Review of Grouper Hatchery Technology

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Larval rearing of groupers

Successful larviculture of groupers has been constrained by generally poor, and irregular, survival. The principal constraints to successful larviculture are:

1. the small gape of the larvae and hence their requirement for small prey at first feed; and
2. the occurrence of high mortality at various stages through the larval rearing phase.

(Kohno *et al*. 1990, 1997, Tamaru *et al*. 1995, Leong 1998, Rimmer *et al*. in press).

This document briefly reviews grouper larviculture technology, and summarises the current status of this technology.

Taxonomic note

Because of the sometimes confused taxonomic status of groupers, particularly the genus *Epinephelus*, the literature pertaining to grouper aquaculture commonly misidentifies the species concerned. There is a voluminous literature on *E. tauvina*, but most of this in fact refers to the estuary cod (or greasy grouper) *E. coioides*. To add to the confusion, the synonym *E. suillus* is also sometimes used to refer to *E. coioides*. The black-spotted cod *E. malabaricus* is sometimes referred to by its synonym *E. salmoides*. However, much of the Thai literature on *E. malabaricus* apparently refers to *E. coioides* (R. Yashiro, pers. comm.). In the following literature review, references to *E. tauvina* and *E. suillus* are assumed to refer to *E. coioides*, and references to *E. salmoides* to *E. malabaricus*. Instances of possible misidentification of *E. coioides* as *E. malabaricus* are noted.

Status of grouper hatchery technology

Eggs of *E. coioides*, *E. fuscoguttatus*, *C. altivelis* and *Plectropomus leopardus* take 15–19 hours to hatch, while the European white grouper *E. aeneus* take around 25 hours to hatch (Rimmer *et al*. in press). Grouper eggs and newly hatched larvae are very sensitive to stress and handling (Predalumpaburt and Tanvilai 1988, Caberoy and Quinitio 1998). Handling mortality is minimised by handling only neurula-stage eggs (after the formation of the optic vesicles) and by stocking eggs into the culture tanks 2

h before hatching so that the larvae need not be handled (Lim 1993, Tamaru *et al*. 1995, Caberoy and Quinitio 1998). Grouper larvae are stocked at relatively high density: 20–30 per litre (Ruangpanit 1993, Duray *et al*. 1996) up to 50 per litre (Lim *et al.* 1986, Aslianti 1996). The larvae are sensitive to light during the early stages of their development and are generally kept in darkened conditions.

Rearing tanks are generally rectangular and range in size from 5 to 30 m^3 (Rimmer *et al*. in press). Tank size, shape and colour may affect the survival of grouper larvae cultured intensively. *E. coioides* larvae cultured in $3m^3$ tanks demonstrated a better survival rate (19.8%) at D24 compared with only 7.4% for those in $0.5m^3$ tanks at D21 (Duray *et al.* 1997). Growth and survival of *E. fuscoguttatus* larvae was improved using cylindrical rather than rectangular larval rearing tanks (Waspada *et al*. 1991b). *Cromileptes altivelis* larvae exhibited higher survival in green or blue coloured tanks than in red or yellow coloured tanks, but growth rate was not affected by tank colour (Aslianti *et al*. 1998). Grouper larval rearing tanks are usually supplied with microalgae (generally *Nannochloropsis oculata*, formerly known as marine *Chlorella*), or *Tetraselmis* sp. at 500×10^3 to $100–500 \times 10^6$ cells per mL ('green water' system) (Ruangpanit 1993, Tamaru *et al*. 1995, Watanabe *et al*. 1996, Leong 1998, Rimmer 1998, Rimmer *et al*. 1998). The microalgae provides a shading effect; provides food for the live prey organisms added to the tanks; and may also be ingested by the larvae (although whether the larvae gain any nutritive value from ingested microalgal cells is unknown). More recently, the use of *Isochrysis* for 'green water' rearing has been shown to improve larval growth and survival (Su *et al*. 1998).

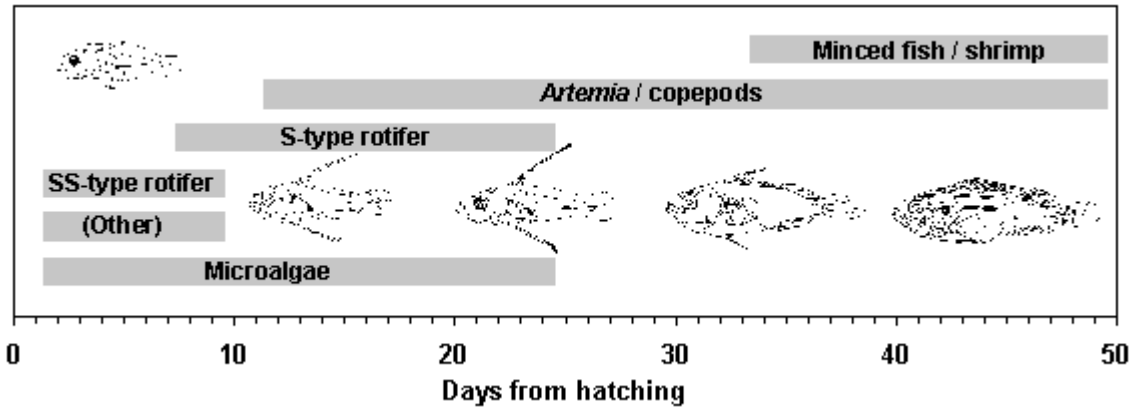

Figure 1 Generalised feeding schedule for intensive 'green water' larval rearing of groupers (modified from Tamaru *et al*. 1995). Other: oyster trochophores, mussel larvae, sea urchin eggs, barnacle nauplii. Representative larval development stages of *E. fuscoguttatus* shown (from Kohno *et al.* 1993); larval stages not to scale.

The mouth of larval groupers generally opens 2–3 days after hatching (D2–3), and the

larvae begin feeding soon thereafter (Kitajima *et al.* 1991, Kungvankij *et al.* 1986, Ruangpanit 1993, Ruangpanit *et al.* 1993, Duray 1994, Doi *et al.* 1997). Kohno *et al*. (1997) described the development of the feeding apparatus in *E. coioides* in detail and suggested that the major difficulties in larval rearing of groupers were attributable to the small size of the bony elements forming the oral cavity, small mouth and body size, poor reserves of endogenous nutrition and lower initial feeding rates.

A generalised feeding schedule for grouper larval rearing is shown in Figure 1. Grouper larvae are initially fed on rotifers, often in combination with oyster trochophores, mussel larvae, sea urchin eggs or barnacle nauplii (Hussain and Higuchi 1980, Lim 1993, Ruangpanit 1993, Tamaru *et al*. 1995, Watanabe *et al*. 1996, Rimmer *et al*. 1998). Oyster trochophores, mussel larvae, sea urchin eggs and barnacle nauplii are around 70μ m in size and are thus small enough to be readily consumed by the larvae (Kungvankij *et al.* 1986, Tamaru *et al*. 1995). Small strain (S-type) rotifers (*Brachionus rotundiformis*) are too large for newly hatched grouper larvae to ingest. Super-small strain (SS-type) rotifers (*Brachionus* sp.), or S-type rotifers screened to <90μ m, are suitable for grouper larvae at first feed (Lim 1993, Tamaru *et al*. 1995, Watanabe *et al*. 1996, Duray *et al*. 1997, Su *et al*. 1997). Optimal prey density during the early larval stages is 10-20 organisms/ml (Ruangpanit 1993, Ruangpanit *et al.* 1993, Tamaru *et al*. 1995, Wardoyo *et al*. 1997).

The use of copepod nauplii as prey during the early larval rearing of groupers has shown considerable promise in improving larval growth and survival (Hussain and Higuchi 1980, Doi *et al*. 1997). Provision of nauplii of calanoid copepods (mainly *Pseudodiaptomus annandalei* and *Acartia tsuensis*) and rotifers in rearing tanks resulted in higher survival and faster growth in *E. coioides* compared with the use of rotifers alone (Doi *et al.* 1997, Toledo *et al*. 1999), and *E. coioides* larvae actively select copepod nauplii in preference to rotifers (Toledo *et al*. 1997).

S-type rotifers are fed from about D7 (Tamaru *et al*. 1995, Su *et al*. 1997), and brine shrimp (*Artemia franciscana*) are introduced from about D10. Brine shrimp are initially fed at 1–3/mL, increasing gradually to 7–10/mL at D25–35 (Ruangpanit 1993). Higher prey densities (2–3/mL) enhance growth and survival (Duray *et al*. 1997), as does frequent feeding of brine shrimp (4–5 times per day) (Ruangpanit 1993, Duray *et al*. 1997). Grouper larvae fed rotifers and brine shrimp enriched with *n*–3 HUFAs demonstrate better growth, survival and greater stress endurance than those fed with normal live feeds (Pechmanee *et al.* 1988, 1993, Pechmanee and Assavaaree 1993, Chao and Lim 1991, Dhert *et al.* 1991, Lim 1993, Quinitio 1996). Most laboratories supplement live prey for grouper larvae with microalgae (e.g. *Tetraselmis*, *Chaetoceros*), home made lipid emulsions (egg yolk blended with cod liver oil), or commercially available lipid emulsions or microencapsulated supplements (Ruangpanit 1993, Rimmer *et al*. in press). Minced fish or shrimp may be introduced from about D35 to wean the larvae (or juveniles) from live to inert feeds (Hussain and

Higuchi 1980, Ruangpanit 1993, Tamaru *et al*. 1995).

Survival of groupers to metamorphosis is generally low: usually <10% and commonly <1%. Recent reports of survival in experimental conditions are: 1–10% (average 4%) for *E. coioides* (J. Toledo, pers. comm.) and 1–5% for *C. altivelis* (K. Sugama, pers. comm.). In addition to low average larval survival, survival is highly variable, and a (relatively) successful larval rearing 'run' can be followed by several runs which have negligible or zero survival. It is this both these aspects, low and irregular survival, that have constrained the application of the existing fingerling production technology to commercial hatchery production.

The major causes of mortality in grouper larviculture are:

1. High mortality associated with the commencement of exogenous feeding. This mortality may be associated with the provision of live prey organisms of unsuitable size and nutritional composition, but even when 'suitable' prey types are used, there is generally high mortality at this stage (Ordonio-Aguilar *et al*. 1995, Duray *et al*. 1997).
2. Several mortality syndromes have been described for grouper larvae. A commonly reported mortality syndrome is the 'shock syndrome' that occurs in late stage larvae from about D25 (Lim 1993, Duray *et al*. 1997). This problem may be related to nutritional deficiencies in the live prey organisms used to feed the larvae, since shock syndrome is symptomatic of low levels of HUFAs in the diet (Cowey and Sargent 1972).
3. Cannibalism is a major cause of mortality during the later stages of larval rearing, i.e. from D30–35 (Lim 1993, Tamaru *et al*. 1995, Rimmer *et al*. in press). Although cannibalism can be controlled by grading larvae and juveniles into similar size classes, grading is often associated with high mortality because handling commonly induces the shock syndrome seen in late stage larvae (Rimmer *et al*. in press). Provision of shelter is reported to reduce cannibalism in juvenile *E. malabaricus* (*E. coioides*?) (Rimmer *et al*. in press).

Nutritional requirements of larval groupers

The few studies that have been carried out on the nutritional requirements of larval groupers suggest that supplementation of the larval diet with HUFAs improves growth and survival. Waspada *et al*. (1991c) found higher growth rates of *E. fuscoguttatus* larvae when they were fed rotifers supplemented with bakers yeast with sardine oil or bakers yeast with cod oil. These treatments had high levels of EPA (8.8–8.9% respectively) but highly variable levels of DHA (0.1–5.5% respectively) (Waspada *et al*. 1991a). Su *et al*. (1997) found that growth and survival of larval *E. coioides* was associated with the fatty acid composition of the larvae. Larvae with high levels of total fatty acids grew faster than those with lower levels, and larvae with high DHA or

EPA content (> 2mg/g DW) exhibited better survival (>10%) than those with lower HUFA content.

Ruangpanit *et al*. (1993) reported improved survival of *E. malabaricus* (*E. coioides*?) larvae when brine shrimp used for larval rearing were enriched with a cod liver oil / egg yolk emulsion (*n*–3 HUFA = 450 mg/g) or were supplemented with copepods or the freshwater cladoceran *Moina*. Dhert *et al*. (1991) found that supplementation of brine shrimp with an emulsion containing high levels of DHA had little effect on growth or survival of *E. coioides* larvae, and concluded that application of DHA could be delayed until D25 before mortality due to nutritional deficiency affected *E. coioides* larvae.

Thyroid hormone treatment

Lam (1994) and Lam *et al.* (1994) reported that the levels of the thyroid hormone are higher in buoyant than in non-buoyant *E. coioides* eggs. The buoyant eggs are more viable than non-buoyant eggs, suggesting a relationship between levels of thyroid hormone and viability. Application of 0.01–1.0 ppm triiodothyronine (T_3) or thyroxine (T_4) is effective in increasing larval survival and hastening the resorption of the dorsal and anal fins in 2–6 days in contrast to 2–3 weeks in fish not so treated (Tay *et al.* 1994, de Jesus 1996, de Jesus *et al*. 1998). Thyroid hormones can be applied by immersing eggs or larvae in a bath, or by bioencapsulation using rotifers or brine shrimp. De Jesus *et al*. (1998) concluded that a dose rate of 0.01 ppm T4 is appropriate for accelerated metamorphosis and improved survival in 3- to 4-week old grouper larvae.

Commercial grouper larviculture in Taiwan

The only commercial hatchery production of grouper fingerlings that has been identified is from Taiwan. Larviculture of groupers (as well as other marine finfish species) in Taiwan is undertaken using either the 'indoor method' or 'outdoor method', i.e. in concrete tanks indoors or in outside ponds (Rimmer 1998).

Indoor method

The indoor method utilises large fibreglass or concrete tanks up to about 100 m^3. The rearing tanks are circular or rectangular in shape, flat-bottomed and with a white or light coloured interior. Larviculture is undertaken using either greenwater or clearwater techniques. The algal density used for greenwater culture ranges from 50,000 to 500,000 cells/ml. Variables such as algal density are only measured in research hatcheries – commercial hatcheries just add algal cells until the desired shade of green is reached (Rimmer 1998).

Generally, eggs are added directly to the larval rearing tanks. Grouper larvae are fed oyster trochophores from first feed (usually D4) for 2 days. Rotifers are also added to the rearing tanks, generally commencing from first feed. Recent research at Taiwan Fisheries Research Institute's Tungkang Marine Laboratory (TML) indicates that a combination of oyster trochophores and small rotifers (either SS-strain, sieved S-strain, or neonates) is the best initial feed (Su *et al*. 1997). Rotifer densities are maintained at about 2–3/ml until the grouper larvae are large enough to eat brine shrimp or adult copepods, which is when the dorsal and pectoral spines reach the end of the caudal fin. Generally, grouper larvae are able to feed on adult copepods from D16 (water temperature >26^{o}C) or D22 (<26^{o}C) (Rimmer 1998).

Outdoor method

The outdoor method of larval rearing is undertaken in concrete or earthen ponds ranging in size from 200 m^2 to 0.5 ha, and, less commonly, to 1 ha. The ponds are filled only 1–2 days before they are stocked with eggs. The inlet is screened with a fine mesh 'sock' filter to exclude potential predators and nuisance species. Stocking density for grouper ranges from about 1 kg of eggs (i.e. *c*. 1.5 million eggs) in 0.1 ha to 2 kg (*c*. 3 million eggs) in 0.2–0.5 ha larval rearing ponds (Rimmer 1998).

One or two enclosures formed by a tarpaulin set around a floating support structure are set up in the pond, usually with shadecloth overhead to reduce light intensity, and mild aeration to ensure adequate dissolved oxygen and mixing of the water within the enclosure. The enclosures range in size from 5m^3 in small concrete ponds, to 8–10m^3 in earthen ponds 0.2–0.5 ha in area. The enclosures are pumped full of pond water, and fertilised grouper eggs are added to the enclosures. Oyster trochophores are added to the enclosures from first feed (usually D4) for 2 days, then the larvae are released into the pond. The enclosures allow smaller quantities of oyster trochophores to be fed while retaining relatively high prey densities. They also allow the farmer to visually estimate larval survival after the first few days of culture which is the period when most mortality occurs. If survival is very low, the farmer may choose to restock the enclosure with another batch of larvae, rather than release the survivors into the pond (Rimmer 1998).

Rotifers (and, incidentally, other zooplankters) are cultured in small concrete or earthen ponds, usually about 0.05–0.1 ha. The rotifers are cultured using trash fish placed in fertiliser bags and left to decompose in a corner of the pond, or by the addition of organic wastes. A paddlewheel aerator is placed in the pond to assist with aeration and to generate a current in the pond. Zooplankton is harvested using a fine (c. 85 μ m) mesh net that is set downstream from the paddlewheel aerator for 1–2 hours. The collected zooplankton is added to the larval rearing pond. Farmers attempt to maintain rotifer density at about 3–4/ml, but like other aspects of pond management,

rotifer density is not measured, but is maintained 'by eye'. Later in the larval rearing cycle, adult copepods are harvested from the zooplankton production ponds using the same technique, although with a larger mesh (c. 210 μ m) mesh net. Some farms pump water from the rotifer production ponds into the larval rearing pond, and may also pump zooplankton-rich water from growout ponds into the larval rearing ponds (Rimmer 1998).

The larvae are reared in the ponds until they reach 2.5–3 cm total length (TL), when they are harvested. In the case of grouper, this takes about 4 weeks. Pond temperatures need to be above 20^{o}C to ensure any larval survival for grouper; if pond temperatures drop below 18^{o}C, the grouper larvae will die. For this reason, some farms will not purchase grouper larvae until April, even though eggs are available from early March. In addition, farmers feel that the quality of eggs produced early in the season is inferior to those produced later in the season. Survival of groupers using both indoor and outdoor larval rearing methods is highly erratic, but generally low: 7% survival is regarded as good. Researchers at TML report that a major constraint to grouper aquaculture is the irregular nature of larval survival. The major problem, according to TML researchers, is high mortality at first feed, although there is often low-level mortality throughout the larval rearing process (Rimmer 1998). Because of the generally low survival of groupers in larviculture, prices for grouper fingerlings are relatively high: US$2–3 per fingerling (Tamaru *et al*. 1995).

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