caused by a nodavirus and is also known as viral encephalopathy and retinopathy. It is difficult to entirely eliminate VNN from hatcheries, but the incidence of VNN outbreaks can be reduced by following the 'best practice' guidelines in this manual. The source of VNN in hatcheries has not been established: it may be transmitted vertically (from the broodstock, via eggs and sperm) or horizontally (in water introduced to flush tanks, or in live-food cultures).

Current research suggests that most outbreaks of VNN in tropical marine finfish hatcheries are due to horizontal transmission (Hick et al. 2011; Manin and Ransangan 2011). Strict biosecurity is the best defence against VNN outbreaks (Hick et al. 2011).

The most obvious symptom of VNN is disorientation of the fish, which may swim in a 'spiralling' pattern. This is often accompanied by a change in skin colour, with the fish usually becoming darker. An outbreak of VNN can cause substantial mortality within a few days, and in the worst case will wipe out an entire production run.

A definite diagnosis of VNN can be made only with the aid of both a histopathological examination and a polymerase chain reaction (PCR) test. A PCR test alone is not sufficient to diagnose VNN as the cause of the disease outbreak—it confirms only the presence of the virus. An additional histological examination is required to confirm that the disease is VNN. Histological examination should focus on the eye, the brain and the spinal cord. VNN will show as heavy vacuolation of the retina, brain and spinal cord tissue.

If an outbreak of VNN occurs, ensure that the affected tanks are strictly quarantined and there is no transfer of fish or equipment between affected and unaffected tanks. Personnel accessing the infected fish should disinfect their hands and footwear and change clothes before accessing non-infected areas. If the outbreak is severe and will likely result in the loss of most fish in the tank, it is recommended to kill the fish in the affected tank and disinfect the tank as well as associated equipment (nets, buckets, airstones, airlines etc.) to reduce the chance of the outbreak spreading to other tanks. If the outbreak is mild, remove dead or moribund larvae regularly (several times per day) and kill/disinfect the larvae before disposal using chlorine or a similar disinfectant (see Appendix 1). Disinfect the nets and other equipment after each tank check.

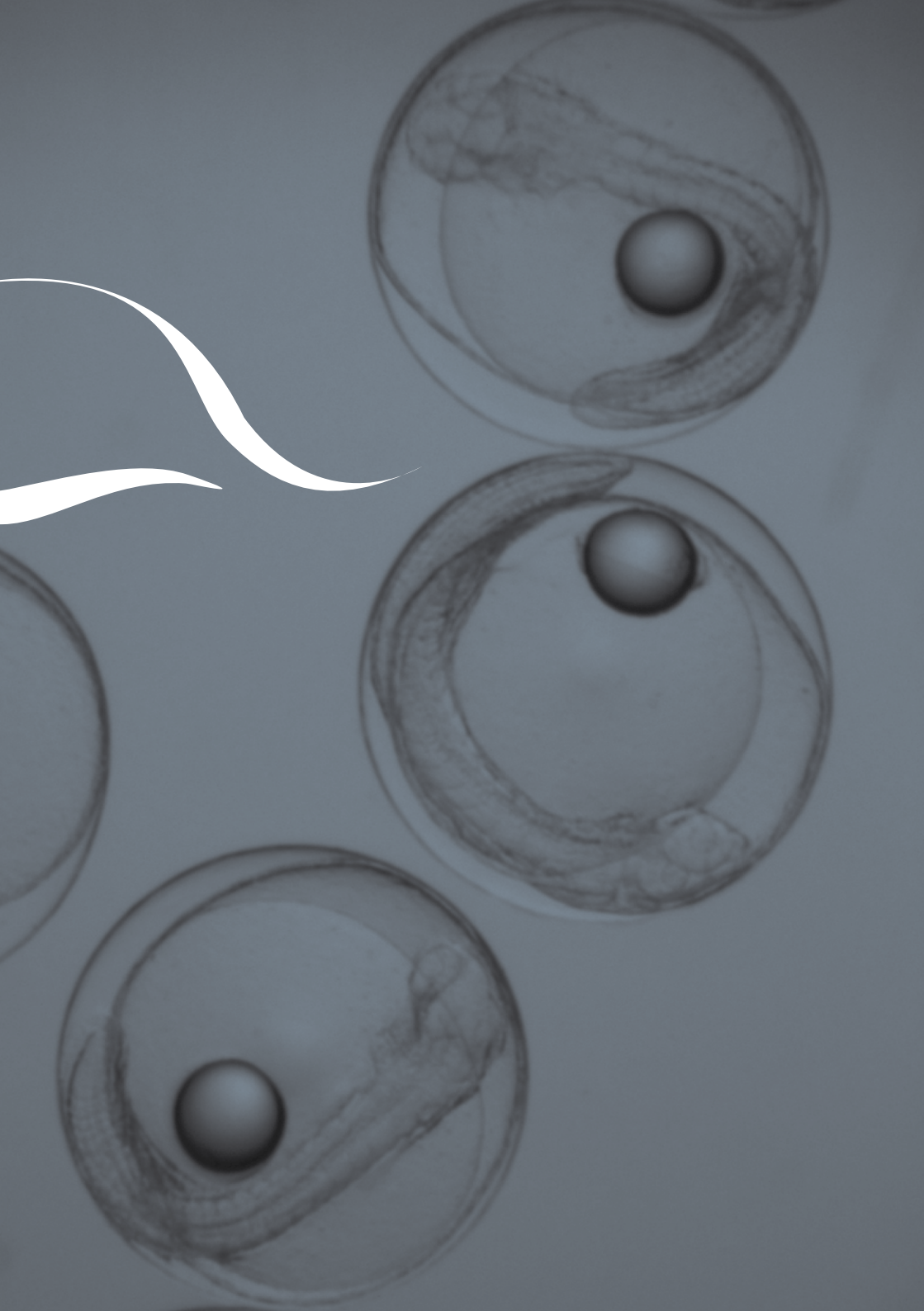
'Shock syndrome'

Another problem that is seen in grouper larval rearing is 'shock syndrome'. This typically occurs from about 20 DAH, increasing in prevalence around 25 DAH. Our research has demonstrated that improving the nutritional composition of the live food fed to green grouper (*E. coioides*) by using larval nutritional supplements can dramatically reduce the incidence of 'shock syndrome' in cultured larvae. This result suggests that 'shock syndrome' is related to nutritional deficiencies, particularly in essential fatty acids. The use of nutritional supplements high in essential fatty acids, particularly DHA, will reduce the incidence and severity of 'shock syndrome' in larval groupers.

Cannibalism

During the later stages of larval rearing, cannibalism may begin to become a problem in the larval-rearing tanks. Cannibalism in groupers is discussed in more detail in the 'Nursery management of grouper' publication in this series (Ismi et al. 2012). However, in general, to reduce cannibalism:

- ensure that food is available to the larvae every morning at first light. If particulate (pellet) feed is being used, the first feed each day should be immediately after dawn. If live food is being used, ensure that densities of the live-food organisms are high at dawn, or feed immediately before dawn each day
- feed particulate feed frequently, i.e. every 1–2 hours
- for late-stage larval groupers, maintain light levels around 600 lux
- do not grade groupers until they have reached metamorphosis, when they are fully scaled and robust (usually 2.0–2.5 cm TL).



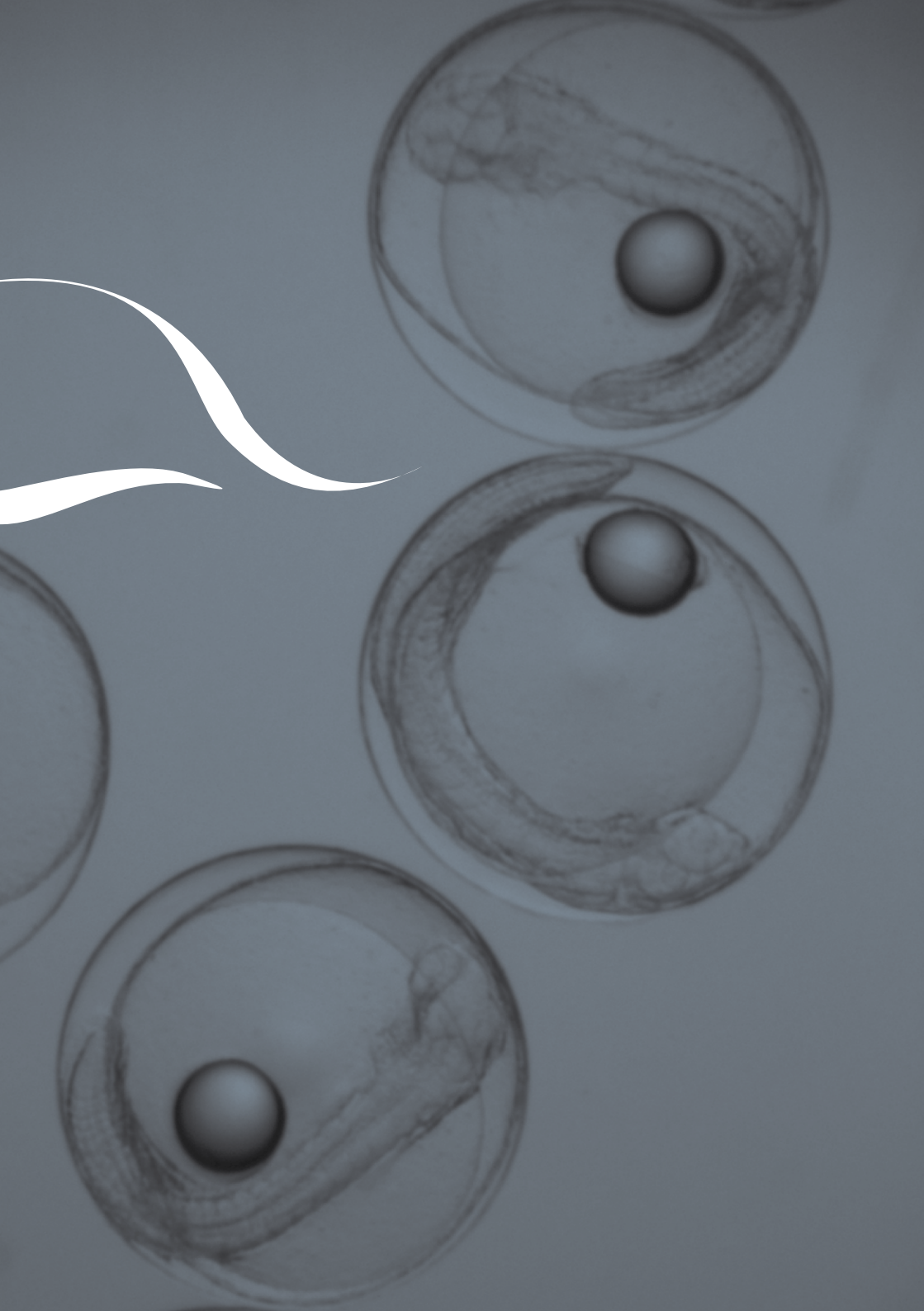
Fingerling production

Based on experiences at RIM Gondol, by 45 DAH, almost all tiger grouper larvae have metamorphosed into juveniles ranging from 2.0 to 2.8 cm TL. The survival rates of tiger grouper at 45 DAH range from 5 to 40% and in most cases are between 15 and 25% when the initial stocking density of newly hatched larvae is about 10 larvae/L. For a 10 m^3 larval-rearing tank, initially stocked at 10 larvae/L, the hatchery can expect to harvest around 20,000 fingerlings.

Juveniles harvested from larval-rearing tanks are still too small and not strong enough to be introduced directly into sea cages. Instead, the juvenile grouper are cultured in the nursery (Figure 22), as described in another publication in this series: 'Nursery management of grouper' (Ismi et al. 2012).

We recommend that marine finfish hatcheries operate on a 'batch' basis; that is, each batch of larvae is treated as a separate production cycle, and the hatchery is shut down between each production cycle. During this period, hatchery equipment should be disinfected (see Appendix 1 for further information on appropriate disinfection techniques) and cleaned to reduce the chances of disease outbreaks in subsequent production cycles.

Figure 22 Juvenile tiger grouper ready for nursery culture (Photo: M. Rimmer)



Appendix 1

Disinfection procedures for marine finfish hatcheries

This appendix provides guidelines on the use of chlorine for disinfection of marine finfish hatcheries because this is one of the most commonly used disinfectants (due to its ready availability and low cost). Other disinfection options are also listed.

Disinfection using chlorine

1. Use at 100–250 mg/L available chlorine.
2. Soak all used hatchery paraphernalia (e.g. handling nets, aeration lines, buckets) overnight.
3. Scrub tank bottom and side walls with the freshly prepared disinfectant.
4. Drain disinfectant. Rinse thoroughly with clean fresh water several times.
5. Allow to dry in the sun and let stand for several days.

Procedure for disinfecting rearing water using calcium hypochlorite (70% chlorine activity)

The following table provides a guide to determining the amount of calcium hypochlorite (g) for water disinfection.

	Weight (g) of calcium hypochlorite required for chlorine concentration of:			
Volume of water	**5 mg/L**	**10 mg/L**	**15 mg/L**	**20 mg/L**
500 L (0.5 m^3)	3.6	7.1	10.7	14.3
1,000 L (1 m^3)	7.1	14.3	21.4	28.6
3,000 L (3 m^3)	21.4	42.9	64.3	85.7
5,000 L (5 m^3)	35.7	71.4	107.1	142.9

For example, if the water volume is 1 m^3 (1,000 L) and the desired chlorine concentration is 20 mg/L, the amount of calcium hypochlorite needed is 28.6 g.

The amount of calcium hypochlorite can be multiplied by different factors to obtain other chlorine concentrations. Example: To obtain 100 mg/L chlorine solution in 1 m^3 water, multiply 28.6 g by 5 or 14.3 by 10. To obtain 250 mg/L chlorine solution in 1 m^3 water, multiply 28.6 g by 12.5 or 14.3 by 25.

Methodology

1. Dissolve the required amount of calcium hypochlorite powder for the desired volume of water in 500 mL water.
2. Fill the tank with the desired volume of water then add the dissolved calcium hypochlorite solution.
3. Keep chlorinated water for at least 12 hours (up to 24 hours) then check the residual chlorine level using a commercial kit. Neutralise residual chlorine with an equal amount of sodium thiosulfate ($Na_2S_2O_3$) before using the water. This should be used within 6 hours after neutralisation since bacterial load increases within 24 hours.

Disinfection options for marine finfish hatcheries

Compiled by Dr John D. Humphrey, University of Sydney

Application	Agent	Concentration	Procedure
Footbaths	Iodophor*	200–250 mg/L available iodine	Replenish footbath daily
	Hypochlorite	50–100 mg/L available chlorine	Brush boots before immersion
	Chloramine-T	50 g/L	Leave to dry on boots
Nets	Hypochlorite	200 mg/L available chlorine	Dip for >2 minutes then rinse
	Iodophor*	200–250 mg/L available iodine	Dip for >10 minutes
Equipment, buckets, trays	Hypochlorite	100–200 mg/L available chlorine	Follow by rinse in fresh water
	Iodophor*	100–250 mg/L available iodine	Spray on or rinse previously cleaned and dried equipment
	Boiling water		Short dip
Handwash	Benzalkonium chloride	0.1–1 g/L	Apply for 1 minute
	Chlorhexidine	4% weight/volume (w/v) chlorhexidine	Apply and rinse for 1 minute
	Iodophor*	200 mg/L available iodine	Apply for a few seconds
	Antiseptic soap		Thoroughly wash and rinse
Hard surfaces and holding tanks (cleaned first with soap and hot water)	Benzalkonium chloride	2–5 g/L	Apply for >15 minutes
	Iodophore*	200–250 mg/L available iodine	Apply for 1–2 minutes
	Hypochlorite	100–250 mg/L available chlorine	Apply for 3 hours
	Steam cleaning	115–130 °C	

Application	Agent	Concentration	Procedure
Transport vehicles	Hypochlorite	50–100 mg/L available chlorine	
	Steam cleaning	115–130 °C	
Protective clothing	Laundering	50–60 °C minimum with detergent	Commercial laundering
	Iodophor*	200–250 mg/L available iodine	
Boots and footwear	Iodophor*	200–250 mg/L available iodine	Scrub boots before treatment
	Hypochlorite	50–100 mg/L available chlorine	
Solid/ semi-solid wastes	Incineration		
	Burial		Limit access by birds and vermin
	Heating	Minimum 60 °C for 1 hour	
	Rendering as fertiliser	Approved process	
Water and washings	Hypochlorite	100 mg/L active chlorine	Hold >24 hours before discharge
	Ozone	Levels of 0.08–1.0 mg/litre	Caution: significant occupational health and safety (OH&S) issues
	Heat	60 °C for 10 minutes, 70 °C for 6 minutes, 75 °C for 5 minutes, or 80 °C for 4 minutes	

* Suitable products include Wescodyne®, Betadine® or Phoraid®

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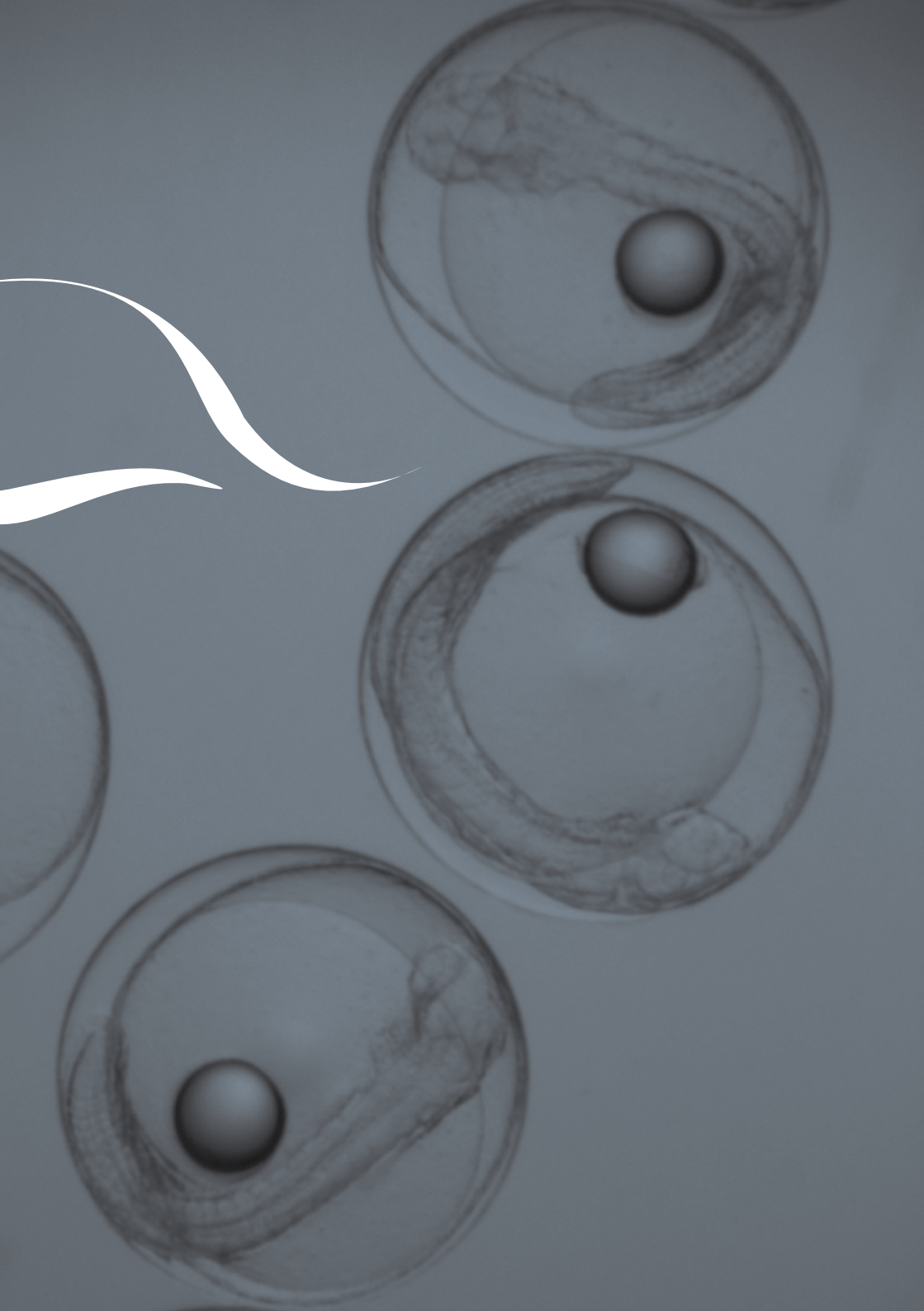


Appendix 2

Example data sheets for marine finfish hatcheries

Note: Collection and examination of production data are important aspects of 'best practice' in hatcheries. These data sheets are provided as a guide to the type of information that should be collected routinely. However, they should be modified to the specific needs of each hatchery.

If a hatchery has computer access, the data should be collected and maintained on 'hard copy' data sheets, and the data transferred to a spreadsheet program. This enables comparison of seasonal and annual data, and graphing data to visualise any trends.

Example data sheet for recording larval-rearing tank water quality

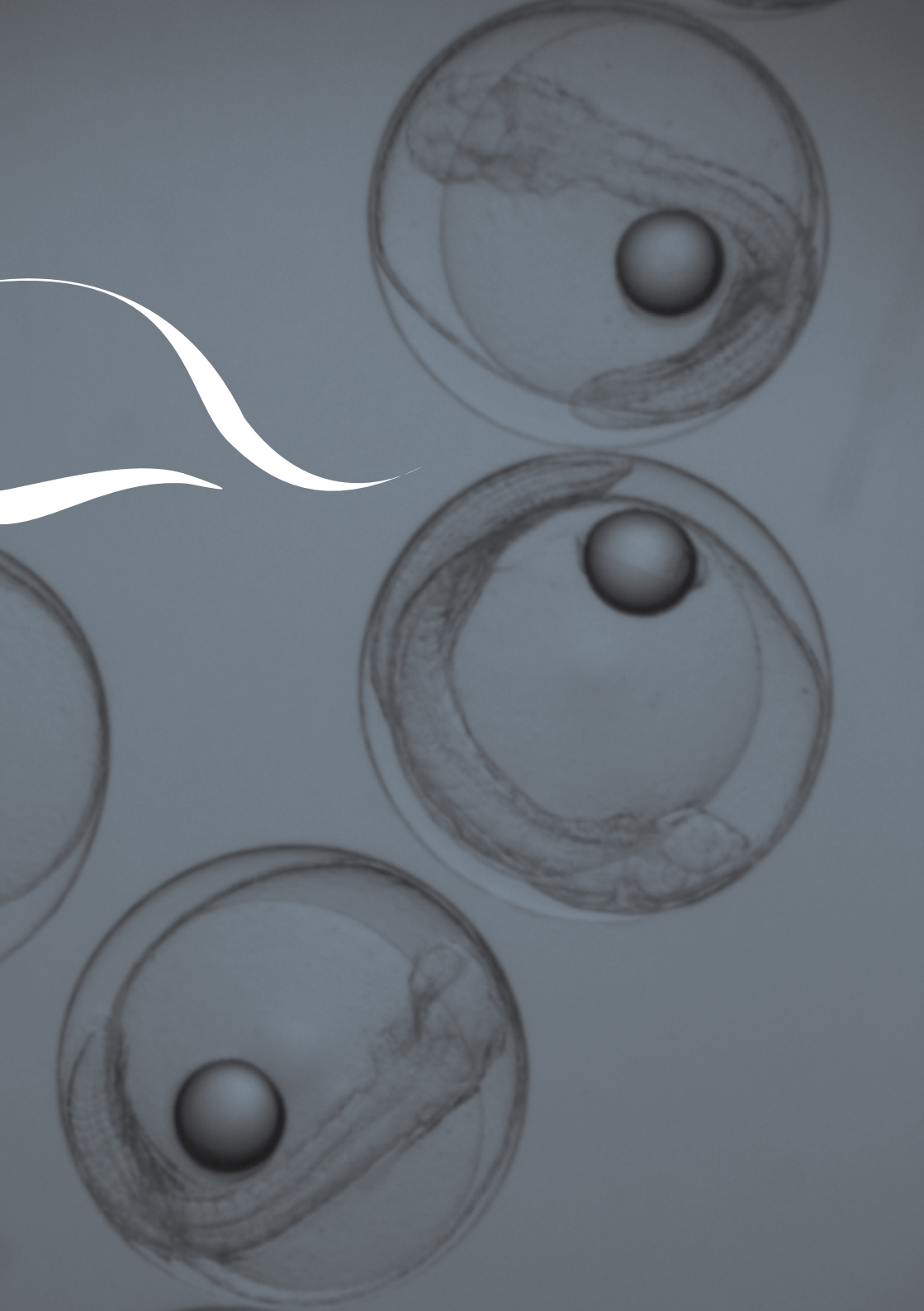
Tank no.: Date:

Temperature (°C)		Salinity (ppt)		Dissolved oxygen (mg/L)		pH		Water exchange (%)	Notes
am	pm	am	pm	am	pm	am	pm		

Example data sheet for recording live-food densities and feed management

Hatchery name: Date:

Tank	Rotifers (ind/mL)		Artemia (ind/mL)		Copepods (ind/mL)		Rotifers added (no.)		Artemia added (no.)		Copepods added (no.)		Pellet feed (size, g)	Notes
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm		



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