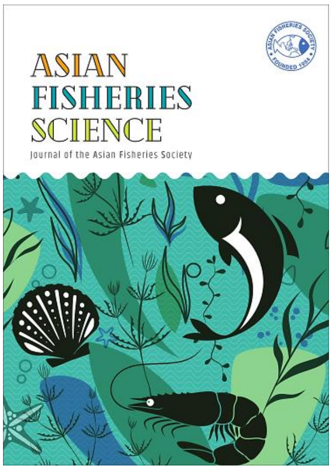

Simplified Hatchery Protocols for Culture of Orange-Spotted Spinefoot *Siganus guttatus* (Bloch, 1787) in Palawan, Philippines

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Abstract

Three hatchery experiments for orange-spotted spinefoot, *Siganus guttatus* (Bloch, 1787), were carried out in Puerto Princesa City, Palawan, Philippines using larvae and fingerlings produced through induced spawning. The first experiment (E1) involving larvae raised in six 5,000-L concrete tanks until 39 days post-hatch (dph) comparing two stocking densities (T1: 3; and T2: 5 larva.L^{-1}), obtained survival rates (SR) of 6.00 and 7.85 %. The second experiment (E2) monitored the growth and survival of 47 dph juveniles for 3 weeks, raised in 25-L plastic basins, fed with a commercial diet at three stocking densities with five replications. The initial stocking densities (6, 12, 18 ind.L^{-1}) were reduced during the second (4, 8, 12 ind.L^{-1}) and third (2, 4, 6 ind.L^{-1}) week, respectively. The weekly SR for all treatments ranged between 99.2 and 100 %. Weekly final total lengths (TL) were not significantly different except during the second week. The third experiment (E3) evaluated the effects of two types of commercial feeds (T1: grouper feed; T2: milkfish feed) on the growth and survival of 47 dph juveniles in plastic basins for 3 weeks, at similar densities reduced on a weekly basis. The SR (96.2 to 99.9 %) were not significantly different, but the TL of fish in T1 (4.39 cm) were significantly bigger than in T2 (3.52 cm). While there is a need to improve the low and irregular survival of *S. guttatus* larvae for cost-effective large-scale production, we recommend using small basins in the intensive rearing of juveniles.

Keywords: aquaculture, blue-basin, fishery resource, growth, hatchery-produced

Introduction

The orange-spotted spinefoot, *Siganus guttatus* (Bloch, 1787), is a rabbitfish (siganid) commonly found in shallow waters with seagrass beds and coral reefs in Southeast Asia. The fish can reach up to 45 cm total length (TL), although on average, it reaches 25 cm (Carpenter and Niem, 2001). While this species is of aquaculture importance within its distribution range (Duray, 1998; Stattin, 2012; BFAR, 2018; Seale and Ellis, 2019), the supply of *S. guttatus* in Palawan, Philippines, mostly comes from the wild. Rabbitfishes are caught at night and are sold either fresh, semi-dried or dried, served in high-end restaurants, and as popular take-home food items among tourists.

The increasing demand for fish and many other marine resources and the use of destructive fishing methods have damaged habitats and caused a decline in fish populations (Fabinyi, 2010; Seale and Ellis, 2019; Wolanski et al., 2020). In Indonesia, the seagrasses and their role in the ecosystem are in a critical state of decline due to shifting environmental conditions, coastal development, overfishing and pollution (Unsworth et al., 2018). In the Philippines, about 30-50 % of the seagrass ecosystems are damaged due to industrial development, ports and recreation (Kirkman and Kirkman, 2002). Employed conservation approaches to avert the damage to the ecosystems are met with many challenges (Weeks et al., 2010). Sustainable aquaculture using closed-cycle species helps reduce the pressure on wild fish populations and ecosystems.

Sustainable siganid cage farming in Palawan requires a stable supply of hatchery-produced fingerlings. Studies on the propagation and aquaculture of *S. guttatus* dates back to the early 1980s. Hatchery trials by Juario et al. (1985) for *S. guttatus* larvae at high densities ranging from 16.67 to 120 ind.L^{-1} fed with mixtures of natural and artificial diet had highly variable survival rates (SR), ranging between 0.7 and 24.7 %, upon reaching metamorphosis or 35 dph. In contrast, Hara et al. (1986) obtained 4.5 to 30.1 % SR for *S. guttatus* larvae raised for 21 days at densities ranging between 8.26 to 57.48 ind.L^{-1}. Lower larval densities resulted in higher SR for Asian sea bass (Salama, 2007) and Cobia (Hitzfelder et al., 2006). Hence, reducing the larval stocking density for *S. guttatus* is expected to favour higher SR.

Studies on the effect of density on juvenile, however, is scarce. Only Syah et al. (2020) reported the effects of density (50 to 200 ind.m^{-3}) on the growth and survival of juvenile *S. guttatus*. In the first stage of the experiment, which involved smaller juveniles, density did not significantly affect the growth, but had slight negative effects on survival. This suggests that with proper water management and adequate feeding, the density could be increased without affecting growth and survival. Higher densities (10 to 40 ind. per 52-L aquaria translated into 192 and 769 ind.m^{-3}) were tested for *Siganus rivulatus* Forsskål & Niebuhr, 1775 (Saoud et al., 2008), which could also be tested for *S. guttatus*. The maximum utilization of space through increased stocking density could make hatchery operations cost-effective.

Many siganid grow-out studies in ponds and cages had promising results (Duray, 1998; Stattin, 2012; Visca and Palla, 2018; Syah et al., 2020). However, the siganid aquaculture in Palawan or even in the Philippines remained in its infancy stage, as reflected in the country's national statistic records. As of 2018, although total siganid production from cages ranked second to milkfish, it only constituted 0.12 % of the total volume (BFAR, 2018).

The usual practice of rearing high larval density with highly variable SR, and the rearing of juveniles in tanks at low stocking density needs further improvement. In this study, three experiments (E1, E2, and E3) were conducted to develop simplified protocols for rearing *S. guttatus* larvae and juveniles under hatchery conditions. Specifically, E1 looked into the survival of larvae at two stocking densities until complete metamorphosis. E2 evaluated the growth and survival rates (SR) of juveniles subjected to the gradual reduction of densities. Lastly, E3 monitored the growth and survival of juvenile *S. guttatus* fed with two types of feeds and having the density reduced on a weekly basis. This was the first attempt in Puerto Princesa City, Palawan, to artificially produce fry and fingerlings of *S. guttatus* for cage aquaculture, which could be an effective fisheries and ecosystem management strategy for the immediate benefit of coastal communities.

Materials and Methods

Study site

The experiments were conducted at the hatchery facilities of the South Sea Exclusive Philippines in Sta. Lucia, Puerto Princesa City, Palawan, Philippines. The company also maintained a floating fish cage structure for the culture of different fish breeders.

Spawning, incubation, and larvae collection

Twenty individuals (ind.) of wild-sourced cage-cultured, orange-spotted spinefoot breeders (average TL: 27 ± 4.36 cm; weight: 0.46 ± 0.11 kg) were purchased (11 January 2016) from a nearby private cage farm. The fish were conditioned in floating cages for three weeks and fed with floating pellets. For further conditioning, the fish were then brought to the hatchery and distributed in three 800-L capacity blue fibreglass tanks (7, 7, and 6 ind.tank^{-1}). A day before the first quarter of the moon (15 February 2016), all fish were injected with 500 IU human chorionic gonadotropin (HCG). No feeding was employed until spawning, which occurred on the 3rd and 4th day. Daily water exchange was at 50 %. The spent breeders were immediately transferred to a different tank before being transferred back to the sea cages. The eggs were retained in the tanks and were provided with aeration, continuous water flow at ambient water temperature (27–29 °C). The eggs hatched after 24–28 h, and the larvae were used in E1.

Experiment 1 (E1): Survival of *Siganus guttatus* larvae at two stocking densities

The SR of the newly hatched fry was monitored at two different stocking densities, 3 ind.L^{-1} (T1) and 5 ind.L^{-1} (T2), with three replications arranged in a completely randomized design (CRD). A control group containing higher densities was not included as a treatment considering the numerous trials involving high larval densities from other studies (Juario et al., 1985; Hara et al., 1986). The experiment was carried out until complete metamorphosis using six 5,000-L capacity concrete tanks (Table 1). Before introducing fish

Table 1. Treatments (T) and replications (R) involving day-old hatched *Siganus guttatus* larvae raised at two stocking densities for 39 days fed with Rotifer, Artemia and commercial feed.

T	R	Density (ind.L^{-1})	Number per 5000-L tank	Duration (Day)
1	3	3	15,000	39
2	3	5	25,000	39

larvae, each tank received 100-200 L of cultured microalgae *Nannochloropsis oculata* (Droop) D.J. Hibberd, 1981 to provide shade and serve as immediate food to the fish larvae. The density of microalgae was not measured; instead, volume (in Litres) was adjusted depending on greenish colour of the water which was visually determined. The cultured algae were added to the tanks every other day until the 25th day. The first feeding using vitamin-enriched rotifers occurred on the 3rd day post-hatch (dph) when the larva's mouth was large enough to consume rotifer (L type). The rotifer density in the larval rearing tanks was monitored twice a day (0700 and 1500 h) to maintain the desired number until the 30th dph (Fig. 1). Newly hatched vitamin-enriched *Artemia salina* (Linnaeus, 1758) were added twice daily (0700 and 1500 h) between the 12th and 30th dph. A pinch of artificial feed was given every hour between 0600 and 1700, starting on the 12th until 39th dph. Flow-through water exchange (1 $L.min^{-1}$) and removal of settling dirt were implemented starting at 8 dph. The larval rearing was done in filtered UV-treated seawater (T1: 27.91 ± 0.51 and 27.76 ± 0.52 ppt) at ambient water temperatures (T1: 28.13 ± 0.89 and T2: 27.99 ± 0.95 °C), pH (T1: 8.36 ± 0.22 and T2: 8.34 ± 0.23) and ammonia (T1: 0.75 ± 0.61 and T2: 0.86 ± 0.67 ppm). The study was terminated after 39 dph when all the larvae had completed their final metamorphosis into the juvenile stage. The harvested juveniles in E1 were conditioned for 7 days in a large concrete tank fed with artificial diet to have similar nutritional status before randomly choosing the test animals for both E2 and E3, which started at 47 dph.

Experiment 2 (E2): Growth and survival of juvenile Siganus guttatus subjected to gradual density reduction fed with commercial diet

This three-week experiment involved 47-day old larvae (2.68–2.74 cm) raised in 25-L blue plastic basins using CRD with five replications in a semi-indoor structure with black sack roofing. The three (6, 12, 18 $ind.L^{-1}$) stocking densities (treatments) were reduced during the second (4, 8, 12 $ind.L^{-1}$) and third (2, 4, 6 $ind.L^{-1}$) week, respectively, to avoid overcrowding as the fish increased in size, and to reduce size variation (Table 2). In reducing the density, larger and smaller individuals from the majority were removed, leaving behind individuals of similar sizes. A total of 4,500 juveniles were used during the first week, which were reduced into 3,000 and 1,500 ind. for the second and third week, respectively. Imported grouper pellets were given *ad libitum* 3–5 min every hour between 0700 and 1800 h. The basins received gentle aeration, continuous water flow (at about 1 $L.min^{-1}$), and 2–3 times daily waste siphoning. The basins were covered with a black net to prevent the escape of cultured fish.

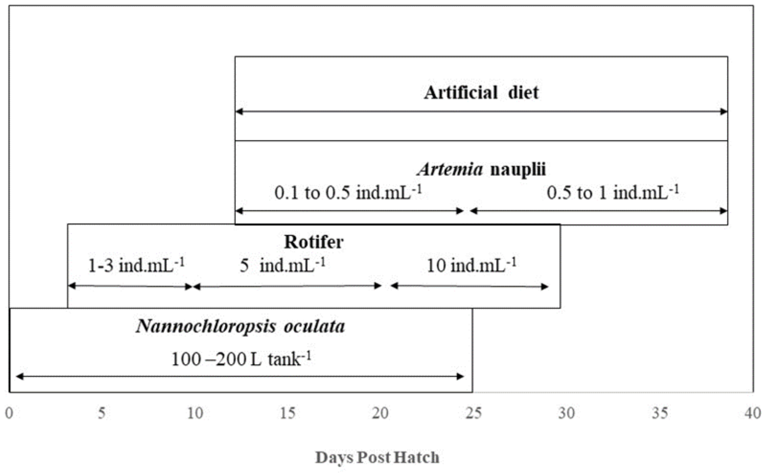

Fig.1. Feeding schedule for the *Siganus guttatus* larvae from hatching until 39 days.

Table 2. Treatments (T) and replications (R) indicating the weekly (W) change in density, initial total length (TL) and age of *Siganus guttatus*.

T	R	Density ($ind.L^{-1}$)			Number per 25-L basin			Initial TL (cm)	Starting age (dph)
		W1	W2	W3	W1	W2	W3		
1	5	6	4	2	150	100	50	2.74 ± 0.25	47
2	5	12	8	4	300	200	100	2.68 ± 0.23	47
3	5	18	12	6	450	300	150	2.73 ± 0.22	47

Experiment 3 (E3): Growth and survival of juvenile Siganus guttatus *fed with two different commercial feeds*

This experiment was carried out using two treatments with five replications arranged in CRD in a semi-indoor structure with black sack roofing. The two treatments had the same densities which were reduced on a weekly basis, while the feed varied between treatments: grouper feed for T1 and milkfish feed for T2. The density during the first week (12 ind.L^{-1}) was reduced during the second (8 ind.L^{-1}) and third (4 ind.L^{-1}) week, respectively (Table 3). A flow-through system at approximately 1 L.min^{-1}, gentle aeration, and 2–3 times daily waste removal was employed to maintain good water quality. The feeds were given *ad libitum* for 3–5 min every h from 0700 to 1800 h. The basins were covered with a net to avoid the escape of fish. A total of 3,000 juveniles were used during the first week.

Sampling

For E1, the number of newly hatched larvae were counted by volumetric method before transferring in the assigned tanks. While at the end of the study, all the juveniles were manually counted. For E2 and E3, sampling for TL was done daily for 10 randomly selected individuals from each replicate (basin). At the end of each week, all surviving individuals were counted. After that, larger and smaller individuals were removed to reduce the density and to have a uniform size group in the following week. All individuals removed from the experiment were placed in an extra rearing tank.

Data analysis

The variables between treatments in E1 (survival) and E3 (growth and survival) were compared using t-test. While the growth and survival among treatments in E2 were compared using analysis of variance and Scheffe post hoc tests. All analyses were performed using SPSS trial version.

Results

Experiment 1 (E1)

The SR (0.69–12.64 %) of *S. guttatus* varied largely between tanks (Table 4) but were not significantly different ($P > 0.05$) between treatments. At the end of the culture period, the fry measured 1.4 ± 0.1 cm TL. A total of 8,590 fry produced from both treatments were used in the succeeding two experiments.

Experiment 2 (E2)

The weekly increase in TL was visible for each treatment (Fig. 2). During the first and second week, the initial lengths did not significantly differ among treatments ($P > 0.05$). In contrast, the weekly final lengths for all treatments were not significantly different ($P > 0.05$) during the first and third weeks. Significant differences were noted on the final TL on the second week and the initial TL on the third week, where T1 significantly differed from other treatments ($P < 0.05$). Despite these variations, the weekly TL increments (Table 5) did not significantly vary ($P > 0.05$). The weekly SR were high, ranging between 99.2 and 100 %, and were not significantly different among treatments ($P > 0.05$).

Table 3. Treatments (T) and replications (R) for the *Siganus guttatus* juveniles indicating the type of feed, weekly (W) density, initial total length (TL) and age at the start of the study.

T	R	Feed pellet	Density (ind.L^{-1})			Number per 25-L basin			Initial TL (cm)	Starting age (dph)
			W1	W2	W3	W1	W2	W3		
1	5	Grouper feed	12	8	4	300	200	100	2.68 ± 0.14	47
2	5	Milkfish feed	12	8	4	300	200	100	2.46 ± 0.15	47

Table 4. Survival rates (%) of *Siganus guttatus* larvae raised at two different stocking densities.

Replicate	Survival (%)	
	Treatment 1	Treatment 2
1	10.95	8.87
2	0.69	12.64
3	6.37	2.05
Mean (± SD)	6.00 (± 5.14)	7.85 (± 5.37)
Total juvenile produced	2,701	5,889

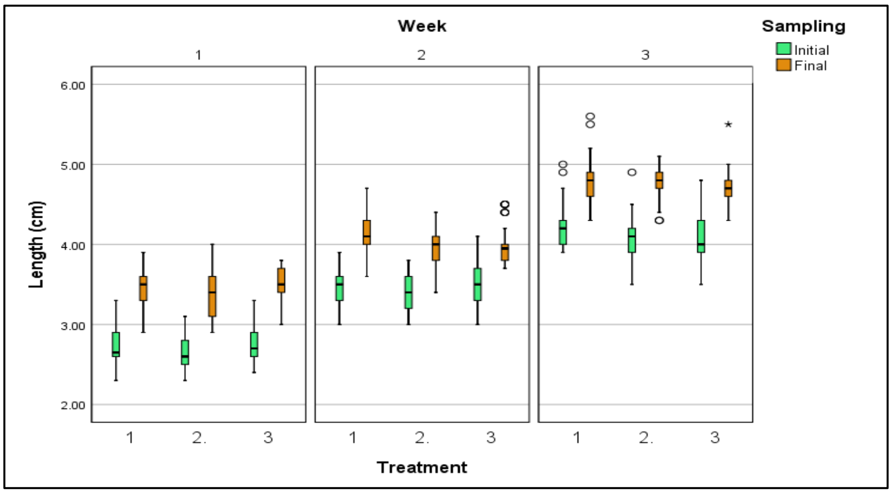

Fig. 2. Weekly initial and final total lengths (TL) of *Siganus guttatus* in the three treatments. The final TL of T1 during the second week, and the initial TL of T1 during the third week were significantly higher than the other treatments.

Table 5. Average total length (TL) weekly increment (cm) of *Siganus guttatus* at different stocking densities. Weekly TL increments did not significantly vary among treatments.

Treatment	Week 1 Average TL (cm) increment (± SD)	Week 2 Average TL (cm) increment (± SD)	Week 3 Average TL (cm) increment (± SD)
1 (n = 50)	0.72 (± 0.16)	0.67 (± 0.09)	0.54 (± 0.15)
2 (n = 50)	0.73 (± 0.43)	0.57 (± 0.13)	0.70 (± 0.06)
3 (n = 50)	0.78 (± 0.15)	0.57 (± 0.15)	0.64 (± 0.12)

Experiment 3 (E3)

The final TL for each week (Fig. 3) were in favour of T1, and these were significantly higher than in T2 ($P < 0.05$). Considering the significant variations on the initial weekly lengths at each treatment, the weekly length increments (Table 6) were compared, and it was found that TL in T1 increased nearly twice faster than T2 ($P < 0.05$). The observed weekly SR (96.20 ± 3.96 to 100 %) were not significantly different.

Discussion

Experiment 1 (E1)

The SR of hatchery-produced *Siganus* spp. larvae were generally low and unpredictable in spite of the reduced density. In our study, it was difficult to explain the low SR (0.69 and 2.05 %) in one of the replications of each treatment (Table 4). Anomalous SR for the species was reported by Juario et al. (1985), who obtained an average of 24.7 % SR during the first trial in 1981, but SR gradually declined to reach 0.7 % on the 8th trial in 1983. In general, after conducting several trials from 1981–1983, the average SR was 8.56 ± 6.46 %. Hara et al. (1986) obtained 3.5 to 16.6 % (mean: 7.52 %) SR, while Ayson (1989) reported 0.6 to 37.2 %. For other species, Bryan and Madraisau (1977) reported 16 % survival for *Siganus lineatus* (Valenciennes, 1835) reared from hatching through metamorphosis in 35 days, while for *Siganus canaliculatus* (Park, 1797), May et al. (1974) obtained approximately 0.3 % SR from hatching to metamorphosis 30 days after hatching.

The larvae in this study underwent complete metamorphosis on the 39th dph, and measured 1.4 ± 0.1 cm. The TL was a little shorter than in the report of Juario et al. (1985) where *S. guttatus* reached about 1.8 cm in 35 days. Size and period of metamorphosis for *Siganus vermiculatus* (Valenciennes, 1835) was 2.52 cm and 37 dph (Anuraj et al., 2021). Whilst larvae of *S. canaliculatus* metamorphosed at 2.03 cm SL and 35 dph (Bryan and Madraisau, 1977).

Major factors affecting the variation in survival and period required to complete metamorphosis include timing of feeding, nutrition, size of feed and size of tanks. Bagarinao (1986) found that a delay in initial feeding of more than 24 h after eye pigmentation and opening of the mouth were found fatal for the larvae of *Chanos chanos* (Forsskål, 1775), *S. guttatus* and *Lates calcarifer* (Bloch, 1790). The onset of feeding for *S. guttatus* is at 48 h after hatching (Duray, 1998) and should be fed by day 3 or 4 to avoid mortalities (Juario et al., 1985). Ayson (1989) attributed the low SR to the great size variability of the rotifers used as feed for the larvae. Cabanilla-Legaspi et al. (2021) accelerated the metamorphosis of *S. guttatus* by feeding the larvae

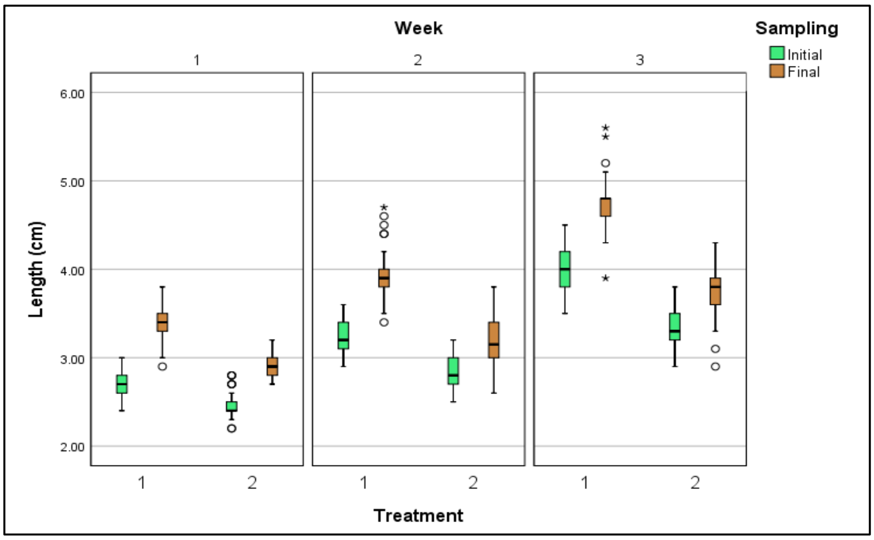

Fig. 3. Weekly initial and final lengths (TL) of *Siganus guttatus* in treatments 1 and 2. Significant variation existed between treatments on the weekly initial and final TL of the species.

Table 6. Weekly average total length (TL) increments of *Siganus guttatus* fed with two types of diets. The weekly TL increments in T1 were significantly higher than in T2.

Treatment	Week 1 Average TL (cm) increment (± SD)	Week 2 Average TL (cm) increment (± SD)	Week 3 Average TL (cm) increment (± SD)
1 (n = 50)	0.75 (± 0.08)^a	0.70 (± 0.13)^a	0.78 (± 0.16)^a
2 (n = 50)	0.45 (± 0.04)^b	0.39 (± 0.07)^b	0.41 (± 0.03)^b

with sodium iodide supplemented *Artemia*. Juario et al. (1985) reported lower survival (Range: 0.9–8.4 %; Mean: 4.23 %) in 3-ton concrete tank than in 600-L tank (Range: 0.7–37.4 %; mean: 9.86 %). However, compared to the average survival (4.23 %) of Juario et al. (1985) in 3-ton tanks, we obtained a higher average SR for both treatments (6.00 and 7.85 %) despite using a much larger tank (5,000-L). Hara et al. (1986), also obtained higher survival in large tanks (≥5 m^3) than in small tanks (500-L).

Experiment 2 and 3 (E2 and E3)

The growth of *S. guttatus* is dependent on the type of feeds and rearing systems. *Siganus guttatus* in E2 fed with the same brand of feed showed a consistent weekly increase irrespective of variations in density (Fig. 1). The weekly final TL were not significantly different except during the second week. However, the weekly TL increments were not significantly different, hence it is presumed that the observed variations were due to sampling error. Stocking density had no significant effect on the growth rate, as Saoud et al. (2008) reported for *S. rivulatus* raised at much lower densities.

In E3, the growth in T1 was consistent with treatments in E2 where the fish received the same brand of feed. The fish in T2 of E3, however, which received a floating milkfish "bangus" feed had slower growth. This variation could be attributed to the nutritional content of the feed, as also observed by Villanueva et al. (2021) for Tiger grouper *Epinephelus fuscoguttatus* (Forsskål, 1775). Visca and Palla (2018) reported better growth of *S. guttatus* provided with high crude protein (CP) containing food. Sallam et al. (2020) significantly improved the growth of wild-caught *S. rivulatus* juveniles by adding zinc-containing compounds on three experimental diets. In addition, Cabanilla-Legaspi et al. (2021) also improved the growth of *S. guttatus* larvae when fed with iodide-supplemented brine shrimp. The grouper feed we used contained 44 % CP as indicated on the label, while the packaging of milkfish feed did not contain any nutritional information. However, it is presumed that it has a lower CP because milkfish fry requires feed containing 40 % CP (Benitez, 1984). The growth could have also been influenced by the weekly reduction in density as also observed in grouper (Villanueva et al., 2021).

The growth of *S. guttatus* also depends on the rearing system and level of management. Andam et al. (2016) raised 30 dph juveniles for 60 days in hapa net cages set in pond conditions at densities ranging between 250 and 1,000 ind.m^{-3} (or 0.25 to 1 ind.L^{-1}). However, the obtained average lengths (3.99 to 4.49 cm) and SR (43.29–68.83 %) were lower compared to the fish in this study which were raised in small basins at much higher densities (2–18 ind.L^{-1} equivalent to 2,000 to 18,000 ind.m^{-3}) and for a shorter amount of time (21 days). The juveniles at the end of this study were only 68 dph, while in the research of Andam et al. (2016) they were 90 days old. Wide variation in initial sizes, uncontrolled environmental factors, and food shortage was pointed out as factors affecting the slow growth of *S. guttatus* in ponds compared to tanks (Aypa, 1990).

The high SR in E2 (99.2 to 100 %) and E3 (96.20 to 100 %) could have been attributed to the gradual reduction in density, the provision of net cover preventing fish escape, and the continuous water flow maintaining high water quality. These protocols were also found effective for the Tiger grouper *E. fuscoguttatus* (Villanueva et al., 2021). Saoud et al. (2008) also obtained >95 % SR for *S. rivulatus* juveniles by providing the rearing aquaria with a cover.

The absence of variation in the SR at different densities suggests that with proper feeding and water management, juvenile *S. guttatus* can be raised even at a higher stocking density without affecting the SR. The tested stocking densities in E2 and E3 (2–18 ind.L^{-1}) translated into 2,000 to 18,000 ind.m^{-3} were much higher than those of Syah et al. (2020) who only raised *S. guttatus* juveniles in floating net cages at densities between 50 and 200 ind.m^{-3}. For *S. rivulatus*, Saoud et al. (2008) only tried 10 to 40 ind. per 52-L aquaria (translated into 192 and 769 ind.m^{-3}). The high SR (96.2 to 100 %) and the good growth of *S. guttatus* juveniles at such extremely high densities in both E2 and E3 is a significant contribution to the protocol in raising siganids.

Conclusion

Despite using lower stocking density, the survival of larvae in the hatchery remained unpredictable and required further investigation before applying technology to large-scale production. However, our results in E2 and E3 suggest that high densities of *S. guttatus* in the small basin have no apparent negative effect on growth and survival. The imported feed significantly enhanced the growth of juveniles, nearly twice faster than the locally available feed. Hence, the juveniles could be intensively raised in a simplified set up of small blue basins until ready for grow-out. The use of small basins prior to transfer to the grow-out facility could be tested for other finfish species. Small basins could be easily managed and would not need a big investment, making it more practical and adaptable for small-scale hatcheries and nurseries. However, for large-scale facilities, the use of bigger tanks at higher density could be tested following similar feeding and water management schemes. An economic analysis will be important in determining the feasibility of this approach.

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Conflict of interest: The authors declare that they have no conflict of interest.

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