Protocol development for the improved hatchery propagation of Tiger grouper *Epinephelus fuscoguttatus* (Forsskål, 1775) in Palawan, Philippines

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ABSTRACT

The expanding grouper cage farming in Palawan, Philippines requires stable seed supplies from hatcheries to sustain the demands of fish cage operators. To improve the current hatchery practices for Tiger grouper *Epinephelus fuscoguttatus* (Forsskål, 1775), four experiments were undertaken. The first experiment (E1), involving three different temperatures ranges (T1: 24-26°C; T2: 27-29°C; and T3: 30-32°C) revealed significantly higher hatching rates at 27-32°C. The second experiment (E2) found that survival rates after 38 days from hatching were inversely proportional with density (T1: 3; T2: 5; and T3: 10 larvae L^{-1}). The third experiment (E3) found that the growth and survival of fry raised at three different stocking densities for three weeks (from 21 to 42 days after hatching) were not significantly different. The fourth experiment (E4) compared the growth and survival of fingerlings (from 70 – 91 days after hatching) fed with two brands of commercial feeds. After three weeks, both treatments had comparable total lengths. These desirable results are attributed to the weekly thinning or reduction in the density of juveniles in E3 and E4 and the use of small rearing containers.

Keywords: aquaculture, hatchery production, survival, growth, diet, stocking density, hatching rate.

INTRODUCTION

In Southeast Asia, the main target reef species are groupers (subfamily *Epinephelinae*), which are mainly destined for international live reef-fish trade. The large demand and high price for groupers have led to severe overfishing. Even more worryingly, to obtain their catch alive, fishers often employ cyanide to temporarily immobilize their catch (Wilcox 2016). The use of cyanide has a deleterious impact on the reef, including the health and productivity of other reef-dwelling organisms (Halim 2002).

To bring a halt to the overfishing and destruction of coral reefs across Southeast Asia, there is a need to provide alternative livelihoods to fishermen (Heeger et al. 2001). In addition, ensuring future food security and inclusive development in rural areas. The sustainable aquaculture of high-value marine finfish is often identified as a potential solution that meets these requirements (Haylor et al. 2003).

In Palawan, a local non-government organization (NGO) has been pioneering the hatchery production of different grouper species to support the livelihood of coastal inhabitants. To further refine its hatchery protocols, the organization teamed up with the Western Philippines University (WPU) to optimize the production and expand the volume and diversity of hatchery produced fingerlings.

One of the focal species for this joined research project was the Tiger grouper *Epinephelus fuscoguttatus* (Forsskål, 1775), locally known as "Lapulapu Baboy" or Kugtong Baboy". This reef-dwelling species was selected because of its high demand in the local and international markets. This fish grows relatively fast reaching 500 g in nine to 10 months. However, this fast growth rate is accompanied by severe cannibalism, which can lower survival rates (SR). Therefore, improved hatchery and nursery protocols need to be established to support future grow-out culture in coastal areas. This paper included four studies which dealt with the following: 1) hatching rates (HR) at different temperatures, 2) larval SR at different stocking densities, 3) growth and survival of fry, and 4) growth and survival of fingerlings subjected to weekly reduction in densities.

METHODS

Egg Collection

Eggs were collected from 15 *E. fuscoguttatus* breeders (each weighing 15 to 20 kg) from floating sea cages (4 x 4 x 7 m) by the Bureau of Fisheries and Aquatic Resources (BFAR) – Inland Sea Ranching Station for over 5 years along the Sta. Lucia cove of Puerto Princesa Bay. The upper half of the inner circumference of the cage holding the breeders (~20 individuals) were lined with fine-meshed net to retain any floating eggs inside the cage. The breeders were monitored between 2300 and 0100 hours on the night of new moon and up to three consecutive nights thereafter. When spawning occurred, eggs were collected with a fine meshed net 10 minutes after the main spawning activity to ensure proper fertilization. Eggs were then transported from the broodstock cage to the land-based hatchery using 10-L buckets with gentle aeration.

Egg Cleaning and Incubation

Newly collected eggs were first rinsed with fresh seawater to remove algae and other foreign materials. After this, the eggs were rinsed in water with an iodine solution for 1 min. Viable floating eggs were separated from the non-viable suspended eggs. Then the eggs were incubated in conical 50-L incubators with an upwelling flow-through system. Eggs typically hatched between 24 to 28 hours after spawning. Undeveloped eggs and other debris which sank at the bottom of the tanks were regularly removed by opening the bottom drain valve of the conical tank. The produced larvae were used in E2, E3 and E4, respectively.

Experiment 1 (E1): Hatching rates of *E. fuscoguttatus* Eggs at Different Temperature Regimes

In this experiment the HR at three temperature regimes (3 replications), T1: 24-26°C; T2: 27-29°C; and T3: 30-32°C were evaluated for a 24-hour period in nine 15-L capacity cylindrical plastic containers. For each treatment-replicate, 12,000 newly collected eggs were stocked in each cylindrical container (Table 1). Seawater ice (in double-layered plastic bags) and water heaters were used to maintain the desired temperature ranges. The temperature was monitored every five hours, and additional ice were added when necessary. After 24 hours, subsamples from each treatment-replicate were taken to determine the ratio between larvae and un-hatched eggs.

Table 1. Density of tiger grouper *Epinephelus fuscoguttatus* eggs subjected to different temperature ranges for 24 h. T-treatment; R-replicate.

<table>
<tr><th>T</th><th>R</th><th>Temperature Range (°C)</th><th>Duration (h)</th><th>Egg Density/Container</th><th>Water Volume (L)</th></tr>
<tr><td>1</td><td rowspan="3">3</td><td>24-26</td><td rowspan="3">24</td><td rowspan="3">12,000</td><td rowspan="3">15</td></tr>
<tr><td>2</td><td>27-29</td></tr>
<tr><td>3</td><td>30-32</td></tr>
</table>

Experiment 2 (E2): Larval Rearing of *E. fuscoguttatus* at Different Stocking Densities

For this experiment the SR of newly hatched fry was monitored at three different stocking densities (individuals per liter or ind L^{-1}) or treatments (T1: 3 ind L^{-1}; T2: 5 ind L^{-1}; and T3: 10 ind L^{-1}) with three replications. This was carried out for 38 days (from the first day after hatching or DAH) in nine

5,000-L capacity concrete tanks (Table 2). The larvae were fed with a combination of rotifer, *Artemia* and imported commercial grouper feeds.

Table 2. Density of tiger grouper *Epinephelus fuscoguttatus* larvae at different stocking densities fed with a combination of rotifer, *Artemia* and commercial feed. T-treatment; R-replicate.

T	R	Feeding	Density (ind L^{-1})	Density/Tank	Duration (day)	Tank Volume (L)
1	3	rotifer, *Artemia,* commercial feed	3	15,000	38	5,000
2			5	25,000		
3			10	50,000		

The first feeding in E2 occurred on the 3rd DAH when the mouth of the larvae was large enough to consume rotifers (L type). The rotifer density in the larval rearing tanks was monitored twice a day (0700 and 1500 h) to maintain the desired number and size until the 25th DAH. On the 12th DAH, a pinch of artificial pellet was introduced every hour between 0600 and 1700. *Artemia* were added two times daily (0700 and 1500 h) between 12 DAH and 30 DAH, starting with newly hatched *Artemia*. As the grouper larvae increase in size, larger *Artemia* were fed. *Artemia* were enriched with vitamins for 4 h before feeding to the larvae. Water temperature, salinity, pH and ammonia were monitored in the morning (0800 h) and afternoon (1500 h).

Experiment 3 (E3): Growth and survival of *E. fuscoguttatus* Fry at Different Stocking Densities Fed with Commercial Feed

The experiment was carried out for three weeks in 15 plastic 25-L capacity blue basins to monitor growth and survival for 42-day old juveniles at different stocking densities. Three different stocking densities (treatments)

Table 3. Weekly density of tiger grouper *Epinephelus fuscoguttatus* juvenile at different treatments fed with commercial feed raised in small basin (25-L) for 21 days. T-treatments; R-replicates; W-week; DAH-days after hatching.

T	R	Feeding	Density (ind L^{-1})			Number per Basin			Starting Age (DAH)	Water Volume per Basin (L)
			W1	W2	W3	W1	W2	W3		
1	5	*ad libitum,* 3-5 min every hour from 0700 - 1800	6	4	2	150	100	50	42	25
2			12	8	4	300	200	100		
3			18	12	6	450	300	150		

were reduced each week for a period of three weeks by manually removing the largest and the smallest individuals (Table 3). Each treatment was replicated five times. The juveniles having the following initial average total length (TL): 2.66±0.34 cm (T1); 2.99±0.40 cm (T2); and 2.89±0.60 cm (T3) were fed *ad libitum* throughout the day using imported commercial grouper feed. Wastes that settled on the bottom of the basin were siphoned 2-3 times a day.

Experiment 4 (E4): Growth and Survival of *E. fuscoguttatus* Fingerlings Subjected to Weekly Reduction of Densities and Fed with Two Different Commercial Diets

This experiment monitored the growth and survival of fingerlings fed with two different commercial diets: an imported (T1) and a locally manufactured pelleted feed (T2). The experiment with nine replications was carried out in 18 plastic 25-L blue basins. To maintain uniform size and prevent the occurrence of cannibalism, the densities were manually reduced on a weekly basis (Table 4). The 70-day old fingerlings initially measured 6.07±0.70 cm (T1) and 6.17±0.70 cm (T2), respectively. The fingerlings were fed *ad libitum* throughout the day. The treatments received continuous water exchange at 2 - 4 L hr^{-1} and gentle aeration. Waste was siphoned 2-3 times daily.

Table 4. Weekly density of tiger grouper *Epinephelus fuscoguttatus* fingerlings fed with two commercial feed. T-treatment; R-replicates.

T	R	Feeding	Feed Pellet	Density (ind L^{-1})			Actual Number/Basin			Starting Age (DAH)	Water Volume per Basin (L)
				W1	W2	W3	W1	W2	W3		
1	9	*ad libitum*, 3-5 min every hour from 0700 - 1800	Imported	4	3	2	100	75	50	70	25
2			Local								

Data Analyses

The HR in E1, the SR in E2, E3 and E4, and the growth rates in E4 were all compared separately using analysis of variance and Scheffe post hoc tests. The TL and SR in E4 were compared using T-test. All analyses were performed at 5% significance level using SPSS 19.0 trial version.

RESULTS

Experiment 1 (E1): Hatching Rates of *E. fuscoguttatus* Eggs at Different Temperature Regimes

Treatment 3 had the highest HR (80.32±8.37%) although it was not significantly different (P>0.05) from T2 (75.43±10.64%). The HR (54.66±23.87%) in T1 was significantly lower (P<0.05) compared to the other treatments (Figure 1).

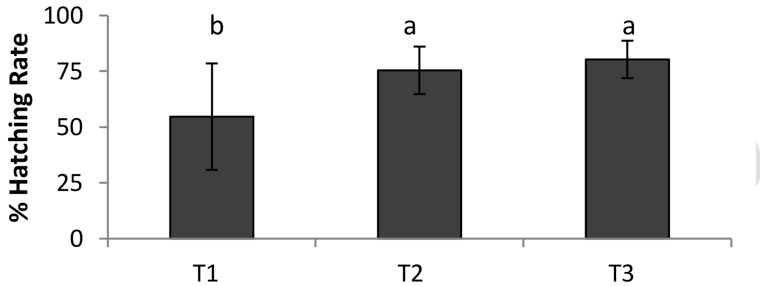

Figure 1. Average (±SD) hatching rates of *Epinephelus fuscogutatus* eggs at three temperature regimes (T1: 24-26^{o}C; T2: 27-29^{o}C; and T3: 30-32^{o}C).

Experiment 2 (E2): Survival of *E. fuscoguttatus* at Different Stocking Densities

The SR was inversely proportional to density (Figure 2) and was significantly different (P<0.05) among treatments. The average (±SD) SR in T1 (6.86 ±0.54%) was significantly higher than in T3 (1.33±1.42%) but not in

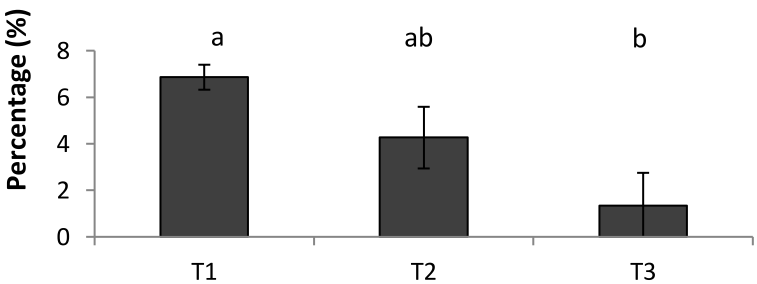

Figure 2. Average (±SD) survival of *Epinephelus fuscoguttatus* fry at three different stocking densities (T1: 3 ind L^{-1}; T2: 5 ind L^{-1}; T3: 10 ind L^{-1}) raised in concrete tanks from day 1 to 38 days after hatching.

T2 (4.26 ±1.32%). At the end of the rearing period, the fry measured 20.7 (± 0.52) mm, an average 18.7 mm TL increment. The range of average water temperature: 28.37°C (±0.58) to 28.71°C (±0.51); salinity: 26.14 (±0.63) to 26.29 (±0.70) ppt; pH: 8.50 (±0.18) to 8.67 (±0.19); and ammonia: 0.43 (±0.16) to 0.45 (±0.15) did not significantly differ among treatments.

Experiment 3 (E3): Growth and Survival of *E. fuscoguttatus* Fry at Different Stocking Densities fed with Commercial Feed

The weekly increase in TL was about 1 cm for all treatments. In week 1, the average (±SD) final TL in T2 (4.03±0.54 cm) was significantly bigger than the other two treatments (Table 5). The SR (Table 6) for the first week were significantly different than the second and third weeks ($P<0.05$). However, SR among treatments were not significantly different ($P>0.05$).

Table 5. Initial and final total length (cm) of *Epinephelus fuscogutatus* at different stocking densities. The same letter superscript (per column) means not significant at 5%. n-number of measured samples per treatment; TL-total length; w–week.

Treatment	Weekly Average (±SD) Total Lengths (cm)					
	W1		W2		W3	
	Initial TL (cm)	Final TL (cm)	Initial TL (cm)	Final TL (cm)	Initial TL (cm)	Final TL (cm)
1 (n=50)	2.65^{a} (±0.34)	3.70^{a} (±0.49)	4.39^{a} (±0.35)	5.38^{a} (±0.37)	5.86^{a} (±0.24)	6.94^{a} (±0.30)
2 (n=50)	2.98^{b} (±0.40)	4.03^{b} (±0.54)	4.35^{a} (±0.41)	5.33^{a} (±0.38)	5.84^{a} (±0.31)	6.92^{a} (±0.34)
3 (n=50)	2.89^{b} (±0.60)	3.64^{a} (±0.44)	4.33^{a} (±0.45)	5.22^{a} (±0.42)	5.92^{a} (±0.32)	6.92^{a} (±0.42)

Table 6. Weekly survival rates (%) of *Epinephelus fuscogutatus* at different stocking densities. T-treatment; w-week.

T	Weekly Survival Rates (%)		
	W1	W2	W3
1	95.73	99.20	100.00
2	94.47	99.40	99.20
3	94.84	98.73	99.73

Experiment 4 (E4): Growth and Survival of *E. fuscoguttatus* Fingerlings Fed with Different Commercial Feeds

The weekly increase in TL was less than 1 cm for both treatments. Those fed with imported commercial feed (T1) were larger on the third week and were significantly different ($P<0.05$) to the other treatment (Table 7). Survival rates (Table 8) varied between 91.67% and 99.78% and were not significantly different between treatments ($P>0.05$).

Table 7. Initial and final average (±sd) total lengths (cm) of juvenile *Epinephelus fuscoguttatus* fed with two brands of commercial grouper feed. T-treatment; n-number of samples; w-week. TL-total length.

T	**Weekly Average (±sd) Total Lengths (cm)**					
	W1		**W2**		**W3**	
	Initial TL (cm)	Final TL (cm)	Initial TL (cm)	Final TL (cm)	Initial TL (cm)	Final TL (cm)
1 (n=90)	6.07 (±0.70)	6.69 (±0.65)	7.03 (±0.72)	7.47 (±0.66)	7.67 (±0.47)	8.51 (±0.61)
2 (n=90)	6.17 (±0.70)	6.62 (±0.63)	7.09 (±0.64)	7.41 (±0.69)	7.50 (±0.71)	8.04 (±0.53)
remarks	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05

Table 8. Survival rates of *Epinephelus fuscoguttatus* fed with two different commercial feed. w-week.

Treatment	**Weekly Survival (%)**		
	W1	**W2**	**W3**
1	91.67	98.22	99.78
2	92.78	96.74	99.11

DISCUSSION

Experiment 1 (E1): Hatching Rates of *E. fuscoguttatus* Eggs at Different Temperature Regimes

The average HR for *E. fuscoguttatus* in T2 (75.43%) and T3 (80.32%) were comparable to the following reported HR for other grouper species: 83±10.12% for *Epinephelus polyphekadion* (James et al. 1997), 84.3% for *Epinephelus akaara* (Okumura et al. 2002), and 75.7% for *E. malabaricus* (Ruangpanit et al. 1993). The high HR in T3 and T2 showed a large temperature range (27°C and 32°C) for incubating *E. fuscoguttatus* eggs. This large range is comparable to that of *E. coioides* (Kawahara et al. 1997;

Fouroofghifard et al. 2012; Table 9). Ideal temperature for fertilization and hatching for other species may be narrow (28 and 30°C) as for the case of *Heterobranchus bidorsalis* (Okunsebor et al. 2015). The observed wide range for *E. fuscoguttatus* is therefore an advantage when breeding the species.

Table 9. The hatching rates of *Epinephelus fuscoguttatus* at three different temperature ranges compared with similar studies. T-treatment.

Species	T (°C)	Stocking density/ Liter	Culture System	Duration (h)	Hatching Rate (%)	Source
Epinephelus fuscoguttatus	24-26	800	Cylindrical plastic containers (15 L)	24	54.66	This study
	27-29				75.43	
	30-32				80.32	
Epinephelus coioides	22	428.6	Beaker (7 L)	33	2.1 - 93.2	Kawahara et al. 1997
	24				87.2 - 96.5	
	26				86.4 - 98.3	
	28				85.1 - 98.2	
	30				67.3 - 93.8	
	32				45.7 - 84.8	
	34				1.2 - 9.6	
Epinephelus polyphekadion	29	300 – 500	Fiber glass tanks (2,000 to 2,800 L)	19	83	James et al. 1997
Epinephelus akaara					84.3	Okumura et al. 2002
Epinephelus malabaricus	28.15	1,500-1,600	Flow-through Pan-like tanks (30 L)	24	75.7	Ruangpanit et al. 1993
Epinephelus coioides	23 – 24	25	Polyethylene tanks (300 L)	24	0	Fourooghifard et al. 2012
	26 -27				23	
	28-29				75	
	31-32				23	

Experiment 2 (E2): Larval Rearing of *E. fuscoguttatus* at Different Stocking Densities

Survival of grouper larvae up to metamorphosis is generally low across all species. James (et al. 1997) recorded a survival of 1.73% – 2.98% for *E. polyphekadion*. Survival for *E. fuscoguttatus* reported in Reyes (undated) was between 0.109% and 0.91%. In this study, the SR were inversely proportional with density. However, these SR (1.33%, 4.26% and 6.86%) in three treatments were higher compared to the 0.9% survival of *E. fuscoguttatus* raised at 30 ind L^{-1} for a period of 45 days (James et al. 1998; Table 10). This shows that density greatly affect the survival of *E. fuscoguttatus* fry, and is also reported for many other species such as *Clarias gariepinus* (Jamabo and Keremah 2009), *Rachycentron canadum* (Hitzfelder et al. 2006), and *Soiea soiea* (Schram et al. 2006). While lower stocking densities in indoor tanks may result in higher SR, this requires increased space and resources in hatcheries. However, the use of other culture facilities, such as outdoor concrete tanks for *Epinephelus fuscoguttatus* x *lanceolatus* at 8 ind L^{-1}, had 26.9% survival (Anita and Dewi 2020). If available space permits, a trial on outdoor rearing of fry could be performed for *E. fuscoguttatus* to possibly increase SR.

Table 10. Average total lengths (TL) and survival rates (SR) of *Epinephelus fuscoguttatus* fry compared with other similar studies. T- treatments.

Species	T	Density (ind L^{-1})	Duration (day)	Mean TL (mm)	Feed	SR (%)	Culture System	Source
Epinephelus fuscoguttatus	1	3	38	20.7	Natural/ commercial feed	6.86	Concrete tank (5,000 L)	This study (E2)
	2	5				2.26		
	3	10				1.33		
Epinephelus fuscoguttatus	1	30	45	34.40	Natural/ artificial diet	0.9	Fiberglass tank (2,800 L)	James et al. 1998
Epinephelus polyphekadion	2			19.77		1.7		
Epinephelus fuscoguttatus x lanceolatus	1	8	30	28	Natural/ artificial diet	26.9	Concrete pond (10,000 L)	Anita and Dewi 2020
Epinephelus fuscoguttatus	1	15	30	7.47 – 9.70	Rotifer/ *Artemia*	0.38 - 0.91	Concrete tank (3,000 L)	Reyes Undated
	2		43	15.7–17.39		0.109 – 0.115	Concrete tank (5,000 L)	
Epinephelus polyphekadion		30	50		Natural/ artificial feeds	1.7 – 2.98	Round fiberglass tank (2,800 L)	James et al. 1997

In commercial hatchery operations, it is not only the SR that is of importance, but also the overall performance of the hatchery (the combination of survival, and growth rate). The grouper in this study completed the development into juveniles and reached 20 mm average TL at 38 DAH. This is much faster than in the study of Sugama et al. (2012), where *E. fuscoguttatus* completely metamorphosed at 45 DAH (TL reached 20–28 mm), and juvenile *E. lanceolatus* metamorphosed at 45 DAH at 35.4 mm TL (Garcia-Ortega et al. 2013). It could be noted however that Sugama et al. (2012) suggested a density of 10 ind L^{-1} which could attain 5 to 40% survival. These variations in larval development and survival could be related to water conditions and nutrition. The early occurrence of complete metamorphosis in this study could be attributed to the lower stocking densities which promoted faster growth. Reduced stocking density promotes good water conditions, and minimizes the chances of serranid larvae of becoming entangled with each other via their elongated dorsal and pelvic spines (Sugama et al. 2012).

Experiment 3 (E3): Growth and Survival of *E. fuscoguttatus* Fry at Different Stocking Densities fed with Commercial Feed

The results in this study were comparable to that of Salari et al. (2012) where stocking density did not significantly affect the survival of *E. fuscoguttatus* juveniles (Table 11). Severe cannibalism often occurs at this stage, but this was not observed even when the densities were much higher than the recommended density by Ismi et al. (2012).

The culture systems and stocking densities affect the growth performances, feed utilization and water quality (Samad et al. 2014). The rearing of fish at higher densities optimizes productivity of the facilities but increases the demand for dissolved oxygen, higher chances of cannibalism and disease outbreaks. However, the survival in this study (99.2 to 100%) are higher than the estimated 60% survival for *E. fuscoguttatus* upon reaching 7 cm TL (Ismi et al. 2012). The weekly manual thinning which was carried out by removing the smallest and the largest individuals to reduce both the density and variations in sizes could have efficiently prevented cannibalism. Although frequent grading has been reported to cause stress which may lead to disease outbreak (Ismi et al. 2012), this did not occur during the experiment. The use of small basins and manual sorting could have facilitated efficient cleaning, water exchange, faster sorting, and reduced disturbance to the fish. The low mortality could have been caused by other factors, but this was not investigated due to limited laboratory facilities and equipment. Future studies may deal on these unknown aspects in the nursery rearing of this species.

In terms of growth, the fish reached 6.94 cm from an initial of 3.65 cm TL after three weeks or 21 days, which was comparable to the estimates of Ismi et al. (2012), that *E. fuscoguttatus* juveniles from an initial TL of 3 cm, could reach a final TL of 7 cm in 30 days. However, it is worth reiterating that our study used small basin with densities several times higher than the recommended density in tanks and cages (Ismi et al. 2012).

Factors affecting fast fish growth includes the maintenance of optimum water conditions, good nutrition and reduced stress (Ismi et al. 2012; Hien et al. 2016). The use of small basin instead of concrete tanks could further significantly reduce the cost in setting up a large hatchery, making this better suited for backyard or small-scale hatcheries. The results suggest that hatchery-produced *E. fuscoguttatus* could be raised at higher stocking densities in plastic basins without affecting their growth and survival. Other higher stocking densities maybe tested to optimize the use of space in small-scale hatcheries.

Table 11. Average total lengths (TL) and survival rates (SR) of *Epinephelus fuscoguttatus* in Experiment 3 in comparison with a similar study. CP-commercial pellets.

T	Starting Density (ind L^{-1})	Final Density (ind L^{-1})	Duration (day)	SR (%)	Final TL (cm)	Final Weight (g)	Feed	Culture System	Source
1	6	2	21	100.00	6.94	6.16	CP	Basin (25 L)	This Study
2	12	4		99.20	6.92	6.00			
3	18	6		99.73	6.92	5.82			
1	1	1	42	80.83	7.615	7.77	CP	Flow through Round Fiberglass tanks (80 L)	Salari et al. 2012
2	3	3		82.77	8.492	10.45			
3	5	5		80.91	7.852	8.90			
1	1	1		78.33	7.211	6.92			
2	3	3		84.86	6.942	6.90			
3	5	5		79.58	7.212	6.74			

Experiment 4 (E4): Growth and Survival of *E. fuscoguttatus* Fingerlings Fed with Different Commercial Feeds

In this experiment, the fish in T1 having an initial 6.07 cm TL on the first week reached 8.51 cm TL during the final sampling on the third week (Table 12), suggesting 0.81 cm weekly average increase. Treatment 2 on the other hand performed a little slower having 0.62 cm average increase per week. This variation could be sampling or nutrition related. Samples were unmarked and were randomly taken each week for size measurement thus causing possible variations. However, the variation due to sampling could be minimal with 90 fish samples per treatment per week, and the relatively similar SD for both treatments. The faster growth in T1 could have been mainly influenced by the quality of the feed. The imported commercial feed used in T1 listed nutritional information (11% moisture, 44% crude protein, 7% crude fat, 16% ash and 3% crude fiber) in its label and manufactured to industry standards. By contrast, the label of local commercial feed used in T2 did not contain such information. The use of good-quality pelleted feed is one of the best practices during the nursery phase as low-quality feeds result in poor nutrition and increase the chance of cannibalism (Ismi et al 2012). In addition, Alvarez-González (2001) and Hien et al. (2016) reported the significant effect of the quality of feed on the grouper growth. The limited laboratory facilities hindered the conduct of independent proximate analysis for both feeds, which should be considered when doing future growth studies involving the use of commercial feed.

For *E. fuscoguttatus* measuring between 5 cm and 9 cm TL, Ismi et al. (2012) recommended stocking densities between 400 and 1000 ind m^{-3} (equivalent to 0.4 to 1 ind L^{-1}) for both tanks and cages. Our study however, showed that this could be increased up to 2-4 ind L^{-1} with high SR (99.11% to 99.78%) when small basins were used as rearing containers. Information on the survival of grouper having similar size with the fish we used are limited. Ahmad et al. (1999) reported 85.5% to 93.8% survival for *E. coioides* raised in tanks and cages at 1 ind L^{-1}, while James et al. (1998) did not mention the survival of *E. fuscoguttatus* raised in fiberglass tanks for 30 days (Table 12). The densities used in this study were much higher than the recommended number in tanks and cages, but we obtained a much higher SR compared to the 60% estimate of Ismi et al. (2012).

Table 12. Average total lengths (TL) and survival rates (SR) of *Epinephelus fuscoguttatus* in Experiment 4 compared with other similar studies. T-treatment.

Species	T	Starting Density (ind L^{-1})	Final Density (ind L^{-1})	Duration (day)	SR (%)	Final TL (cm)	Final BW (g)	Feed	Culture system	Source
Epinephelus fuscoguttatus	1	4	2	21	99.78	8.51	12.1	Pellet Brand A	Basin (25 L)	This Study
	2				99.11	8.04	10.06	Pellet rand B		
Epinephelus coioides	1	1	1	30	93.80		10.9	Pellet	Tank (2,800 L)	Ahmad et al. 1999
	2				85.50		10.6		Cage (1,000 L)	
Epinephelus fuscoguttatus	1	0.2	0.2	30			14.28	Pellet	Rounded fiberglass (2,800 L)	James et al. 1998
Epinephelus polyphekadion	2						8.84			

In aquaculture, cannibalism can cause considerable problems in larval culture (Liu et al. 2017). *Epinephelus fuscoguttatus* tend to eat other fish very close to their own size (Ismi et al. 2012), but the absence of cannibalism in this study proved that the use of basin is a good practice in hatchery management to facilitate fast and effective grading of grouper fingerlings. Basins are also less expensive and easier to manage than concrete tanks and could easily be adopted in small-scale hatcheries.

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