## Journal of Aquaculture & Livestock Production

### **Research Article**



# The Right Time to Add A Commercial Feed for Rabbitfish (*Siganus Guttatus*) from 10 to 25 Days of Age

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#### ABSTRACT

A study on the timing of the start of supplied commercial feed for rabbitfish fry (*Siganus guttatus*) to determine the optimal survival rate and growth in terms of length and weight at 30 days. Four trials, 3 replicates each, and 4 batches in 2020 – 2022 differed when supplied commercial feed to fish fry at 10, 15, 20, and 25 days old before provided commercial feed, all larvae fed by zooplankton and phytoplankton. The results showed that the rabbitfish fry supplemented with commercial feed at 20 days old (NT3 (20)) showed the fastest growth in length, an average of 18.53 mm at 30 days old, average daily increase of the size of 0.65 mm/day and growth rate of size of 5.95% and 36.98 to 38.01g/head of trial 3 (TN3) (20) in 4 feeding batches, and the highest survival rate of 71.85% and weight at the final feeding stage at 30 days of age. There were significant differences in weight growth for the fish fry from at last feeding, and during the fry stages, fish tends to grow in length continuously, then increase in weight later. There was a significant impact by stomach volume at 30 days of the age of NT3 was much higher than in other trials. The other three timing trials of commercial feeding did not have any differences and showed less effectiveness than the treatment of fish at 10, 20, and 30 days old in terms of growth in length, weight, and survival rate. The stomach volume of Fries was stimulated by feedstuffs and early supplied feed to fries when they could eat; trial 3 (NT20) was the biggest stomach size, with a circumference of 11,76 mm<sup>2</sup>.

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Received: August 04, 2023; Accepted: August 08, 2023; Published: August 18, 2023

Keywords: Siganus guttatus, Length, Weight, Survival Rate and Stomach

#### Introduction

In the wild, Siganus guttatus fries, in their early stages, are floating fish and are usually found near the surface of the water above the reef. Juveniles then move into lagoons and estuaries, seasonally spawning, into forest and swampy mangrove ecosystems or places with sandy or rocky bottoms along the coast. Free neurons first observed and recorded in S. guttatus were described by [1]. It is visible that they appear after 6 hours of hatching and disappear after 39 hours. Their presence makes fry very sensitive, and difficult to fix their morphology and position of movement [2]. Showed that in S. guttatus, the dorsal surface and ventral fin rays begin to develop at 3.93 mm, the unordered flexion is completed at about 6.5 mm, and the mature complement of the fin rays is achieved at about 8.0 mm. The anterior molars appear at 4.12 mm, which when in adulthood, occupy the majority of the oral cavity at about 6.5 mm. The jaws and pharyngeal teeth begin to grow at 4.0 - 4.5 mm, so there is a change in the feeding habits of fry at 7.0 - 8.0 mm. Larval stage: they hatch and gradually metamorphose rapidly, already fully characterizing the spines and fin rays of adults of the species. Juveniles are also nearly identical to adults in body shape and color, not living in the same habitat as adults. The period by which the adult fish reaches

size, about 22.0 mm after 45 days from hatching in *S. guttatus*, has a size of 20-24 mm. In other studies, Auntie fish was also shown after 21 days in Auntie fish (*S. channeliculatus*), which has a size of 9.5 mm, and Auntie fish (*S. fuscescens*) after 11 days of hatching. The rate of transformation is influenced by temperature, type of feed. The morphological variation of *S. guttatus* is described in Figure 1 [1,2]. In the early stages of development of the fish, after hatching and 10 days of starting to nurse the fish and release the fish into the pond, the Discus fish stage begins to turn into incense fish. They eat the natural food available in the pond and adapt to the new environment while matching the ability of the fish to find prey in the early stages.

The period from 10 to 30 days after the hatching of the fish has high nutritional requirements and mainly phytoplankton ingestion and intestinal development, especially stomach volume. While they depend on natural food in the culture pond and will be in short supply, the addition of synthetic pellets of small size (1mm) and easy digestion is necessary, but it is also very risky if the fish does not eat after 1 day, the fish will sink (die). Many studies when feeding fish to industrial or homemade food outside, after hatching 12 showed the whole dead fish (Thua Thien Hue Fishery Extension, 2004 and Le van Dan and Le Duc Ngoan, 2005), from which the results of artificial reproduction of Auntie fish have not been successful. In 2019, with the research team's

efforts when exploiting the gene source of Auntie fish successfully reproduced artificially and semi-artificially in Phu Thuan, the survival rate of farmed fish reached 60-70%, Nguyen Quang Linh et al. 2019 and measured stomach volume and feedstuff in size of the stomach. Promoting that result, continue to invest in perfecting the nursery process in 10-30 days, thereby transferring experimental models and transferring to produce fish varieties to meet the actual production needs in Tam Giang - Cau Hai. After about 3-6 days of discus fish rapidly metamorphosing, the fry stage for the fish starts from 11 days. They must be fed high nutritional value, combining phytoplankton and fresh food in the hatchery pond resulting in high survival rates of 50-60% up to 30 days [3]. Determining when to add a commercial feed to improve fish's survival and growth rate between 10 and 30 days of age is essential as the key to breeding incense fish on fingerlings. Since then, the process has carefully studied the diet of fish appropriately, the nutritional content, as well as the biological value of fish feeds to determine the right time to add a commercial feed to help improve the survival rate of Auntie fish from 10-30 days old based on available feed sources available on the market so that when transferring the model to successful farming households. The feeding characteristics of the aunts vary with each stage of development, fry feed on zooplankton but switch to plants in juvenile and adult stages (Suyehiro, 1942).

#### **Materials and Method**

Day feeding 2 times (at 8:00h AM and 15:00h PM). The amount of feed is equal to 2.5 % of body weight. Zooplankton and Phytoplankton: A mixture of three algae species used, Chaetoceros muelleri, Isochrysis galbana and Nannochloropsis oculata Zooplankton and Phytoplankton: A mixture of three algae species used, *Chaetoceros muelleri, Isochrysis galbana* and *Nannochloropsis oculata*, rotifers and Copepoda were introduced into the fry nursery starting from day 10 to day 30 with a density of 3-5,103 cells/mL and enough the live food for larvae.



**Figure 1:** Nursery tanks for larvae eating by phytoplankton and zooplankton and fries at 10 days old, provided by the project, have an average length of 5.5 - 5.6 mm and 1.15 - 1.25 g/head.



**Figure 2:** (Commercial feed) NRD 2/3 (INVE, Thailand), supplied by Pearl Trading and Service Company, has nutritional ingredients including Protein ( $\geq$  55%); Lipids:  $\geq$  9%; fiber:  $\geq$  1.9 per cent; humidity  $\leq$  8%

#### **Experimental Setting - Up**

The experiment consisted of 4 trials in 4 batches of 3 years (2000 -2022), with 3 slots for each trial and repeat by batches the same experimental factors and conditions starting days, as below:

- NT1 (10): Basal *Chaetoceros muelleri*, rotifer + feed supplementation from the 10th day after nursery till 30 days

- NT2 (15): *Isochrysis galbana*, rotifer + feed supplementation from the 15th day after nursery till 30 days

- NT3 (20): Copepoda + feed supplementation from the 20th day after nursery till 30 days

- NT4 (25): *Nannochloropsis oculata*, rotifers + feed supplement from day 25 after nursery till 30 days

Composite tank with volume  $1.5 \text{ m}^3$  (water = 1200 L). The placement experiment was repeated 3 times/experiment/batch. A total of 3 rounds of monitoring of nursery fish in 4 different sizes, with first 5 days of age, the density of 7 head/mL and then stocking density 15 head/L and maintenance.

#### **Studying Variables**

The survival and growth rates at different ages after hatching fish with industrial feed. Monitoring fluctuations in environmental factors, growth in length and body weight, determination of survival rate and herd coefficient

#### **Study Site and Species Identification**

Nursing batch 1 (2020; 2021); batch 3,4 (2021; 2022); each by 10, 15, 20, and 25 days after hatching for commercial feeding starting and final at 30 days of age.

Location of Huy Son, Phu Thuan, Phu Vang, Thua Thien Hue aquatic hatchery and Center for Research and Application of Fishery Technology Transfer, Faculty of Fisheries, University of Agriculture and Forestry, Hue University.

- Siphon and water change 20-30% starting 5 to 10 days; replace 50%. The replacement water is clean seawater filtered through a sand filter, replaced at 10 litres/minute to avoid shocking the fish. The first stage of aeration is slightly opened, and then aeration is adjusted gradually, increasing with fish age.

#### Measurements

The 10-day-old fry was transferred to laboratory tanks with 10 individuals per litre stocking density. Maintaining the water conditions: Temperature  $28 - 29^{\circ}$ C, salinity 30‰, pH 7.8 - 8.5; and DO > 5 mg/L. Daily examination of fluctuations in environmental factors (pH, temperature, oxygen, ammonia, salinity), instruments and time is shown in Table 1.

Table 1: Used Tools and Methods for Water Condition
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	Variables	Tools	Time	Frequency
1	Temperature (°C)	Thermometer	8: 00 h 15:00 h	2 times/day
2	рН	pH Machine hand	8:00 h 15:00 h	2 times/day
3	DO (mg/L)	Test KIT Sera	8:00	2 times/day
4	NH <sub>4</sub> <sup>+</sup> /NH <sub>3</sub> (mg/L)	Test KIT Sera	8:00	2 times/day
5	Senility (‰)	Hydrometer	8:00	2 times/day

Fish growth is determined when the fish reaches 30 days of age. For each collection of 30 fish/tank to determine the mass using the portable electronic scale APTP453 -  $(100g \times 0.01g)$  (Japan), a ruler with a bar determines the length. The norms are determined

after the end of the experiment. + Weight (g/day): ADGW (g/day) = (W2 - W1) / (t2 - t1) (1)(1) + Length (mm/day): ADGL (mm/day) = (L2 - L1) / (t2 - t1) (2)

(2) Notation: L2, L1, W2, W1.

+ Survival rate (%): SR (%) = (Survival numbers/initial numbers)  $\times 100$  (%) (3)

+ Intestinal length (mm) and stomach size (cm) by the pan-mer ruler, calculation of the stomach circumference was selected in batch 4, with 2 trials measured by intestinal length and 2 trials for stomach volume.

Growth assessments are conducted on 10-day-old and 30-day-old fish. The evaluation value is the average of 30 random samples in each tank.

#### **Dat processing Method**

The figures are processed and analyzed based on the SE (standard error)  $\pm$  M (mean) using Microsoft Excel 2010 and biostatistically processed using SPSS 16.0.

#### Results

#### Water Quality and Maintenance

Water quality management practices are extremely important and decisive to the success of aquaculture activities. Environmental factors greatly affect aquatic organisms' growth, development, and reproduction. Each species of organism has a suitable habitat. Biological activity will be affected when environmental factors are no longer within the appropriate threshold. Therefore, monitoring fluctuations in environmental factors in aquaculture systems helps us to take appropriate technical measures to optimize habitats for organisms. Environmental factors such as temperature, salinity, pH, and DO are the basic parameters for the preliminary assessment of water quality in the system to know whether the environment is suitable for farming objects, hereby taking appropriate impact measures. The results of fluctuations in environmental factors are shown in Table 2.

Variables Times Trials (M ± m) 2020 - 2021 2021 - 20227:00 h  $29.60 \pm 0.42$ Water temperature  $23.60 \pm 0.62$ T (°c) 15:00  $32.10 \pm 0.55$  $33.10 \pm 0.25$ 7:00  $8.06 \pm 0.09$  $8.56 \pm 0.19$ 15:00  $8.28 \pm 0.04$  $9.28 \pm 0.14$ pН DO (mg/L) 7:00  $4.60 \pm 0.42$  $5.60 \pm 0.62$ Sanility (‰) 7:00  $29.40 \pm 1.14$  $30.40 \pm 1.44$  $NH_4$ - $NH_2$  (mg/L) 7:00  $0.00 \pm 0.00$  $0.00 \pm 0.00$ 

#### Table 2: Changes in Environmental Factors (n = 30 \*4 = 120)

#### Growth of Fish after Nursery 30 Days of Age

The growth results by mass of the fish after 20 days of incubation at different times of industrial feed supplementation are shown in Table 3.

Table 5. Growth Weight $(n - 50 - 4 - 120)$						
Trails	W10 (mg)	W30 (mg/con) Final				
	Initial	$1 (M \pm m)$	2 (M ± m)	3 (M ± m)	4 (M ± m)	
NT1 (10)	$2.15 \pm 0.13$	32.96ª±0.24	$32.97^{\text{a}} \pm 0.91$	$31.37\pm0.25$	31.77ª±0.4	
NT2 (15)	$2.15 \pm 0.13$	32.38ª±0.22	$32.52^{a}\pm0.63$	32.69 ±0.51	31.86ª±0.46	
NT3 (20)	$2.15 \pm 0.13$	38.01 <sup>b</sup> ±0.12	37.94 <sup>b</sup> ±0.37	$37.98^{b} \pm 0.45$	36.98 <sup>b</sup> ±0.04	
NT4 (25)	$2.15 \pm 0.13$	37.92°±0.32	$36.69^{\circ} \pm 0.41$	36.85° ±0.43	34.42°± 0.43	

Table 3: Growth Weight (n = 30 \* 4 = 120)

a, b, c in the same column, were significant differences with P < 0.05

Table 3 showed that the average growth in the mass of discus fish at the age of 30 days of the 4 experiments after 30 rounds of experiments has an overall mean value in the mass of the 1 experiment; 2, 3, and 4 scored 34.77, respectively; 34,87; 34.98 and 34.42 mg/head are equivalent (p < 0.05). Although the growing movement in each experimental batch fluctuated in volume growth, it was not significant, from which we see that the addition of industrial feed at different times did not show a difference in volume. The initial reason can be explained: because the duration of the experiment is quite short (20 days in total), and each time of addition is separated by 5 days, so the difference has not been shown (P < 0.05), and at the same time the growth of the fry of Auntie develops also according to the Behaviours of development of the species, initially increasing rapidly in length, then growing rapidly in mass.

#### Fish Length Development

Table 4: Length growing (mm, n= 30 \* 4 = 120)

No.	W10 Initial (mm/	W30 (mm/con) Final			
	con)	1 (M ±m)	2 (M ±m)	3 (M ±m)	4 (M ±m)
NT1(10)	$5.33 \pm 0.12$	11.46ª ±0.34	17.14ª± 0.26	16.92ª± 0.22	$17.30^{\mathrm{a}}\pm0.49$
NT2(15)	$5.49\pm0.09$	$17.46^{\mathrm{a}}\pm0.34$	17.05ª± 0.31	17.95ª± 0.29	$17.15^{b} \pm 0.27$
NT3(20)	$5.53\pm0.29$	$18.57\pm0.78^{\text{b}}$	$18.72 \pm 0.46^{b}$	$18.30^{\mathrm{b}}\pm0.7$	$18.53^{\text{b}}\pm0.21$
NT4(25)	$5.56\pm0.19$	$17.16^{\circ} \pm 0.40$	17.18°± 0.53	17.45°± 0.49	$17.26^{\circ} \pm 0.16$

Letters a, b, and c in the same column have significant differences, with P < 0.05



Figure 3: Length growth

Table 4 and Figure 3 shows that the length of development and growth was an important criterion for evaluating the development of nursery fish. The results of the study showed that the overall average growth in the length of the fish from 10 to 30 days of age in the trials was different, specifically in the NT2 (the time of feed supplementation was 15 days), reached 18.15 mm/head and NT3 (the time of feed supplementation was 20 days) reached 18.53 mm/animal, which was similar (p > 0.05) but higher than 2 experiments NT1 formula (feed supplementation time is 10 days) reached 17.30 mm/animal and NT4 test (feed replenishment time was 25 days) reached 17.26 mm/animal (P < 0.05). The absolute growth in length of trials 1, 2, 3 and 4 at 30 days of age was 18.3 to 18.7 mm/ head, equivalent to 0.63, 0.64 and 0.58 mm/day. NT3 had a longest than others at 4 trials; also, difference was statistically (P < 0.05) but not different from NT2, NT1 and NT4.

#### Survival Rate Table 5: Survival rate

NT	(%)			
	(M ± m)	2 (M ± m)	3 (M ± m)	4 (M ± m)
NNT1(10)	57,92ª±6,13	55,17ª±3,16	$56,63^{a} \pm 4,77$	$56,57^{a} \pm 1,37$
NT2(15)	52,78ª±2,97	57,02ª±4,73	$55,33^{a} \pm 2,73$	$56,71^{a} \pm 3,78$
NT3(20)	72,38 <sup>b</sup> ±5,01	70,17 <sup>b</sup> ± 2,57	$71,80^{b} \pm 0,26$	$71,45^{b} \pm 1,15$
NT4(25)	51,37ª±4,73	52,37ª±2,71	52,27° ± 2,48	$51,31^{a} \pm 1,01$

Letters a, b, and c in the same column have significant differences, with P < 0.05

The survival rate is an important indicator for assessing the ability of fish to develop and the effectiveness of technical measures applied. In addition, determining the survival rate is important in determining and adjusting the appropriate amount of feed during the culture process. Follow-up results of fish survival from 10 to 30 days of age are shown in Table 5 and Figure 4.



Figure 4: Survival Rrate

Food plays a decisive role in the survival rate of discus fish at 30 days of age. The clear difference in survival in the trials affected by different feed addition timing showed that NT3 survival was highest at 71.45% in all of 4 nursery batches, higher than NT1 trials at 56.57%; NT2 reached 56.71% and NT4 reached 55.31% (p < 0.05). The survival rate of Red-necked fish after 30 days ranged from 21.80 to 75.50 %, of which *N. oculata* (106 cells/mL), *B. plicatilis* rotifers (5-7 individuals/mL) and Copepoda (5-7 individuals/mL) had the highest survival rate (75.50 %), among

them No. 2 is the test of using food such as fresh algae, rotifers, Copepoda reached 70.80 %.



Figure 5: Fries at 10 days of age



Figure 6: Fish at 30 days of age

S. guttatus or orange-spotted spine foot, is fished using set nets and fish cages. It is common in markets and attached by consumers through intestinal cleaning and stomach as rabbit stomachs contain more plant as marine weed and phytoplankton. The herbivorous diet gives it the potential to be an inexpensive source of protein for mass consumption. It also appears in the aquarium trade

#### Intestinal and Stomach Volume

At the final stage of 30 days of age, table 6 shows the intestinal length differences between NT20 and 10, 15 and NT25, with a dimension of stomach and length, so stomach volume is bigger in NT20 of fries supplied artificial feed.

NT	Intestine length (mm)		Stomach circumference (cm)	
	1 (M ± m)	2 (M ± m)	3 (M ± m)	4 (M ± m)
NT1(10)	5.22ª±1.13	5.17ª±1.16	$0.63^{\mathtt{a}} \pm 0.17$	$0.57^{\mathtt{a}} \pm 1.15$
NT2(15)	5.38ª±1.97	5.02ª±1.73	$0.33^{\mathtt{a}} \pm 0.13$	$0.41^{a} \pm 3.12$
NT3(20)	6.98 <sup>b</sup> ±1.01	6.17 <sup>b</sup> ±1.57	$0.50^{\rm b}\pm0.26$	$0.85^{\rm b}\pm1.15$
NT4(25)	5.37°±1.73	5.37ª±1.71	$0.27^{\circ} \pm 0.18$	$0.31^{a} \pm 1.01$

Table 6. Intestinal length and stomac	h circumference	(n = 5)	*4 = 20	) in last hatch
Table 0. Intestinal length and stomac	ii circumierence	(II – J	4 - 40	) III last Datell

Letters a, b, and c in the same column have significant differences, with P < 0.05

#### Discussions

Table 2 shows that the environmental factors during the nursery period are appropriate for developing fry. Morning and afternoon water temperature results range from 29.60 - 32.100C. According to Boyd, 1998, a temperature of 25-320C is the right for brackish fish species in warm waters. The pH index has little fluctuation between morning and afternoon, respectively 8.06 - 8.28 and is in the pH range suitable for saltwater fish from 8.00 - 8.50. Dissolved oxygen ranges from 4.00 - 5.00 mg/L, and the average reaches 4.60 mg/L. Salinity ranges from 28 - 30‰. Total aminiac 0 mg/L. Thus, the monitoring results on environmental indicators are consistent with assessment and are always within the appropriate threshold for the growth and development of discus fish [2].

Table 3 shows at the time of supplementation of commercial feed on the 20th day of age, and it shows the mass of Auntie fish. The overall mean was 34.98 mg/head. According to the research results of Juario (1985), when raising Auntie fish (S. guttatus) in addition to maintaining fresh food (algae species Chlorella sp., Isochrysis sp.; rotifer Brachionus sp.) is maintained from 0 to 30 days, as grouper larvae (Crassostrea iredalei) from 1 to 3 days of nursery and Artemia (from day 12), artificial feed is also added at the 20th to 35 days of age. In the study by Hara et al. (1986), artificial diet (TP-2) with a size of 280 µm was added from 23 to 33 days of age, and from 27 to 44 days of age, was supplemented with completed artificial feed with a size of 560 µm (TP-3). The weight of the fish on the 30th day reached 27.40 mg/ head, corresponding to a length of 12.90 mm/fish [4]. Similarly, the absolute growth in volume (Table 3.4 and Figure 3) of trials 1; 2; 3 and 4 are 1.63, respectively; 1,64; 1.64, and 1.61 mg/day are equivalent. At the same time, when considering the special growth at 30 days of age, the trials were: 36.98 to 38.01 g/head and 18.30 to 18.72mm/head compared with feed supplied at 10, 15 and 25 days of age (P < 0.05) in 4 batches of 2020 till 2022. Research on red-necked fish showed that fish length at 30 days of age ranged from 16.50 to 19.00 mm/head [3]. the absolute growth of discus fish from 20 to 40 days of age ranges from 0.07 - 0.12 cm/day [1,5,6]. The interval for reaching the juvenile stage varies by species: 22.0 mm after 45 days of hatching in S. guttatus, 20-24 mm at 21 days compared with S. channel insulates, and 9.5 mm at 11 days or S. fuscescens.

The survival rate in artificial reproduction depends on many factors, as the farm's environmental conditions, the brood's quality and especially the source of food and when to start supplementing with food. Trials in 2021 and 2022 were using dried algae, rotifers, Nauplius Artemia reached 65.50%, and the lowest was the trial using fresh algae, synthetic food, and dried algae, reaching 21.80%. While, the trial resulted that the time of adding commercial feed, played an important role in improving the ratio from 10 to 30 days of age. For a high survival rate and enhanced growth rate in fry rearing, it is necessary to provide rotifers at a density of 7 head/mL in the first 5 days, then gradually add Nauplius Artemia or Copepoda in the following days [5]. With resulted adding the artificial feed at 20 days of age shown a best survial rate, more than 70% compared with 60 - 63 % of 10, 15 and 25 days of age. In an experiment feeding Auntie (S. guttatus) artificial feed containing about 40, 45, 50 and 55% crude protein for 21 days, Artemia nauplii were administered as a control feed. Discia flour performed equally well on all artificial diets with characteristic growth rates of 7.80 - 8.35 - 95.35 transition rates of 95.2 - 97.9% and survival rates reached 59.9 - 70.3 %. In contrast, fry using Artemia feed exhibited poorer growth (5.03) and low survival (51%), which could be attributed to inadequate feeding levels or poor nutritional quality of Artemia. Many fish in the genus Siganus, idea including S. guttatus, accept a variety of feeds under feeding conditions (Carumbana and Luchavez, 1979). Therefore, diets with 40% protein and an estimated energy content of 3,971 kcal/kg can be used with greater results in producing Auntie Fish [7]. In hatcheries, marine fish and shellfish larvae production still depends on live food supplies, such as rotifers and Artemia. However, the cost of producing fresh feed is high and labour-intensive (Dhert et al., 1995), encouraging seed production facilities to find suitable and cost-effective alternatives.

When feed was supplied at 20 days, compared with supplementation at 10, 15 and even 25, they also did not get better development of the digestive tract, so they needed food, but the suitable time for giving the artificial feed needed to well understand and feeding correction [5]. Enrique M. Avila, Jesus V. Juario, 1987 stated that the artificial feed stimulates the intestinal tract in the completed development.

#### Conclusions

The appropriate time to add commercial feed (INVE, Thailand) to fish is 20 days old, twice daily, and 2.5% live-weight, has enhanced growth and has a survival rate of more than 70%. The growth rate of the fish is from 10 to 30 days old, with a length of 13.68 - 13.95 mg/day. Artificial diets stimulated the stomach volume and intestine of larvae and fried them during nursery stages [8-17].

#### **Competing Interests**

The authors have no competing interests to declare relevant to this article's content.

#### Acknowledgements

This work was supported by a grant NVQG-2019/DA.18 (Ministry of Science and Technology, Vietnam). We would like to thank Nguyen Duy Quynh Tram, Dean of the Faculty of Fisheries and Nguyen Van Huy, Hue Institute of Biotechnology (Hue University), for their assistance during the sampling period in the station and mechanism support. The authors thank Mr Nguyen Khoa Huy Son and Dr Nguyen Anh Tuan (Hue Uni. Research and Training station) for materials and tools.

#### **Authors' Contribution**

Tran Nguyen Ngoc & conceptualization, methodology, investigation, revision and editing; Nguyen Quang Linh: conceptualization, methodology, investigation, project administration; Tran Vinh Phuong: data analysis, writing – original draft; Tran Nguyen Ngoc investigation,

#### **Informed Consent Statement**

Not applicable

#### **Data Availability Statement**

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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