

Research Report

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## State of Art for Larval Rearing of Grouper

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**Abstract** Groupers belonging to the family of *Serranidae*, are widely distributed throughout sub-tropical and tropic water area. With increasing marketing demands and reduced natural resource, groupers have been considered have high aquaculture potential in tropical and subtropical waters. However, since the first attempt to culture groupers, significant problem exists in fingerling production, particular with survival rates are low and inconsistent. In this review, we will use the life cycle of grouper larvae as a framework to review internal factors regulating ontogenetic development in fish larvae and environmental factors affecting grouper larvae development. To understand the cause of high larvae mortality in early life history, we will review factors related to the ontogenetic development, and then we will focus on issues of first feeding of grouper larvae in intensive aquaculture. At the end, we will review the management strategies of using live feeds in grouper hatcheries.

**Keywords** Grouper; Larvae culture; Live feeds; Growth and survival rate

### Introduction

Groupers are species belonging to the family of *Serranidae* that are very important for sport and commercial fisheries (Glamuzina et al., 1998). They are widely distributed throughout sub-tropic and tropic water area. Because of the high flesh quality and elegant appearance, groupers are very attractive for human consumption. With the increasing demands in the markets, the global production of groupers reached to 198 690 mt in 2007 (Harikrishnan et al., 2011). In the live markets of China, groupers have brought high prices (up to US\$70-100/kg wholesale) (McGilvray and Chan, 2001; Harikrishnan et al., 2011). Increasing marketing request and reduced natural resource made grouper being considered have high aquaculture potential in tropical and sub-tropical waters (Liao et al., 2001; Marte, 2003).

Up to present, reliable fingerling productions are still the issues hindering the development of groupers' aquaculture industries. Since 1970s, efforts have been made to mass produce artificial grouper fingerlings (Lim, 1993). Species such as *Epinephelus tauvina*, *E. salmoides*, *E. akaara*, *E. malabaricus*, *E. striatus* (Chen et al., 1977; Xu et al., 1985; Huang et al., 1986; Maneewong et al., 1986; Tucker et al., 1991) have

been reported for successful spawning. However, as a consequence of massive mortalities in the early development stage, grouper aquaculture remains heavily dependent on the capture and grow-out of wild-caught juvenile. According to the results published in 2003, around 70%~85% of culture groupers were grown out from wild-caught fry (Sadovy et al., 2003). Recently, as a result of successful out-door-ponds culture grouper larvae in China, China has become the biggest grouper fry supplier in Asian. Although artificial breeding has been steadily increased recently, there is a still big gap in the fry market.

Studies show that the early survival rates of groupers are very low when compared to other finfish (Duray et al., 1996; Duray et al., 1997). High mortality during the early life stages has been observed in reared grouper larvae, such as *Epinephelus fuscoguttatus*, *Epinephelus coioides*, *Epinephelus suillus* (Duray et al., 1997; Toledo et al., 1999; Kohno et al., 1990). Although a series of studies have been conducted to explore the optimum rearing protocol for groupers larvae, up to present most of the results are still dissatisfaction as heavy mortalities still often observed within the first two weeks after hatching (Duray and Kohno, 1988;

Kohno et al., 1990; Kohno et al., 1997). Difficulties in rearing early stage larvae of groupers have become the major bottleneck hindering the development of mass fingerling production (Kohno et al., 1997). Marte (2003) summarized the difficulty of rearing grouper into three areas: 1) spawned eggs and larvae are very small and the small mouth gape in early larvae limits the choice of initial live feed; 2) grouper are extremely sensitive to mechanical disturbance; 3) long duration of larval rearing (>60 days).

In this review, we will use the life cycle of grouper larvae as a framework to review internal factors regulating ontogenetic development in fish larvae and environmental factors affecting the general development of grouper larvae. To understand the cause of high larvae mortality in early life history, we will review factors related to the ontogenetic development, and then we focus on issues of first feeding of grouper larvae in intensive aquaculture. At the end, we will review

the management strategies of using live feeds in grouper hatcheries.

## 1 Ontogenetic Development

### 1.1 Eggs and Embryo

The egg size of groupers is generally less than one millimeter (Table 1). Like most marine teleosts, nutrition during the embryonic phase is derived from yolk reserve (Ma et al., 2012). The embryonic period starts from fertilization and ends at the commencement of exogenous feeding. It is divided into three major phases: cleavage egg, embryo, and free embryo (Moyle and Cech, 2003). Figure 1 illustrates the embryonic development of Malabar grouper *Epinephelus malabaricus*. Cleavage egg (A-F), and embryo (G-L) are defined according to Moyle and Cech (2003). Embryonic development is a complex process and egg quality and hatching environments directly affect embryonic development and the sizes of fish at hatching and first feeding (Robin and Gatesoupe, 2001).

Table 1 Comparison of the eggs and larvae of *Epinephaline* Serranids

Species	Egg diameter (mm)	Incubation temperature (°C)	Hatching time (h)	Length at hatching (mm)	Duration of Yolksac absorption (day)	Reference
<i>Epinephelus malabaricus</i>	0.87-0.93	25-31	29-26	1.71-1.98	3-4	Leu et al., 2005; Yoseda et al., 2006
<i>E. tauvina</i>	0.80-0.90	27-30	25-20	1.50-2.4	3	Lim 1993; Hussain et al., 1980
<i>E. fuscoguttatus</i>	0.89	28-30	19-18	1.80-1.90	-	Lim 1993
<i>E. suillus</i>		28-30	20-18	1.62	-	Duray et al., 1996; Duray et al., 1997
<i>E. coioides</i>	0.74-0.85	24.9-28	21-19	1.33-1.86	3	Doi et al., 1991; Zou et al., 2003
<i>E. costae</i>	0.89-0.95	25-25.5	28-24	1.69-1.85	3	Glamuzina et al., 2000
<i>E. akaara</i>	0.70-0.77	25-27	25-23	1.45-1.56	4	Ukawa et al., 1966
<i>E. polyphkadion</i>	0.71-0.83	26-29	21-19	1.70-1.80	2	Rasem et al., 1997
<i>E. marginatus</i>	0.74-0.94	23	33-30	1.40-1.67	3-4	Glamuzina et al., 1998
<i>E. merra</i>	0.71-0.73	26.5-28.3	27-24	1.40-1.60	2	Jagadis et al., 2006

#### 1.1.1 Egg quality

Egg quality is generally derived from broodstock nutrition (Izquierdo et al., 2001; Mazorra et al., 2003; Sawanboonchun et al., 2008; Ma et al., 2012). Since protein, lipoprotein, glycogen, and enzymes contents in yolk reserve directly affect embryonic development (Gunasekera et al., 1995; Harrell and Woods, 1995; Sargent et al., 1999), proper controlled broodstocks nutrition is essential in breeding marine fish. For instance, Dhert et al. (1991) showed that *E. tauvina*

broodstock given trash fish injected the emulsified enrichment diet Marila diet significantly increased oil globule diameter, total lipids, eicosapentaenoic acid, docosahexaenoic acid, and larval survival at day 7. Nutrients such as essential fatty acids (EFA) (Fernández-Palacios et al., 1995), vitamin E (Fernández-Palacios et al., 1998), carotenoids (Craik, 1985), vitamin C (Blom and Dabrowski, 1995), dietary protein, vitamin B<sub>1</sub>, and vitamin B<sub>6</sub> (Izquierdo et al., 2001) in broodstock diets have been considered as essential

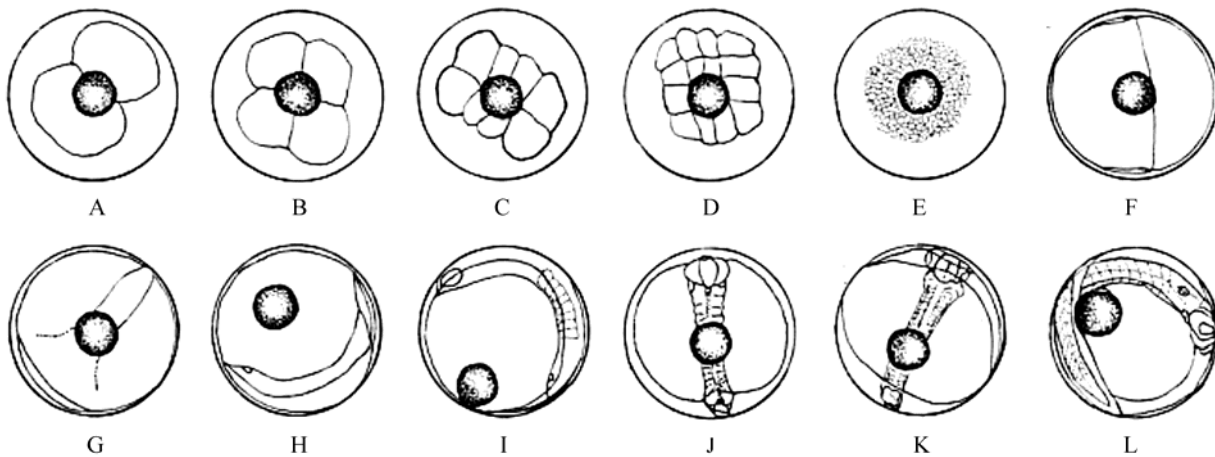


Figure 1 Embryonic development of *Epinephelus malabaricus*

Note: A: Two-celled stage; B: Four-celled stage; C: Eight-celled stage; D: 16-celled stage; E: morhula stage; F: gastrula stage; G: appearance of embryonic shield; H: closure of blastopore; I: appearance of optic vesicles and mytomes; J: 18-myomere stage; K: beginning of motility; L: before hatching (Leu et al., 2005)

for the normal development of embryos (Ma et al., 2012). Evidence indicate that the percentage of normal eggs increases with the increase of  $n-3$  highly unsaturated fatty acids (HUFA) in broodstocks diets of gilthead seabream (*Sparus aurata*) (Fernández-Palacios et al., 1995). Therefore, proper control of broodstocks nutrition can improve egg quality and enhance survivorship in marine fish larvae (Izquierdo et al., 2001).

### 1.1.2 Temperature

Temperature can also affect embryonic development of grouper larvae. Watanabe et al. (1995) found that the variations in water temperatures within an ecological range can markedly influence development rates and survival of pre-feeding Nassau grouper (*Epinephelus striatus*) larvae. Table 1 shows the eggs diameter, incubation temperature, hatching time in 10 grouper species. Clearly, during the grouper embryonic development, with the increased incubation temperature, the hatching time was decreased. Similarly, studies in other species have been demonstrated that higher temperature accelerates development rate (Bermudes and Ritar, 1999; Das et al., 2006; Moran et al., 2007; Kazuyuki et al., 1988; Miranda et al., 1990). For instance, the development rate of mackerel (*Scomber scombrus*) eggs at 17.8 °C is almost three times faster than at 8.6 °C (Figure 2, Figure 3). A similar result has also been reported in striped trumpeter (*Latriss lineata*) when compared with incubation temperatures between 16.2 °C and 8.1 °C (Bermudes and Ritar, 1999). However, high temperature over a

tolerable range may lead to heat shock and fish mortality (Hopkins and Dean, 1975; Kiyono and Shinshima, 1983).

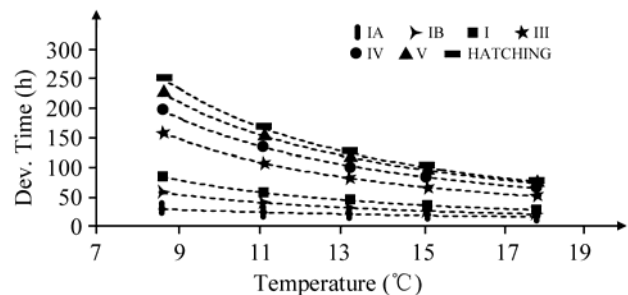


Figure 2 Functional relationship between developmental time, temperature and embryonic stage of mackerel eggs

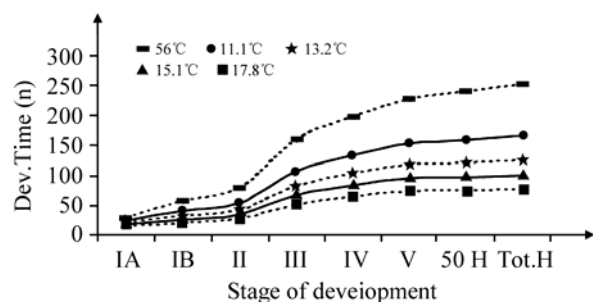


Figure 3 Fitted relationship between developmental time and temperature at each embryonic stage (Mendiola et al., 2006)

Not like other species, grouper eggs and newly hatched larvae (such as *Plectropomus leopardus*) are very sensitive to stress and handling (personal communication with Mr Zhang, Xincun, Hainan Province, P.R. China 2013). Therefore, to minimize the

handling related mortality, stocking activity are recommended to conduct at neurula-stage (after the formation of the optic vesicles) and by stocking eggs into the culture tanks 2 h before hatching so that the directly handle larvae can be avoided (Lim, 1993; Tamaru et al., 1995, Caberoy and Quninitio 1998). Furthermore, The larvae are sensitive to light during the early stages of their development and are generally kept in darkened conditions.

### 1.2 Larvae

Newly hatched grouper larvae are generally less than two millimeters (Table 1), with different structure, morphology and function from adult (Figure 2) (Bone

et al., 1995). Similar with most fish larvae, special larval structure relevant to respiration may develop to increase the area to volume ratio for gas exchange (Houde, 2001). Grouper larvae are delicate and have a large yolk sac and an undeveloped mouth, fins, and eyes (Figure 4). By 3-4 day post hatching, the yolk and oil globule will be absorbed completed (Table 1). The larval stage starts from exogenous feeding and ends with completing metamorphosis. Like most marine fish, massive mortality normally occurs during larval stage because of vulnerability of larvae to predation, starvation, unfavorable environmental conditions and prevailing pathogens (Kamler, 1992; Moyle and Cech, 2003).

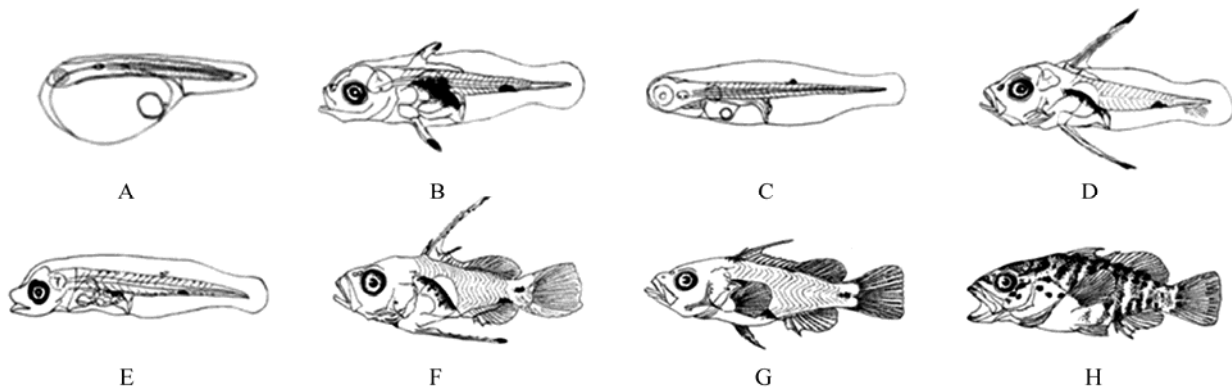


Figure 4 Development of larvae and juveniles of *Epinephelus malabaricus*

Note: A: Newly hatched larvae, 1.92 mm total length (TL); B: two days after hatching, 2.74 mm TL; C: three days after hatching, 2.80 mm TL; D: nine days after hatching, 4.11 mm TL; E: 14 days after hatching, 5.38 mm TL; F: 20 days after hatching, 7.15 mm TL; G: 30 days after hatching, 22.05 mm TL; H: 40 days after hatching, 33.16 mm TL (Leu et al., 2005)

#### 1.2.1 Feeding and temperature

Like most marine finfish, heavy mortalities that occur in early-stage of grouper larvae have been considered to be related to the initial feeding stage (Blaxter and Hempel, 1963a; Kohno et al., 1997; Kohno, 1998). The timing to supply feed with appropriate nutritional composition is a key consideration in marine larval fish culture (Cahu and Infante, 2001; Koven et al., 2001). After yolk sac is depleted, fish larvae rely on food from exogenous sources (Shan et al., 2008). At this point, a delay of live food supply can result in low survival, slow development and alimentary tract degeneration (Heming et al., 1982; Chen et al., 2007; Yoseda et al., 2006). Furthermore, if larvae cannot access suitable food for an extended period (defined as Point of no return by Blaxter and Hempel (1963) PNR) after yolk sac depletion, they may lose the ability for food ingestion and digestion (Blaxter and Hempel, 1963b;

Kamler, 1992). During onset of exogenous feeding, fish mortality is likely to occur if the provision of first feeding is beyond the PNR (Blaxter and Hempel, 1963b). Therefore, the time at first feeding and live food provision is crucial for the growth and survival of postal larvae.

The PNR is closely related to temperature, as low temperature prolongs the time for larvae to reach the PNR and high temperature shows the opposite effect (Dou et al., 2005; Blaxter and Hempel, 1963b; Yin and Blaxter, 1987). Dou et al. (2005) suggested that the high temperature shortens the period for the first feeding larvae to learn ingesting food before the onset of irreversible starvation is a cause for mortality. Similar result also has been found by Ma et al. (unpublished), they found higher temperatures reduced the time for yellowtail kingfish larvae to

reach PNR, thus fish at 25 °C and 27 °C had less time to establish their feeding capability than at 21 °C and 23 °C. This may explain why massive mortality occurred earlier at high temperature than at low temperature. In grouper larvae such Malabar grouper (*E. malabaricus*) are very vulnerable to starvation, the feeding windows for *E. malabaricus* cultured at 28 °C are only 24 h after mouth opening (Yoseda et al., 2006). In order to increase survival and reduce starvation, it is necessary to reduce the culture temperature within the first feeding stage as evidence from previous studies indicate that lower temperature can delay exhaustion of yolk reserve and starvation in other species (Dou et al., 2005; Blaxter and Hempel, 1963b; Yin and Blaxter, 1987). After compared the hatching success and survival rate in a previous study, Watanabe et al. (1995) suggest that a lower temperature may be advantageous to higher temperature for incubating eggs and for rearing first feeding *E. striatus* when prey concentrations are limiting. Similarly, an operating protocol has been proposed by Ma et al. (unpublished) that use low temperature at the initial feeding stage to improve survival and increased temperature to promote feeding and growth in later stage in yellowtail kingfish larvae culture.

### 1.2.2 Light intensity and tank color

Light intensity and tank color in the rearing tank may affect the successful grouper larvae feeding as most of marine fish larvae are visual feeders, and light plays an important role in the foraging behavior of fish larvae (Monk et al., 2008). Previous study in *E. suillus* indicate that no significant preference between black and tan color regarding to the food intake and growth (Duray et al., 1996). However, more and more evidence indicates that tank color preference is more species dependent. For example research in species such as herring and turbot indicate that black wall tanks give a good contrast between food and background (Blaxter, 1968; Howell, 1979), while haddock (*Melanogrammus aeglefinus*) larvae did not grow and survive well in the tank coated with black wall with low light intensity (Downing and Litvak, 1999). More recent study in juvenile barramundi (*Lates calcarifer*) indicate that color preference changed as an effect of the ambient light environment (Ullmann et al., 2011). Therefore research should looking into both factor may improve the feeding rate of fish larvae. Surprisingly, little information can be found in

literature regarding to grouper larvae preference. Therefore, future research should also towards to obtain the optimal tank color and ambient light environment.

### 1.2.3 Water surface death

In the larval culture of several marine teleosts, large numbers of dead larvae are often observed on the water surface around the time of first feeding (Kaji et al., 2004). The cause of surface death has been considered as a consequence of being trapped on the water surface by the water surface tension when body surface is exposed to the air (Kawabe and Kimura, 2007; 2008). Surface death is a heavy mortality factor in yolk-sac grouper larvae such as *E. akaara* (Kaji et al., 1995; Yamaoka et al., 2000), *E. septemfasciatus* (Tsuchihashi et al., 2003), *E. bruneus* (Sawada et al., 1999), *E. fasciatus* (Kawabe and Kohno, 2009). In order to overcome this problem, the addition of an oil film on the water surface is normally used to reduce water surface tension and prevent the occurrence of mass surface deaths of fish larvae (Yamaoka et al., 2000; Kawabe and Kohno, 2009). However, evidence indicates that the outcome of using oil film to prevent surface death varied among hatchery and the removal of oil film from rearing tank was difficult (Yamaoka, 2001). As an alternative substitute, egg white has been used to prevent surface death (Kaji et al., 2003).

## 2 Feeding Protocol and Live Feeds

### 2.1 Feeding Protocol

At hatching, the size and mouth are very small in groupers such as *E. coiodes*, *E. malabaricus*, *E. fuscoguttatus*, *E. suillus* and *C. altivelis* (Table 1). The mouth gape at first feeding of *E. suillus* was about 150-180 µm (Maneewong et al., 1986), and was about 250-300 µm in *E. marginatus* (Glamuzina et al., 1998). As the prey selection criteria are more by size than by taste or other senses (Yufera and Darias, 2007; Fernandez-Diaz et al., 1994; Planas and Cunha, 1999), the small mouth gape in early grouper larvae period limits the choice of initial live food to minute zooplankton such as copepod nauplii (Marte, 2003), eggs and trochophore larvae of mussel or oyster (Lim, 1993; Doi et al., 1991). Figure 5 shows a feeding management plan for *Epinephelus fasciatus*. Not like feeding protocol used in other marine fish larvae, the rotifer and *Aretmia* nauplii feeding periods in the grouper species such as *E. tauvia*, *E. fuscoguttatus*, *E.*

*fasciatus* and *E. suillus* are relative long (30-35 days) (Lim, 1993; Duray et al., 1997; Kawabe and Kohno, 2009). The cause of this kind long feeding period in grouper larvae may relate to the small size of body and narrow mouth gap at hatching (Table 1). In order to improve survival rates during the first feeding period, some small zooplanktons such as copepod nauplii, egg and trochophore of bivalve have also fed to larval grouper along with rotifers (Marte, 2003; Lim, 1993).

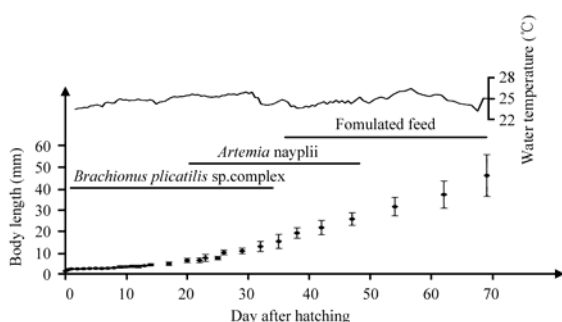


Figure 5 Body length, diet, and water temperature during the rearing of *Epinephelus fasciatus*. Vertical lines indicate mean  $\pm$  SD (Kawabe and Kohno, 2009)

## 2.2 Live Feeds

The production of live feeds with small size characteristics is an important hatchery operation in grouper breeding (Lim, 1993). Since groupers have extremely small mouth gap, under some particular situation, rotifer may not fulfill the size of requirement for the first feeding grouper larvae. Therefore, lots of efforts have been to find the suitable first feeding feeds for grouper live (Russo et al., 2009; Doi et al., 1997; Reyes et al., 2011).

### 2.2.1 Eggs and trochophore of bivalve

Eggs and trochophore of bivalves such as mussels and oyster have been widely used as first feeding live foods for grouper larvae in the past because of the size advantage. For instance the, size of eggs and trochophore of green mussel *Perna viridis* are 55-60  $\mu$ m and 60-80  $\mu$ m respectively (Lim, 1993), while the egg size of Pacific oyster is about 45-62  $\mu$ m (Pauley et al., 1988). The most efficient way to spawns oyster and mussels is temperature manipulation induction. In our recent study, eggs and larvae of *Pinctada martensi* were used to feed the newly hatched *Plectropomus leopardus* larvae, on 5 day post hatching, the feeding rate reached to 45% (unpublished data).

The spawning of mussels is normally induced through physical, chemical, or biological methods. Because of the efficiency and easy operation, “Temperature shock” is one of the most frequently used physical methods in inducing spawns of green mussels. When applied “temperature shock”, the mussels were immersed in warm seawater which the water temperature was 4-5°C higher than the rearing temperature, then followed by cold seawater treatment which the water temperature was 4-5°C lower than the rearing temperature. Upon complete the induction, the mussels were placed into spawning tank (50L~150L), and the spawning activity normally occurred within 1 h. In order to minimize pollution of seawater caused by deterioration of the milt, male mussels were removed immediately after milt production (Lim, 1993). Fertilized eggs were demersal eggs which can be collected by siphoning the bottom of the tank. After collection, eggs were washed via 20  $\mu$ m screen to eliminate as much as milt before feeding to the larval grouper.

Similar to the method used in mussels, pacific oyster can also be induced by temperature cycling. Temperature cycling method involves placing sexually matured oysters in water at a certain temperature then slowly increasing the temperature over a period of time. For instance, when applied this method to induce oyster, the oysters were exposed to 20 °C, then every 5 minutes increased 1 °C until the water temperature reached to 28 °C (PIRSA, [http://www.pirsa.gov.au/aquaculture/aquaculture\\_industry/oysters](http://www.pirsa.gov.au/aquaculture/aquaculture_industry/oysters)). Apart from temperature induction, eggs and milt can also be obtained directly from sexually matured oysters by dissecting the gonads. Currently, commercial production of trochophore oyster is also available in the market, for example “TrochoFeed” which is actually frozen oyster trochophore-stage Pacific oyster. The products are preserved in liquid nitrogen and can be used directly after thrown.

In practice, grouper larvae are normally feed on eggs and trochophore-stage bivalve for two days during the first feeding period (Rimmer, 2000). A feeding study indicates that a combination of oyster trochophore and small rotifers (either SS-strain, sieved S-strain, or neonates) is the best initial feed (Su et al., 1997; Doi et al., 1997). Although evidence indicates that the combination feeding protocol can improve the larval

performance of first feeding grouper, several issues remain unsolved. For instance, water quality can be easily deteriorated when the un-proper treated eggs and trochophore are in used. Furthermore, how to control the development speed of eggs and trochophore is critical as once they are in the D shape stage, fish larvae cannot digest it. Therefore careful feeding management is important once this kind of live feeds were introduced to the larvae rearing tank.

### 2.2.2 Rotifers

S-type and SS- type of *Brachionus* sp. are the major rotifers used as live feeds for grouper larvae. As the S-type rotifers (*Brachionus rotundiformis*) are too large for newly hatched grouper to ingest, SS-type rotifers (*Brachionus* sp), or S-type rotifers screened to <90  $\mu\text{m}$ , are suitable for grouper larvae at first feed (Duray et al., 1997; Su et al., 1997; Watanabe et al., 1996). Optimal prey density during the early larval stages is 10-20 organisms/mL (Ruangpanit et al., 1993; Tamaru et al., 1995)

Rotifers as the major live feeds for marine fish larvae normally contain low levels of polyunsaturated fatty acid (PUFA) content (Conceição et al., 2010). Evidences indicated that the lack of PUFA contents in rotifers has led to fish slow growth, mass mortality, mal-pigmentation, and deformity (Takeuchi et al., 1998; Avella et al., 2007; Olivotto et al., 2006). Previous study find that the eggs of grouper such as *E. coioides* contained high level of DHA, EPA, and ARA suggesting their important in larval development (Alava et al., 2004). However, the content of EFA such as eicosapentaenoic acid 20:5n-3 (EPA), docosahexaenoic acid 22:6n-3 (DHA) and arachidonic acid 20:4n-6 (ARA) in rotifers are relatively low when compared to other live food (e.g., copepods) in nature (Sargent et al., 1999; Conceição et al., 2010). Therefore, enrichment of rotifers with liquid emulsions containing EFA is necessary to improve its quality as fish food (Rainuzzo et al., 1997).

### 2.2.3 Copepods nauplii

In natural, copepods nauplii are the major food for marine fish larvae. Due to the size advantage (smaller than SS-strain rotifers) and superior nutritional value to rotifers (McKinnon et al., 2003), copepods have been widely used in larval grouper rearing in Asian. The unique characters of copepods nauplii make it

becoming a more valuable live feed in the rearing of grouper larvae. Apart from size advantage, copepods contain higher essential fatty acids for fish larvae than any other live feeds such as rotifers and *Artemia* nauplii (Evjemo et al., 2003; Støttrup and McEvoy, 2003). Furthermore, the nutritional profiles of copepods fulfill the nutrient requirement of fish larvae, especially the content and ratio of PUFA, DHA, EPA, and ARA (Venizelos and Benetti, 1999; Bell et al., 2003; Nanton and Castell, 1998). Up to present, copepod culture in intensive indoor and outdoor systems has been successfully developed, however, due to the technical constraints in rearing copepods, its mass production at a commercial scale has not been achieved (Støttrup, 2000; Hagiwara et al., 2001).

### 2.2.4 Artemia nauplii

*Artemia* is another common used live food following the rotifers for larval grouper rearing. Nauplii instar I and II are the most widely used forms of *Artemia* in aquaculture. In China, adult *Artemia* was also used as a food source for those fish not weaned to artificial diets. Due to the lack of EPA and DHA, it is necessary to enrich *Artemia* nauplii with oil emulsions before feeding fish larvae (Ma et al., 2012). Oil emulsions are general composed of fish oil and fatty acids ethyl esters, providing essential fatty acids to fish larvae in neutral lipid classes (Monroig et al., 2006). Because of the character of inherent catabolism, enriched nutritional components can also be absorbed by *Artemia* nauplii after enrichment resulting in great variation of nutrient contents (Naz, 2008; Triantaphyllidis et al., 1995). Thus, timely harvesting and storage of enriched *Artemia*, and quick feeding to fish are recommended to minimize nutrition loss after enrichment (Ma et al., 2012).

## 3 Summary

The grouper aquaculture industry is expanding at a fast rate, and there is great potential for this industry to be further expanded. A number of key progresses have been achieved in the past few decades, and there have been significant achievements in knowledge of grouper rearing. Progress in grouper larval culture in particular has been rapid over the past decade. In order to improve the production efficiency, future research should towards (1) the understanding of timing of live food delivery to first-feeding grouper larvae, (2) the nutritional requirements of first-feeding larvae and of

fish weaning from live food to formulated diet, (3) the environmental requirements, especially temperature, that are suitable for fish larval survival and growth.

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