



## Effects of stocking density on performance of snubnose pompano juvenile (*Trachinotus blochii*) reared in recirculating system

Tran Ngoc Hai<sup>1\*</sup>, Tran Nguyen Duy Khoa<sup>1</sup>, Kotani Tomonari<sup>2</sup>, Ly Van Khanh<sup>1</sup> and Le Quoc Viet<sup>1</sup>

<sup>1</sup>College of Aquaculture and Fisheries, Can Tho University, Viet Nam

<sup>2</sup>Faculty of Fisheries, Kagoshima University, Japan

\*Correspondence: Tran Ngoc Hai (email: [tnhai@ctu.edu.vn](mailto:tnhai@ctu.edu.vn))

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### ABSTRACT

This study aimed to determine appropriate densities for rearing pompano juvenile (*Trachinotus blochii*) in a recirculating system. Four treatments of stocking density were evaluated including 500, 1,000, 1,500, and 2,000 inds/m<sup>3</sup> in triplicate. The experimental system for pompano rearing was designed with 120-L tanks connected to 1 settling tank, 1 filter tank, 1 stone filter tank (contained 250 L of filter volume for each tank). The system was fulfilled with marine water at 30 ‰ of salinity and aerated continuously. Pompano were initially stocked at 0.07 ± 0.03 g/fish of body weight and daily fed using commercial pellet contained 55% of crude protein. After 30 days of rearing, the fish stocked at 1,000 individuals/m<sup>3</sup> showed the best results in growth rate (0.060 g/day of DWG and 10.97 %/day of SGR) and was significantly higher than the treatment stocked at 1,500 and 2,000 inds/m<sup>3</sup> ( $p < 0.05$ ), however, no significant difference was observed between 1,000 and 500 treatments ( $p > 0.05$ ).

### 1. INTRODUCTION

The rapid expansion of aquaculture sector in recent years leads to several issues in water usage and environmental pollution (Zhang et al., 2011). Recirculating aquaculture system (RAS) has been highlighted as an eco-friendly system in aquaculture production with advantages in increasing fish production while conserving water, improving overall waste capture efficiency, and improving water quality for a variety of freshwater and marine fish species (Timmons et al. 1998; Pfeiffer and Riche, 2011; Badiola et al., 2012). RAS was widely applied in the hatchery and commercial production of several aquatic species such as white leg shrimp (Suantika et al., 2018), rainbow trout *Oncorhynchus mykiss* (Pulkkinen et al., 2018), Nile tilapia *Oreochromis niloticus* (Sri-uam et al., 2016), and cobia

*Rachycentron canadum* (Díaz-Muñoz et al., 2019), whereby the promising results were remarked. However, high stocking density and fish growth resulting in increased metabolic wastes, the exchange rates in RAS are reduced and the concentrations of a variety of water constituents increase; this deterioration of water quality may affect fish growth (Pulkkinen et al., 2018; Shao et al., 2019).

Stocking density is a pivotal factor affecting fish welfare in the aquaculture industry, especially RAS where high densities in confined environments are aimed at high productivity (Espinal & Matuli, 2019). Previous studies have reported that stocking density is one of the most important variables affecting fish growth, health, metabolism and welfare (Costas et al., 2008; Laiz-Carrión et al., 2012; Ni et al., 2014; Shao et al., 2019). Moreover, the inverse

growth in cultured fishes was related to stocking density due to social interactions (Irwin et al., 1999; Ellis et al., 2002). Inappropriate stocking densities might result in reduction of growth and immune competence due to factors such as social interactions and deterioration of water quality, which can affect both feed intake and FCR of the fish (Chavez et al., 2011).

Snubnose pompano (*Trachinotus blochii*) has been considered an economically important fish for marine aquaculture due to high market value, rapid growth and high adaption to formulated feed (Chavez et al., 2011; Teletchea, 2015). This study was carried out to evaluate the effect of different stocking densities on the growth performance, survival rate and biomass of *T. blochii* juvenile reared in RAS.

## 2. MATERIALS AND METHODS

### 2.1. Experimental design and fish culture

Four treatments of stocking density were evaluated including 500 (NT1); 1,000 (NT2); 1,500 (NT2); and 2,000 (NT4) fish/m<sup>3</sup> in triplicate. The RAS system for pompano rearing was designed with 120-L tanks connected to 1 settling tank, 1 filter tank, 1 stone filter tank (each tank contained 250 L of filter volume). The RAS was fulfilled with marine water

at 30‰ of salinity and operated with continuous aeration for one week before stocking fish. The experiment was set up randomly and lasted for 30 days.

The juveniles of *T. blochii* were produced from Marine hatchery in College of Aquaculture and Fisheries, Can Tho University and acclimated for 1 week in RAS system. During the acclimation, the fish were satisfied fed twice a day at 8 AM and 3 PM using commercial pellets (containing 55 % of protein, 9 % of lipid, and 1.9 % of fiber). After 1 week, the active, uniform size and healthy juveniles were selected and transferred to the experimental RAS system at designed densities.

### 2.2. Management and sampling

During the experimental period, the fishes were satisfied fed four times per day at 7 AM, 10 AM, 2 PM and 5 PM following the protocol in Table 1. Dissolved oxygen (DO), temperature, and pH were monitored twice daily (at 7 AM and 2 AM) using a digital meter (HI-98196 Multi-Parameter Waterproof Meter, HANNA Instruments, Ltd.). Total ammonium nitrogen (TAN), nitrite/nitrate and alkalinity were measured every 3 days by Sera test kits (Germany). The light density exposed to the systems was recorded four times daily (at 6 AM, 9 AM, 12 AM, 3 PM, and 6 PM) using an EasyView™ wide range light meter (Extech EA30).

**Table 1. The feeding protocol and proximate compositions of feed**

Day	Code	Size (µm)	Proximate composition
1-10	NRD 3/5	300 - 500	Protein 55 %, Lipid 9 %
10 - 20	NRD 5/8	500 - 800	Fiber 1.9 %, Moisture 8 %
20 - 30	N46L	800 - 1.000	Protein 46%, Fiber 5 % Lipid 10 %, Calcium 3.5 %, Phosphorus 2.5 %.

Random sampling was conducted every 10 days wherein 30 fishes per tank were individually measured body weight and total length. At the end of the experiment, the fish were harvested to determine the survival rate (SR), daily weight gain (DWG), specific growth rate (SGR), daily length gain (DLG) and specific growth rate in length (SGRL) as follows:

$$DWG = \frac{\text{Final weight} - \text{Initial weight}}{\text{Day of culture}}$$

$$SGR (\%) = \frac{100 * (\text{Ln final weight} - \text{Ln initial weight})}{\text{Day of culture}}$$

$$DWG = \frac{\text{Final weight} - \text{Initial weight}}{\text{Day of culture}}$$

$$DLG = \frac{\text{Final length} - \text{Initial length}}{\text{Day of culture}}$$

$$SGRL (\%) = \frac{100 * (\text{Ln Final length} - \text{Ln Initial length})}{\text{Day of culture}}$$

$$SR (\%) = \frac{\text{Final stock} * 100}{\text{Initial stock}}$$

### 2.3. Statistical Analysis

Data were presented as mean ± standard division (SD) and were subjected to one-way ANOVA (SPSS 16.0 for Windows, IBM, Armonk, NY, USA) and Duncan's test was applied. All differences were considered at α = 0.05.

### 3. RESULTS AND DISCUSSION

#### 3.1. Dissolved oxygen (DO), temperature, pH, light density

During the experimental period, the temperature ranged from 28°C to 31.5°C, pH values fluctuated from 7.7 to 8.3 and DO was within 4.5 to 5.4 mg/L. However, there was no significant difference among treatments (Table 2). Previous studies highlighted that the optimal temperature for pompano was within 18 and 31°C, in which the juvenile rearing required an appropriate range from 27°C to 31°C (Hanh, 2007; Ha, 2014). Several studies reported that temperature significantly affected the fish survival, metabolism, food intake, swimming speed and cost of transport (Brest et al., 1996; McCarthy et al., 1998; Claireaux et al., 2006). Therefore, the temperature was an important factor that required maintaining an appropriate range during the

experiment. Besides, the pH value in this study was in line with the optimal range (7.5 to 8.7) for pompano rearing suggested by Hanh (2007) and Dung (2015). Importantly, optimal physicochemical parameters of water quality are essential to a healthy, balanced, and functioning aquaculture system (Kramer, 1987; Makori et al., 2017). In which, the DO level in water is a critical factor that affects the growth, survival, distribution, behavior, and physiology of fish (Kramer, 1987). A range of DO from 4.5 to 5.4 mg/L in this study is acceptable for the rearing phase of fish (Sim et al., 2005; Moretti et al., 2005; Hanh, 2007). However, for intensive aquaculture production operated RAS, the DO level > 5 ppm was essential to support good fish production due to the high DO consumption of fish and micro-community in the system (Makori et al., 2017; Duarte et al., 2019).

**Table 2. The average values of temperature, pH and DO**

Treatment	Temperature (°C)		pH		DO (mg/L)	
	7 AM	2 PM	7 AM	2 PM	7 AM	2 PM
NT1	28.7 ± 0.2	31.2 ± 0.3	8.0 ± 0.2	8.1 ± 0.2	4.9 ± 0.5	4.6 ± 0.3
NT2	28.7 ± 0.3	31.2 ± 0.2	8.0 ± 0.1	8.1 ± 0.2	4.8 ± 0.3	4.6 ± 0.2
NT3	28.8 ± 0.1	31.2 ± 0.1	7.9 ± 0.2	8.0 ± 0.2	4.8 ± 0.3	4.5 ± 0.3
NT4	28.6 ± 0.2	31.2 ± 0.2	8.0 ± 0.1	8.0 ± 0.1	4.9 ± 0.1	4.6 ± 0.1

**Table 3. The average light density exposure to the RAS**

Treatment	Light density (lux)				
	6 AM	9 AM	12 AM	3 PM	6 PM
NT1	51 ± 2	2.914 ± 407	4.953 ± 959	3.042 ± 326	35 ± 1
NT2	51 ± 5	3.100 ± 310	5.067 ± 462	3.420 ± 261	34 ± 2
NT3	55 ± 3	3.355 ± 322	5.854 ± 636	3.575 ± 267	37 ± 2
NT4	54 ± 5	3.415 ± 353	6.168 ± 126	3.748 ± 220	36 ± 1

The light density (lux) among treatments significantly fluctuated during the day-night cycle (Table 3). However, there was no remarkable difference in light density among treatments. According to Batty et al. (1990), and Didrikas and Hansson (2009), most pelagic fish are visual predators, whereby their behavior and activity are strongly affected by the diel light cycle and density. In which, the fish swimming speed and feeding behavior associated with bioenergetics models of fish. Therefore, light density is an important factor that needs to consider for the culture systems, especially for the indoor RAS systems.

#### 3.2. Total ammonia, nitrite and alkalinity

In RAS system operation, water quality is an important function that relates to the stability of the culture systems. In which, the bacterial communities play a central role in maintaining water

quality (Rurangwa & Verdegem, 2015). The results from this study showed low levels and narrow variation ranges of TAN, nitrite during the experimental time. The TAN concentration among treatments ranged from 0.5 to 0.64 mg/L, while nitrite was from 0.9 to 1.15 mg/L (Table 4). However, no significant difference in TAN and nitrite was recorded among treatments. According to Galvan et al. (2016), TAN and nitrite levels in RAS systems should be controlled at lower than 5 mg/L. But a concentration at < 0.1 mg/L of TAN and 1.5-2 mg/L of nitrite for marine hatchery (Sim et al., 2005; Moretti et al., 2005; Kroupova et al., 2005; Jayakumar & Nazar, 2013). Moreover, these levels gradually increased along with the increase in fish age –size, therefore, the fish could adapt accordingly. Low and stable levels of TAN and nitrite in this study also indicated that the RAS was functionally and stably operated during the experiment.

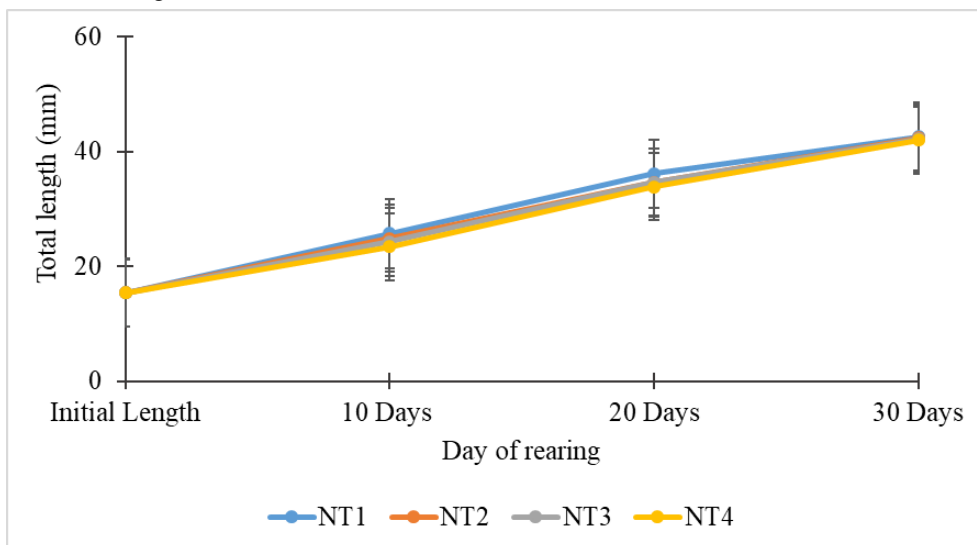
**Table 4. The average values of nitrite, TAN and alkalinity in treatments during the experimental period**

Treatment	Nitrite (mg/L)	TAN (mg/L)	Alkalinity (mg CaCO <sub>3</sub> /L)
NT1	1.08 ± 0.04	0.53 ± 0.01	105 ± 1
NT2	1.10 ± 0.01	0.54 ± 0.01	105 ± 1
NT3	1.14 ± 0.01	0.56 ± 0.04	105 ± 1
NT4	1.14 ± 0.01	0.62 ± 0.02	106 ± 2
Bio-filter input	1.12 ± 0.03	0.56 ± 0.04	105 ± 1
Bio-filter output	0.98 ± 0.02	0.53 ± 0.03	105 ± 2

The bacterial communities in RAS play important roles in nutrient removal, but also consume alkalinity. The gradual decline in alkalinity during shrimp cultivation was the major indicator of nitrification (Rurangwa & Verdegem, 2015). Therefore, the

alkalinity level in the systems requires frequent correction and maintenance within a suitable range for the efficient nutrient removal process.

**3.3. Growth performance of fish**



**Figure 1. The total length of fish among treatments during the experimental period**

**Table 4. Growth in length of fish among treatments**

Treatment	L <sub>0</sub> (mm)	L <sub>t</sub> (mm)	DLG (mm/day)	SGR <sub>L</sub> (%/day)
NT1	15.37 ± 2.40	42.59 ± 0.13 <sup>c</sup>	0.907 ± 0.004 <sup>c</sup>	3.39 ± 0.01 <sup>b</sup>
NT2	15.37 ± 2.40	42.39 ± 0.17 <sup>bc</sup>	0.901 ± 0.006 <sup>bc</sup>	3.38 ± 0.02 <sup>b</sup>
NT3	15.37 ± 2.40	42.15 ± 0.14 <sup>ab</sup>	0.892 ± 0.005 <sup>ab</sup>	3.36 ± 0.01 <sup>a</sup>
NT4	15.37 ± 2.40	41.95 ± 0.13 <sup>a</sup>	0.886 ± 0.004 <sup>a</sup>	3.35 ± 0.01 <sup>a</sup>
P - Value		0.003	0.004	0.004

Alphabetical letters in a column indicate a significant difference among treatments (ANOVA, Duncan's test,  $a < b < c, p < 0.05$ )

After 30 days of rearing, the fish reached to 41.95 – 42.59 mm with 0.886 – 0.907 mm/day of DLG and 3.35 – 3.39 %/day of SGR<sub>L</sub> (Table 4 and Fig.1). In which, NT1 treatment showed a significantly higher total length, DLG and SGR<sub>L</sub> compared to NT3 and

NT4 ( $p < 0.05$ ), but not NT2 ( $p > 0.05$ ). The length of the fish in NT4 was recorded with the lowest increase in length and was not statistically different compared to NT3.

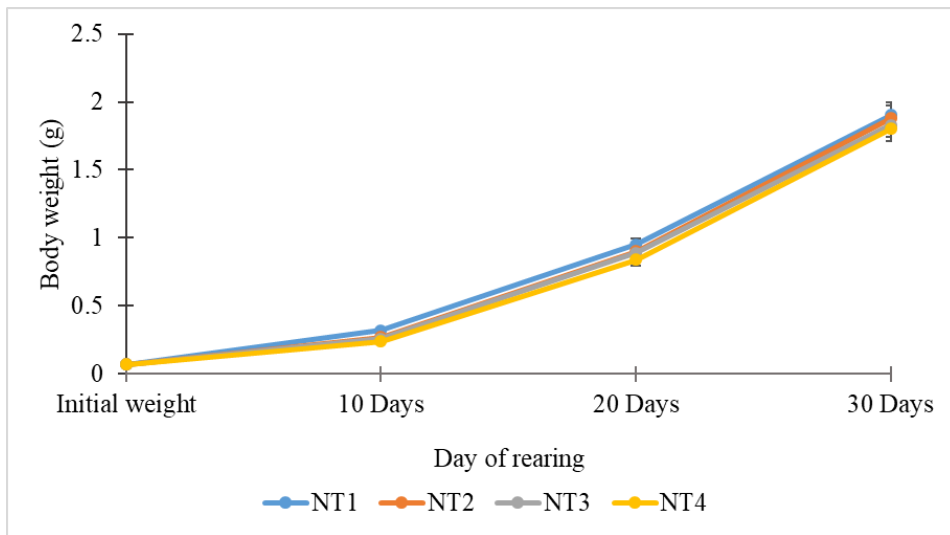


Figure 2. The body weight of fish among treatments during the experimental period

Table 5. Growth in body weight of fish among treatments.

Treatment	W <sub>0</sub> (g)	W <sub>t</sub> (g)	DWG (g/day)	SGR <sub>w</sub> (%/day)
NT1	0.07 ± 0.03	1.91 ± 0.03 <sup>c</sup>	0.061 ± 0.001 <sup>b</sup>	11.01 ± 0.05 <sup>b</sup>
NT2	0.07 ± 0.03	1.88 ± 0.04 <sup>bc</sup>	0.060 ± 0.002 <sup>b</sup>	10.97 ± 0.07 <sup>b</sup>
NT3	0.07 ± 0.03	1.83 ± 0.01 <sup>ab</sup>	0.058 ± 0.001 <sup>a</sup>	10.88 ± 0.02 <sup>a</sup>
NT4	0.07 ± 0.03	1.79 ± 0.02 <sup>a</sup>	0.057 ± 0.001 <sup>a</sup>	10.81 ± 0.03 <sup>a</sup>
<i>p</i> -value		0.004	0.006	0.003

Alphabetical letters in a column indicate a significant difference among treatments (ANOVA, Duncan's test,  $a < b < c$ ,  $p < 0.05$ )

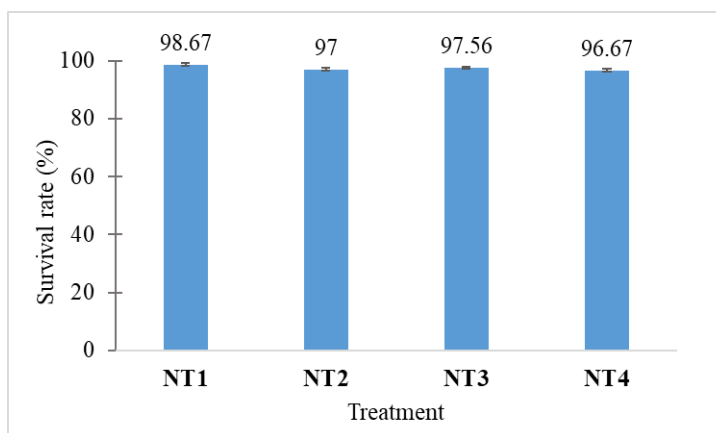
On the other hand, the bodyweight of fish ranged from 1.79 to 1.91 g/fish with 0.057-0.061 g/day of DWG and 10.81-11.01%/day of SGR<sub>w</sub> (Fig. 2 and Table 5). The treatment of NT1 and NT2 showed a significantly higher DWG and SGR compared to NT3 and NT4. However, the final body weight of fish in NT2 and NT3 was not statically different ( $p > 0.05$ ). Remarkably, the NT1 showed the highest body weight (1.91 ± 0.03 g), while the lowest body weight was recorded at NT4 (1.79 ± 0.02 g) ( $p < 0.05$ ).

Several studies have been evaluated the effects of stocking density on the performance of marine juvenile fishes such as red porgy (*Pagrus pagrus*) (Maragoudaki et al., 1999), European sea bass (*Dicentrarchus labrax*) (Sammouth et al., 2009) and gilthead sea bream (*Sparus aurata*) (Parma et al., 2020), in which, most of the fish juveniles could adapt well to the high rearing density condition. This

study showed the high performance of Pompano *T. blochi* at the stocking density of 500 – 1000 juvenile/m<sup>3</sup>, it could be explained by the strong schooling behavior of fish (Chavez et al., 2011). However, the pompano is an extremely active fish, so high stocking density in RAS might induce stress and cause growth inhibition (Chavez et al., 2011).

### 3.4. Survival rate of fish

After 30 days of rearing, the survival rate of fish ranged from 96.67 to 98.67%. However, no significant difference was observed among treatments ( $p > 0.05$ ). Previous studies also reported high survival of pompano juveniles from 95 to 100% (Manomaitis et al., 2007; Lan et al., 2007; Chavez et al., 2011). The high survival rate of pompano juveniles in high stocking density conditions could contribute to mass seed production of this species applied RAS technology.



**Figure 3. Survival rate of fish among treatments after 30 days of rearing**

#### 4. CONCLUSION

Pompano juveniles reared in RAS system proved a hardy and high tolerant fish in the capacity conditions. The RAS system was functionally and stably operated during the experiment lead to the stability of water quality. At 1000 inds/m<sup>3</sup> of stocking density in RAS, *T. blochii* juveniles showed promising results in growth performance and survival rate.

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