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Some Hatchery Parameters of Three Species of Groupers: Tiger Grouper (*Epinephelus fuscoguttatus*), Humpback Grouper (*Cromileptes altivelis*), and Leopard Coral Grouper (*Plectropomus leopardus*)

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ABSTRACT

Three species of groupers, including tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*), have been successfully cultivated. However, the constraints on larviculture that have been described still arise in the hatchery. This study compares several hatchery parameters in these three grouper species representing three different genera (*Epinephelus*, *Cromileptes*, and *Plectropomus*). The egg incubation and larval rearing were carried out in a concrete tank (3 x 3 x 1.2 m^3) filled with 6-7 m^3 seawater. Eggs were stocked with a density of 100,000 eggs/tank. The grouper larvae were reared until they became juvenile. The eggs were incubated until they were hatched in the same container and media as larval rearing. Larvae were reared until 60 dah (days after hatching), and data were taken from the rearing for three cycle's larval culture. Management of grouper larval rearing follows the Good Fish Hatchery Method (Cara Pembenihan Ikan yang Baik/CPIB) based on Indonesian National Standard (Standar Nasional Indonesia/SNI). The experimental parameters were egg diameter, hatching rate, larvae mouth opening width, survival activity index (SAI), larvae development, and total body length growth. The several hatchery parameters in three grouper fish species are significantly different. The tiger grouper has the best larval performance.

1. Introduction

Groupers are highly valued mariculture fish, mainly when sold alive (Ismi and Budi 2020), and one of the leading live reef fish trades (Halim 2001). The grouper aquaculture industry has rocketed in the recent decade, and many grouper species have been farmed widely in China and South-East Asian countries (Guo *et al.* 2015; Pierre *et al.* 2008). Tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*) are some of the grouper species that have high demand and prices in the market (Rimmer and Glamuzina 2019), and belong to the 11 most commonly traded grouper species (Halim 2001). The fry of the three grouper species has been successfully mass-produced in Indonesian hatcheries (Halim 2001; Pierre *et al.* 2008; Rimmer and Glamuzina 2019).

Reliable fry production is still an obstacle in developing the grouper farming industry (Ma *et al.* 2013). Successful grouper's larviculture has been restricted by generally poor and irregular survival (Rimmer 2000). The main limitations for successful larviculture are the small oral opening of the larvae and, therefore, the need for small prey on the first diet, and the presence of high mortality at different stages during the larval rearing process (Kohno *et al.* 1993, 1997; Peixoto *et al.* 2004; Rimmer *et al.* 1999; Seng 1998; Tamaru *et al.* 1995).

Although all three species of groupers (tiger, humpback, and leopard coral grouper) have been successfully cultivated, the constraints on larviculture

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that have been described still arise in the hatchery. This study compares several hatchery parameters in these three grouper species representing three different genera (*Epinephelus, Cromileptes*, and *Plectropomus*).

2. Materials and Methods

2.1. Larval Rearing

This study was conducted in 2019 on CV Jaya Utama Abadi (Kalianget Village, Banyuglugur District, Situbondo Regency, East Java, Indonesia) and Institute for Mariculture Research and Fisheries Extension (Gondol, Buleleng Regency, Bali, Indonesia). Tiger grouper eggs were obtained from the breeding facility of CV. Utama Jaya Abadi, humpback from Ambon Marine Cultivation Fishery Centre (Maluku, Indonesia), and leopard coral grouper from the Institute for Mariculture Research and Fisheries Extension (Gondol, Bali, Indonesia).

The egg incubation and larval rearing were carried out in a concrete tank (3 x 3 x 1.2 m^3) filled with 6-7 m^3 seawater. Eggs were stocked with a density of 100,000 eggs/tank. The grouper larvae were reared until they became juvenile. The eggs were incubated until they were hatched in the same container and media as larval rearing. Larvae were reared until 60 dah (days after hatching), and data were taken from the rearing for three cycle's larval culture. Management of grouper larval rearing follows the Good Fish Hatchery Method (Cara Pembenihan Ikan yang Baik/CPIB) based on Indonesian National Standard (Standar Nasional Indonesia/SNI) (SNI 8035:2014) (Badan Standarisasi Nasional 2014) and follows the existing guidelines (Sugama *et al.* 2012).

After the larvae reach one dah, the water surface is dripped with fish oil as much as 0.1 ml/m^2 in the morning and evening until five dah. Fish oil dripped on the surface of the water close to the aeration point will spread to cover the surface of the water so that the larvae do not die much trapped on the surface due to the entanglement of surface layer cohesion (Ismi *et al.* 2004, 2016).

The media was green by giving *Nannochloropsis* sp. with a density of 300-500 x 10^3 cells/ml starting from two dah. Rotifers were given as feed when the larvae started to open their mouths at three days (Ismi *et al.* 2012). Copepod naupli were only given to leopard coral grouper larvae. Artemia, artificial feed, and small prawns were first fed to tiger grouper larvae, then humpback and leopard coral grouper. Complete feeding management during larval rearing is presented in Table 1.

Table 1. Feeding management for raising tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*) larvae

Feeding management	Age of larvae (dah)													
	2	3	4	7	9	10	11	12	20	30	35	40	50	60
Nannochloropsis sp.														
Tiger grouper														
Humpback grouper														
Leopard coral grouper														
Rotifer														
Tiger grouper														
Humpback grouper														
Leopard coral grouper														
Copepod naupli														
Leopard coral grouper														
Artemia														
Tiger grouper														
Humpback grouper														
Leopard coral grouper														
Artificial feed														
Tiger grouper														
Humpback grouper														
Leopard coral grouper														
Small shrimp														
Tiger grouper														
Humpback grouper														
Leopard coral grouper														

Management of the quality of the maintenance media was carried out by adding plankton and adding seawater slowly with a percentage adjusted for the age of the larvae. Tank bottom tapping was carried out to remove leftover feed and manure sediment starting at 12 dah. Water management and tank bottom cleaning during larval rearing are presented in Table 2. Measured water quality parameters during the study can be seen in Table 3.

2.2. Parameters Observed

The parameters observed were egg diameter, hatching rate, larvae mouth opening width, survival activity index (SAI), development of larvae, and growth in total body length. A total of 20 eggs were observed under a light microscope connected to a video monitor (60×) to measure the diameters of the eggs one by one. The diameters of the ovoid shape of the egg were calculated using the formula $\sqrt{(D \times d)}$. Where D represents the larger section and d represents the smaller section of the egg (Budi *et al.* 2020). The hatching rate was measured after 20 hours approximately (at an incubation temperature of 28-30°C) using the formula (TE/TL) x 100%, where TE is the total of eggs, and TL is the total of larvae. Measurements of larvae mouth opening width in one dah under a microscope (magnification 60x, n = 20 fish) and calculating with the equation $\sqrt{(2AB)}$, where AB represents upper jaw length (Tang *et al.* 2020). One day after hatching, 100 larvae were transferred into 1,000 ml beaker glass, left undisturbed, and starved until they died to determine SAI. The following formula calculated SAI $\frac{1}{2}\sum_{k}^{i=1}(N - hi) \times i$, where N is the total number of larvae, *hi* is the cumulative mortality by the ith day, and k is the number of days elapsed until all larvae were dead because of starvation (showed as survival times in Table 4.) (Forsatkar *et al.* 2017). Development of larvae was observed in 1, 3, 5, 10, 20, 30, 40, 50, and 60 dah, and the growth of total body length was measured every five days starting from one dah.

Table 2. Water management and tank bottom cleaning during larval of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*) rearing

Water management	Age of larvae (dah)									
	1	2	6	12	18	24	30	40	50	60
Water addition										
±10% per day (plankton+seawater)										
±20% per day										
±50% per day										
water circulation ±100% per day (flow trough)										
Cleaning the tank bottom										

Table 3. Water quality parameters of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*) and leopard coral grouper (*Plectropomus leopardus*) larval rearing

Parameters	Tiger grouper	Humpback grouper	Leopard coral grouper
Temperature (°C)	28.7-30.7	28.8-30.2	28.6-30.3
Salinity (ppt)	33.0-34.0	33.0-34.0	33.0-34.0
pH	7.9-8.2	7.6-8.1	7.8-8.1
Dissolved oxygen (mg/l)	4.3-6.5	4.5-6.1	4.4-6.2

Table 4. Egg diameter, the total length of hatched larvae, mouth opening width of larvae of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*) and leopard coral grouper (*Plectropomus leopardus*) (n = 20)

Groupers	Rearing cycle	Parameters		
		Egg diameter (µm)	The total length of hatched larvae (mm)	Mouth opening width of larvae (µm)
Tiger grouper	1	0.819	2.491	0.154
	2	0.821	2.489	0.156
	3	0.817	2.492	0.155
	Mean ± SD	0.819±0.032	2.491±0.211	0.155±0.020
Humpback grouper	1	0.798	2.315	0.134
	2	0.793	2.317	0.137
	3	0.797	2.298	0.141
	Mean ± SD	0.796±0.039	2.310±0.141	0.137±0.018
Leopard coral grouper	1	0.721	2.194	0.125
	2	0.719	2.189	0.127
	3	0.723	2.191	0.126
	Mean ± SD	0.721±0.036	2.191±0.173	0.126±0.013

At 3-15 dah, observation of rotifer intake in larvae was carried out every day. Every 08.00 AM, 20 larvae were collected from each tank, measured, dissected, and observed under a microscope for rotifer intake (modified procedure from Duray *et al.* 1996). Rotifers were given adlibitum by maintaining the amount in the culture medium of more than five individuals per ml at 3-7 dah and more than ten individuals per ml at 7-15 days. Every afternoon (04.00 PM), the rotifer density in the culture medium was counted following Kabir *et al.* (2010). Rotifer, egg, and larvae were collected in the tank using 1,000 ml beaker glass at five location points on the tank, 4 points in each corner and 1 point in the center of the tank.

2.3. Data Presentation and Analysis

All observed data and parameters (egg diameter, hatching rate, larvae mouth opening width, survival activity index (SAI), development of larvae, and growth in total body length) were analyzed descriptively in tables, graphs, and pictures.

3. Results

Early life parameters of three grouper species in three cycle hatcheries can see in Table 4. and Table 5. Based on the observed data, the highest egg diameter, the total length of hatched larvae, and mouth opening width of larvae were observed in tiger grouper (Table 4). The relationship between egg diameter, larval size, and larval mouth opening is proportional (Table 4).

The hatching rate (HR) in three grouper species is almost the same value (Table 5). The highest survival times (ST), survival activity index (SAI), and survival rate (SR); were occurred in tiger groupers (Table 5).

The rotifers number in the stomach of three grouper species rearing for 15 dah can see in Figure 1. The highest number of rotifers was observed in the larvae stomach of tiger grouper larvae. The rotifer density in the culture medium tends to decrease with the length of the rearing period. The lowest number of rotifers was observed in the culture medium of tiger grouper larvae (Figure 2).

The growth of three grouper species rearing in 60 days can see in Figure 3. The total length of three grouper species increased proportionally with the length of the rearing period. The highest total length was observed in tiger grouper. A comparison of larval development of three grouper species can be seen in Figure 4.

4. Discussion

A positive relationship between the egg size and the total length at hatching was observed. The larger diameter of the eggs will produce the more significant total body length and mouth opening size of larvae. The size of eggs varies among teleost species in wide ranges (Kamler 2005; Kashiwagi *et al.* 1987; Knutsen and Tilseth 1985). Egg size is directly related to yolk size, where the yolk is the main component of an egg (Kamler 2005). Egg yolk contributes to larval body size (Keckeis *et al.* 2000). Egg size and total larval length at hatching in various fish species are directly proportional (Miller *et al.* 1988). Logically, the larva's mouth opening will get bigger too.

Meanwhile, the hatching rate (HR) observed is almost the same value. The previous study on Japanese whiting (*Sillago janopica*) showed some correlations between egg size and total hatch, but the egg diameter has significant correlations with the percentage of

Table 5. Hatching rate (HR), survival times (ST), survival activity index (SAI) and survival rate (SR) of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*) and leopard coral grouper (*Plectropomus leopardus*) larvae

Groupers	Rearing cycle	Parameters			
		HR %	ST (days)	SAI	SR (%)
Tiger grouper	1	81.05	5.00	2.96	16.17
	2	85.75	6.00	3.98	21.31
	3	81.39	6.00	3.91	14.60
	Mean ± SD	82.73±2.62	5.67±0.58	3.61±0.57	17.36±3.51
Humpback grouper	1	80.91	4.00	2.65	9.45
	2	84.56	5.00	3.45	12.31
	3	86.06	5.00	3.36	12.50
	Mean ± SD	83.84±2.65	4.67±0.58	3.15±0.44	11.42±1.71
Leopard coral grouper	1	80.24	4.00	1.87	6.11
	2	81.39	4.00	1.93	5.32
	3	86.44	4.00	2.17	6.24
	Mean ± SD	82.69±3.30	4.00±0.00	1.99±0.16	5.89±0.50

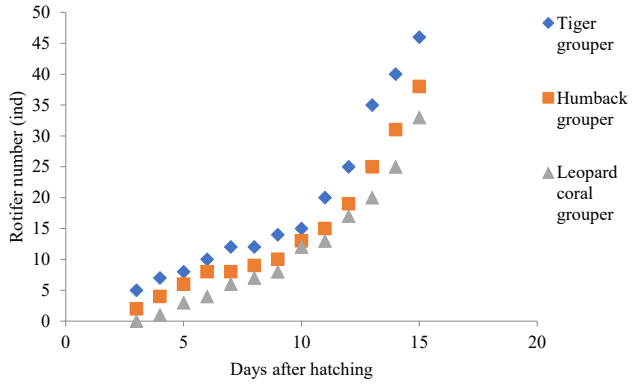

Figure 1. The number of rotifers in the stomach of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*) and leopard coral grouper (*Plectropomus leopardus*) larvae rearing in 15 days after hatching

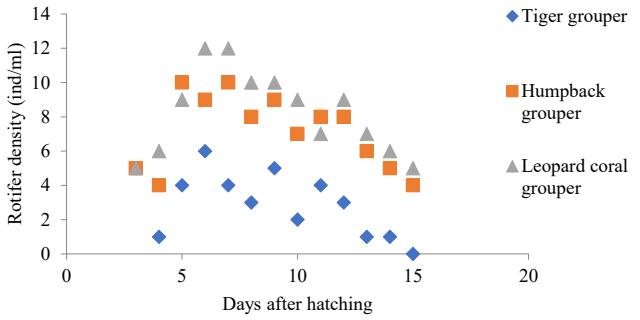

Figure 2. The density of rotifer in the culture medium of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*) and leopard coral grouper (*Plectropomus leopardus*) larvae rearing in 15 days after hatching

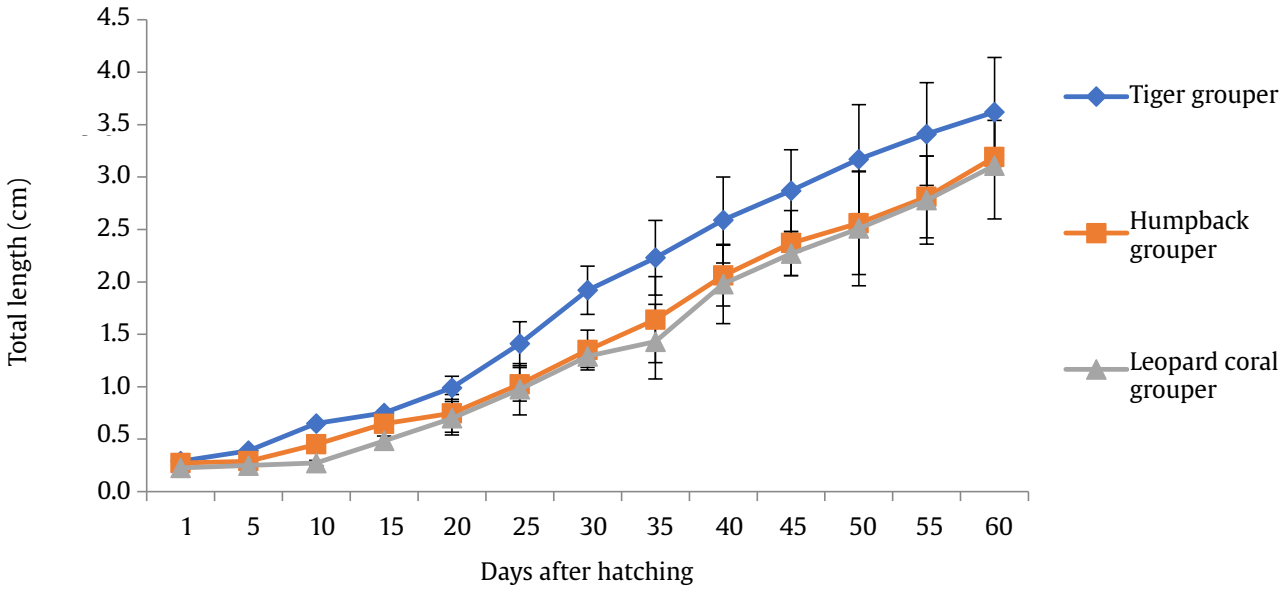

Figure 3. The total length of tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*) larvae rearing in 60 days after hatching

viable hatch (Kashiwagi *et al.* 1987). The same trend in survival times (ST), survival activity index (SAI), and survival rate (SR) was also obtained in Japanese whiting (*Sillago janopica*), where the larger size of eggs produced more viable larvae than smaller eggs (Kashiwagi *et al.* 1987). SAI is defined as an index for larval fish quality because it is expressed as a function of larval tolerance to starvation and is positively connected with larval survival (Matsuo *et al.* 2006). The SAI is a widely used metric for assessing the vitality and quality of larvae in marine fish larviculture (Wang *et al.* 2013). The SAI is linked to nutrition storage (Wang *et al.* 2013) in the yolk and oil globule and egg size (Bengtson *et al.* 1987; Knutsen and Tilseth 1985). In this study, the tiger grouper with the largest egg size produced the highest ST, SAI, and SR, followed by the humpback and leopard coral grouper with smaller egg sizes.

The difference in total length and mouth opening of hatched larvae affect feeding capability and capacity, expressed as the rotifer number in the larvae's stomach. Grouper larvae are initially fed on rotifers (Rimmer 2000). The number of rotifers in the larval stomach increased with the length of the rearing period. The amount of feed that enters and is digested will increase

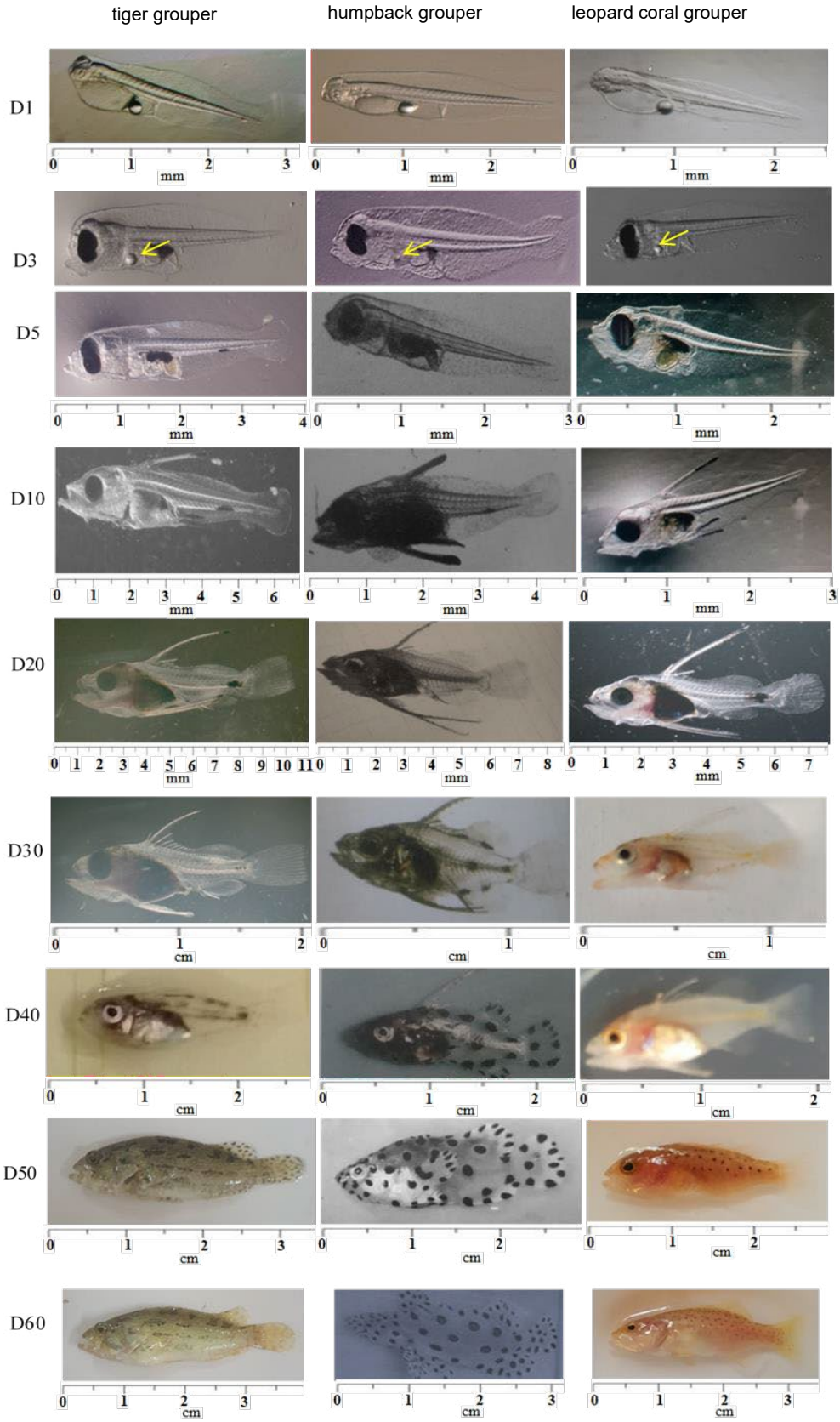

Figure 4. Tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*) larvae and fry develop in 60 days after hatching. The yellow arrow indicated oil globules. D is the day after fertilisation

along with the digestive tract's development, followed by an increase in the production and activity of digestive enzymes (Khoa *et al.* 2021). Besides the rotifers' number in the stomach, feeding capacity is also indicated by the number of rotifers in rearing media, where the rotifers were given adlibitum as a feeding technique. The rotifer density in the culture medium tends to decrease with the length of the rearing period. Commonly, grouper farmers attempt to maintain rotifer density at about 3–4/ml (Rimmer 2000).

The large size of the eggs in the grouper, followed by the high total length and opening mouth of the hatching larvae, and the high SAI and SR values were also followed by the significant growth value. The growth rate of embryos and yolk-feeding larvae, the efficiency with which yolk energy is transferred to body tissues, and the yolk energy content are the critical intrinsic elements contributing to the larval body size obtained from the yolk (Kamler 2005).

All transparent groupers larvae hatched in D1. Hatched larvae still have egg yolk with oil globules in their peritoneal. Larvae hovered in the water column in the rearing tank with the head facing down ±45° from the tail position. In D2, the position of the larva facing down, ±90° from the tail position, and the larvae are mainly in the middle and bottom of the tank so that the larvae are not visible from the surface. The egg yolk has been absorbed and shrinks, and the oil globules. Before the yolk sac is fully absorbed, when the change from endogenous to external nutrition sources occurs, most marine fish larvae acquire a digestive tract, eyes, mouth, and pectoral fins (Yúfera and Darias 2007). In D3, the egg yolks of the three groupers larvae have been wholly absorbed while the oil grains are still slightly left (arrow).

On the other hand, the abdomen has been pigmented, black dots (melanophores) are visible, the eyes are black, and the pectoral fins are growing, actively swimming and looking for food. Grouper larvae have a distinctive colouration that begins to develop early in the larval stage, well before the transition to the juvenile stage (Tucker 1999). Groupers have a distinctive "kite-shaped" body, with the second dorsal-fin spine and pelvic-fin spines dramatically elongating during the larval stage and disappearing immediately before the juvenile stage (Heemstra and Randall 1993; Tucker 1999). Between D4-D5, the three groupers' larvae pectoral and dorsal fins begin to grow marked with minor bumps and continue to elongate. In D7, the dorsal and pectoral fins turn into spines; larvae begin to school in the corners and edges of the rearing tank.

Furthermore, in D8, the tip of the spines began to appear black and increasingly elongated, the larvae movement was more agile in D10, and the larvae spread. Until D20, the larvae continue to grow, but there is no change or performance development. The dorsal and pectoral fin spines continue to elongate, and more active movement. From D21 until D30, the tiger's larval body and humpback grouper have begun to colour, especially the dark head, and the dorsal and pectoral spines begin to thicken and shorten. Fins start to grow from the dorsal to the top and bottom caudal. The larvae have resembled fish, although the dorsal and pectoral spines are still visible and are not fully finned. The colour of round black spots (polka dots) begins to grow from the bottom of the abdomen and then, with increasing age, spreads throughout the body of the humpback grouper larvae from D20 to D30, called Polkadot grouper (Ismi *et al.* 2004, 2016).

Meanwhile, at D30, the red colour of leopard coral grouper larvae began to appear, and the tiger grouper began to have brownish spots spread throughout the body. Some tiger grouper larvae had metamorphosed into small fish/juvenile forms. After 35 days, the larvae begin to develop into juveniles which are characterized by the absence of spines on the dorsal and pectoral. At D40, the tiger grouper larvae have become complete juveniles.

The larval rearing of all kinds of grouper in this study was done following the Good Fish Hatchery Method (Cara Pembenihan Ikan yang Baik/CPIB) based on Indonesian National Standard (Standar Nasional Indonesia/SNI) (SNI 8035:2014) (Badan Standarisasi Nasional 2014) and followed the existing guidelines (Sugama *et al.* 2012). The assessed water quality was consistent with the conditions of life for grouper larvae, allowing the larvae to grow, develop, and survive.

In conclusion, the several hatchery parameters in 3 grouper species are tiger grouper (*Epinephelus fuscoguttatus*), humpback grouper (*Cromileptes altivelis*), and leopard coral grouper (*Plectropomus leopardus*), clearly different. The tiger grouper has the best larval performance.

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