



# Juvenile production technology for tiger shrimp, *Penaeus monodon*, through different stocking density using a recirculation system

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## Abstract

Aquaculture recirculation technology has evolved in recent years, as it can save water use and maintain good water quality during tiger shrimp fry rearing and ultimately increase juvenile production. The recirculation technology in this experiment is expected to be adopted by small-scale tiger shrimp seed farmers. This study aims to develop the technology for producing a juvenile tiger shrimp recirculation system to support shrimp cultivation in ponds. The recirculation system container comprises a biofiltration tank filled with oysters, seaweed, and tilapia. Containers to keep tiger shrimp larvae alive in the recirculation system use a round-shaped container with a volume of 2 m<sup>3</sup>. Tiger shrimp larvae were cared for at; A. density of 2000 individuals/m<sup>2</sup>; B. density of 1500 individuals/m<sup>2</sup>; and C. density

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of 1000 individuals/m<sup>2</sup>. This study found that the survival rate of tiger shrimp in treatments C and B were high, reaching 96.800<sup>b</sup> ± 2.716% and 91.62<sup>b</sup> ± 3.432%, respectively, while survival in treatment A was the lowest, at 81.700<sup>a</sup> ± 4.715%.

#### KEYWORDS

production, seeds, shrimp, water quality

## 1 | INTRODUCTION

Tiger shrimp, *P. monodon* is one of the most popular aquaculture commodities and is in demand by the public for cultivation because it has a high market share and selling value. However, the development of its cultivation is relatively slow compared to vannamei (*L. vannamei*). The problem often encountered in tiger shrimp farming in ponds is the lack of availability of quality and continuous seeds. Farmers prefer juvenile-sized tiger shrimp stockings because juvenile size is more resistant to environmental conditions than post-larval (PL) size seeds. Tiger shrimp juveniles for pond maintenance usually have higher productivity and a faster maintenance time than small fry use (<PL-12). Currently, tiger shrimp juvenile production to meet market demand continues to be developed in the pond community. Farmers choose to keep tiger shrimp fry from PL 10 to PL 12 because the price is cheaper. However, if it is directly stocked in the pond, it is at risk, especially for high mortality. Tiger shrimp juvenile sellers set juvenile prices adjusted to the maintenance age. The longer the maintenance, the more expensive the juvenile selling price. Pond farmers maintain such seeds for 7–30 days in the soil pond, concrete ponds, fiberglass tanks, or hapas in ponds with water sources from rivers without going through treatment first so that they have the potential to bring pathogens to shrimp (Anh, 2017; Chuchird et al., 2023; Hendradjat & Pantjara, 2012; Pantjara et al., 2021).

The problem experienced by farmers when raising shrimp is if the water quality of the pond decreases, mainly under the influence of the season (climate) and water pollution, and tiger shrimp fry are susceptible to disease, primarily by viruses and bacteria, resulting in slow growth and even causing shrimp death (Chaiyapechara et al., 2022; Chen et al., 2022; FFlegel, 2019; Geetha et al., 2022; Kim et al., 2022; Sathish Kumar et al., 2022; Sellars et al., 2019; Senapin et al., 2011; Subash et al., 2022). Good water quality management during seed rearing through a recirculation system can increase tiger shrimp juvenile production (de los Santos et al., 2020; Sfez et al., 2015; Suantika et al., 2018; Xu & Boyd, 2016). A recirculation aquaculture system (RAS) can stabilize water quality so that it remains suitable and can be reused for aquaculture. In addition, the recirculation system can control plankton and algae blooming so that the water remains in good condition (Díaz-Jiménez et al., 2018; Matich et al., 2020; Soares & Henry-Silva, 2019; Yang et al., 2020). The same was reported by Badiola et al. (2012), Martín-Calderón et al. (2015), Xu and Boyd (2016), and Zarain-Herzberg et al. (2010) that the use of recirculation can reduce organic waste and other nutrients so that water quality is in good condition and suitable for use in aquaculture. In the future, the recirculation system will be used more for aquaculture, thereby saving water and being more efficient (Ahmed & Turchini, 2021; Iber & Kasan, 2021; Malibari et al., 2018; Sfez et al., 2015).

Currently, tiger shrimp juvenile cultivation utilizes RAS technology because it requires high capital for its manufacture. However, with a simple recirculation system through filtration, the recirculation system for seed rearing is easy to apply in the community at a cheaper cost. This study aimed to determine the survival of Juveniles at different stocking densities through the recirculation system.

## 2 | METHOD AND MATERIAL

### 2.1 | Experiment location

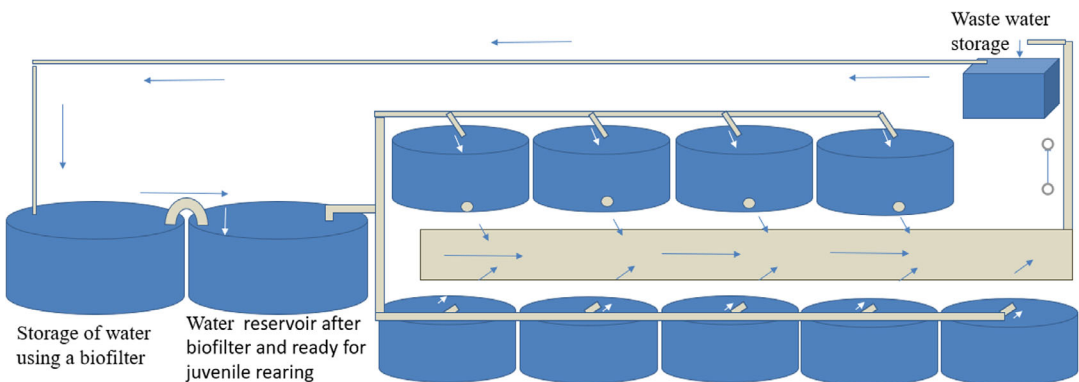
The experiment was conducted in the Experimental Pond Installation (EPI) at the Research Institute for Coastal Aquaculture and Fisheries Extension (RICAPE) in Maros, South Sulawesi, Indonesia, from February to June 2021.

### 2.2 | Preparation and assembly of trial containers

Research preparation began by providing containers with various volume sizes. Then washed it and put the container according to the design placement. The container was equipped with aeration using a blower machine (RESUN LP-200 air pump) of output: 250 lpm, pressure: 0,045 Mpa, freq: 50 HZ, power 200 watts. The recirculation system that was carried out was simple and effective enough to assist in maintaining good water quality during juvenile rearing. Tiger prawn seeds were obtained from a shrimp hatchery in the Barru district, South Sulawesi Province, Indonesia.

The RAS in fiber containers for tiger prawn juvenile production is shown in Figure 1. The container for rearing juveniles consists of nine round fiber containers with a volume of 2 m<sup>3</sup>.

The container for collecting wastewater uses a square fiber tub with a diameter of 1.60 m and a height of 1 m, equivalent to a volume of 2 m<sup>3</sup> or an outlet container (OC). Meanwhile, there are 2 round-shaped containers with a diameter of 2.26 m and a height of 1 m, equivalent to a volume of 4 m<sup>3</sup>, each used to filter water from the OC or water biofilter container (BFC). The other container was used to store a clean water container (CWC) from the biofilter tank. To drain water from one container to another, use a 3/4-inch Polyvinyl chloride pipe (PVC). The water source is the Maros River, and before being used for research, the water was collected for 3 days to precipitate suspended solids. River water collection was carried out in a concrete pool measuring 20 × 5 m<sup>2</sup>. After 3 days, the water in the pool is sucked and flowed to BFC using a Submersible pump (WASSER, WD200EA) with specifications of Head: 6,6 meters and maximum capacity 110 liters/minute. In the BFC container, oysters (*Crassostrea* sp.), seaweed, *Gracilaria verrucosa*, and red tilapia, *Oreochromis niloticus*, were stocked. The oysters stocked as many as 32 individuals/containers (8 individuals/m<sup>3</sup>) and *G. verrucosa* as much as 1.14 kg/container (0.28 kg/m<sup>2</sup>). Oysters in the biofilter container help absorb plankton and microorganisms floating in the water, while *G. verrucosa* serves to absorb excess nutrients from the decomposition of organic residues (Menasveta, 2002). In addition, 3 individuals of red tilapia in size 200–300 g/individual. Tilapia feeds on macroalgae, while shellfish/oysters and seaweed use nutrient-rich organic waste, absorbing nutrients (Iber & Kasan, 2021; Mateo et al., 2020; Mawi et al., 2020). They are



**FIGURE 1** Model of tiger shrimp seed rearing for juvenile production on fiber tanks through recirculation aquaculture systems.

filling water from the CWC to the juvenile rearing container by pumping it through a PVC pipe as much as 1.5 m<sup>3</sup> (1500 L) per container. Water can be added when the water in the juvenile rearing container has begun to decrease and is checked every 3 days, about 10%–15%. This recirculation system can save the use of new water by approximately >80% when compared to conventional because new water is added only to replace water wasted during organic waste disposal and when evaporation occurs.

### 2.3 | Experimental design

Based on the PCR test, the selected post larvae (PL-12) were pathogen-free, and the larvae were healthy and agile. Post larvae were purchased from an Indonesian shrimp hatchery in Takalar Regency, South Sulawesi. The post-larvae were acclimatized before being applied to the larval-rearing container by floating a plastic bag containing the post-larvae until the water temperature and salinity in the larvae bag were set to the same in the larvae-rearing container. Post larvae with an initial average weight of 0.0015 g were transferred to nine fiber containers (2 m<sup>3</sup>). A completely randomized design (CRD) was used in this study, with the following treatment a stocking density of (A) 2000, (B) 1500, and (C) 1000 individuals/m<sup>3</sup> for this treatment, each of treatment with three replicates.

### 2.4 | Feeding management

During rearing, the post-larvae were fed commercial pellets (38% protein content) in crumbles mashed with a blender. The feeding program was regulated through regular check tray monitoring; 40% of the calculated feed rations were given in the morning (07.00 AM), 30% at (12.00 AM), and 30% in the evening (08.00 PM). The feed dose in the first week was reduced from 75 to 60% of body weight, in the second week from 60 to 30% of body weight, in the third week from 30 to 15% of body weight, and in week IV from 12 to 8% of body weight.

### 2.5 | Sample collection and analysis

The juvenile body weight gain was measured every two weeks, and at the end of the study, 100 juveniles were taken using a fine net, and the average weight was measured using a digital analytical scale with a precision of 0.0001 mg and a capacity of 200 gr (OHAUS PX224/e Analytical Balance). Every day, water quality parameters like oxygen (DO meter YSI PRO20), temperature, and pH (Portable pH/ORP Meter HANNA HI98190), as well as salinity (ATAGO MASTER-S/Mill Refractometer), were measured. Meanwhile, total organic matter (TOM), total ammonia nitrogen (TAN), nitrite (NO<sub>2</sub>-N), nitrate (NO<sub>3</sub>-N), and phosphate (PO<sub>4</sub>-P) were measured according to the standard method (USEPA, 2012). The water quality observations were taken every two weeks.

When this study was carried out in conjunction with the transition season from the rainy to the dry season, salinity decreased and increased. Meanwhile, tiger shrimp requires a balance between the ion content in body fluids with the ion content around their environment, so osmoregulation analysis is carried out. The osmoregulatory capacity in hemolymph and larval media were measured every week. Shrimp larval hemolymph was obtained by grinding samples and put in an Eppendorf volume of 1 mL to which anticoagulants had been added prepared from a mixture of solutions (30 mM Na citrate, 26 mM citric acid, 2% NaCl, 0.1 M glucose, 10 mM EDTA) in a ratio of 4:1. Taking the mixture as much as 1 mL using a syringe and then inserted into an Eppendorf tube volume of 1.5 mL and centrifuged using a micro centrifuges (Ohaus, models FC5916) at 5000 rpm for 3 min. A total of one mL of supernatant is taken using a pipette and transferred to the tube provided. To analyze osmolality by inserting as much as 20 µL of supernatant into the Osmometer tube and measuring. In preparation for the analysis of the following sample, the tube is cleaned using a probe cleaner, and drying is carried out. Osmolality was determined by injecting 20 L of

sample into the D-1000 semi-micro model osmometer (Knauer, Berlin, Germany). Osmoregulation capacity (OC) was calculated by the difference in value between hemolymph osmotic pressure and water osmotic pressure, as reported by Lignot et al. (2000).

## 2.6 | Harvesting

Harvesting juvenile was carried out after maintenance (32 DOC) using fine fishing nets, and water was harvested by pouring it through the container's outlet faucet. Growth and survival rate at the end of the study were calculated as follows: Growth rate (GR) = final mean weight-initial mean weight/time (days); Survival rate (SR) = (final number of shrimp/initial number shrimp) × 100%.

## 2.7 | Data analysis

The Survival rate data was transformed to are sin before analysis. The data on tiger shrimp's growth and survival rate were evaluated for normality, and an ANOVA and post-hoc tests of Bonferroni using IBM SPSS program software version 22.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Weight gain and survival rate

The result showed that the weight gain of juveniles in treatment A ranged from 0.19 to 0.28 g/individual. Treatment B achieved a range of 0.210–0.285 g/individual, and treatment C achieved a weight range of 0.25–0.26 g/individual. It appeared that the weight gain of the tiger shrimp juveniles was affected by the growth of shrimp seeds at each treatment. Because of the high stocking density of shrimp, there was competition for space to get food, and these conditions caused shrimp to become stressed, affecting shrimp growth even though it was adequate for nutritional needs. The average weight of tiger shrimps at the start of the study was 0.001 g/individual. After 30 days, the average weight for each treatment was A. 0.24 g/individual, B. 0.25 g/individual, and C. 0.26 g/individual (Table 1).

The length of the nursery, as well as the effect of seed stocking density and competition for food, all affect the weight gain of tiger shrimp juveniles. However, analysis of variance showed no significant difference in weight gain

**TABLE 1** Weight gain and survival of tiger shrimp, *P. monodon* fry at different stocking densities through a recirculation system.

Treatment	Initial weight (g/individual)	Growth		Survival rate (%)
		Range (g/individual)	Final weight (g/individual)	
A. Stocking density of 2000 individual/m <sup>3</sup>	0.001	0.19–0.28	0.24 <sup>a</sup> ± 0.05	81.70 <sup>a</sup> ± 4.71
B. Stocking density of 1500 individual/m <sup>3</sup>	0.001	0.21–0.28	0.25 <sup>a</sup> ± 0.04	91.62 <sup>b</sup> ± 3.43
C. Stocking density of 1000 individual/m <sup>3</sup>	0.001	0.25–0.26	0.26 <sup>a</sup> ± 0.01	96.80 <sup>b</sup> ± 2.72

Note: Mean values with a different letter in the same column indicate significant differences by ANOVA and Bonferroni Tests.

( $p > 0.05$ ) between density treatments. A study of tiger shrimp fry reared in ponds with a density of 1000–2000 ind./m<sup>2</sup> during 30 days obtained a juvenile weight gain of 0.35–0.34 g/individual (Pantjara et al., 2021) and the maintenance of black tiger shrimp up to PL 50 size in net cages in land ponds reaches a weight of  $0.23 \pm 0.06$  g (Utiswannakul et al., 2011). Meanwhile, research conducted by Anh (2017), with the use plastic tank (100 L) filled with 80 L of water and the stocking of tiger shrimp fry as much as 30 PLs (375 individuals/m<sup>3</sup>) for 45 days of maintenance increased weight from 0.03 g / individual to 0.91–0.99 g per individual.

In addition to stocking density, feeding factors can influence juvenile growth. In this study, 38% of the protein contained in pellets could meet the nutritional needs of tiger shrimp growth. The balance of protein in the feed for energy will affect shrimp growth, and non-protein energy, fat, and carbohydrates are used for energy sources. The weight growth of shrimps in this study was better than that reported by (Hendradjat & Pantjara, 2012), who reported an absolute weight of tiger shrimps in the range of 0.12–0.14 g/individual from an average initial weight of 0.001 g/individual in stocking densities of 1000 individuals/m<sup>3</sup> in hapa for 32 days. In addition, other factors, such as environmental factors and food availability, also affect the survival and growth of tiger prawns. Cannibalism, environment (water quality), and feed could affect tiger shrimps' growth and survival rate (Kocsis et al., 2022). Furthermore, the high seed density factor can lead to competition among individuals for food, space, and the use of oxygen. Shrimp that are smaller in size lose when competing for food. Giant shrimp, on the other hand, is easier and faster to obtain food, so the giant shrimp there are, the bigger they become. Meanwhile, good-quality shrimps are uniform in size.

The survival rate of tiger prawns shown in Table 1, and there is a significant relationship between treatment and survival. Treatment C had the highest survival rate for tiger prawns in this study, followed by B, and A had the lowest. The study results showed that the survival rate of tiger shrimps reared in fiber containers of the recirculation cultivation system was  $81.70 \pm 4.71$  percent, lower than treatment B, which was  $91.62 \pm 3.43$  percent, and C was  $96.80 \pm 2.72$  percent after 30 days of rearing. Hendradjat and Pantjara (2012) reported that rearing tiger shrimps in hapa in ponds with a stocking density of 1000 individuals/m<sup>3</sup> for 32 days resulted in a survival rate of 91.33%–95.25%. Meanwhile, Pantjara et al. (2021) reported that tiger shrimp rearing on hapas in ponds with a stocking density of 1000; 1500; 2000, and 2500 individuals/m<sup>3</sup>, respectively can achieve survival of 76.70, 66.42, 63.25 and 54.40 percent after 30 days rearing. In this study, it appears that treatment A has a low survival rate, and this is due to the high stocking density, which caused tiger shrimps to move less freely due to space limitations, so many tiger shrimp are stressed and eventually die. Furthermore, shrimp have the property of eating each other (cannibalism), especially when the shrimp molts or the shrimp's body size is small, and the body is weak, so it is quickly attacked and preyed upon by shrimp that are larger and stronger in size. The high density of shrimp fry also impacts water quality, owing to an increase in dissolved organic matter, TSS, and ammonia.

### 3.2 | The osmoregulation

Osmoregulation is an aquatic animal's attempt to control the balance of water and ions in the body and its environment using osmotic pressure regulation mechanisms (Charmantier & Charmantier-Ôaures, 2001; Lignot et al., 2000). Water's osmotic properties are derived from all water-soluble electrolytes. Table 2 showed the osmolality levels in this study, and it appeared that osmotic levels in all treatments had a value greater than one, indicating that the environmental media were in a hyperosmotic state. Indirectly, high-spreading solids degrade water quality, particularly the influence of organic waste feed waste, excretion, and shrimp metabolites, which can affect the osmoregulation capacity of tiger shrimp. This study discovered that shrimp with densities of 2000 individuals/m<sup>3</sup> have a better osmotic rate than shrimp with a dense spread of 1500 individuals/m<sup>3</sup> or 1000 individuals/m<sup>3</sup>. The high osmotic energy load in shrimp obtained from food for the osmoregulation process will be reduced for the growth process and shrimp survival (Khraisheh et al., 2020). The osmoregulation mechanism allows shrimp to balance their body's osmotic pressure with their life medium. The osmoregulation process usually does not cause stress in shrimp. Osmoregulation is a homeostatic system in shrimp that maintains the stability of body fluids, which are considered the

**TABLE 2** The effect of tiger prawn stocking density on the osmoregulation capacity of recirculating aquaculture systems.

Observation (days)	Treatment	Osmotic pressure (mOsm/l H <sub>2</sub> O)		The osmoregulation capacity (mOsm/kg)
		Water	Hemolymph	
7	A	715.00 ± 6.25	1099.33 ± 76.45	384.33 <sup>a</sup> ± 73.28
	B	718.33 ± 7.37	1067.33 ± 105.51	349.00 <sup>a</sup> ± 98.20
	C	708.77 ± 5.51	873.33 ± 34.82	164.67 <sup>b</sup> ± 31.13
15	A	772.33 ± 22.03	987.00 ± 60.92	214.67 <sup>a</sup> ± 65.73
	B	786.00 ± 6.08	947.33 ± 59.37	161.33 <sup>a</sup> ± 64.30
	C	826.30 ± 5.90	937.67 ± 7.10	111.33 <sup>a</sup> ± 57.45
22	A	764.67 ± 7.57	1122.33 ± 59.35	357.67 <sup>a</sup> ± 63.82
	B	750.33 ± 12.34	1032.33 ± 35.92	282.00 <sup>a</sup> ± 36.17
	C	776.00 ± 20.95	1060.67 ± 21.08	284.67 <sup>a</sup> ± 32.33
30	A	743.67 ± 48.29	1079.33 ± 18.58	335.67 <sup>a</sup> ± 66.52
	B	772.33 ± 22.03	1005.33 ± 47.05	233.00 <sup>a</sup> ± 50.09
	C	828.00 ± 49.37	1028.00 ± 37.60	200.00 <sup>a</sup> ± 19.62

Note: Remark, shrimp density of 2000 ind./m<sup>3</sup> (A); 1500 ind./m<sup>3</sup> (B); and 1000 ind./m<sup>3</sup>(C).

internal environment of body cells. Furthermore, osmoregulation can maintain a balanced state (milieu interior) in blood and other body fluids by regulating the balance of osmotic concentrations between intracellular and extracellular fluids (Brown & Tytler, 1993; Morgenroth et al., 2022; Yamaguchi & Soga, 2020).

The antennae glands, gills, digestive tract, and excretion organs are all involved in the osmoregulation process. Furthermore, according to Lignot et al. (2000), the level of osmotic carried out by shrimp if the conditions of the living medium have a difference in osmotic pressure with the osmotic pressure in their body fluids, which means that the difference between the osmotic pressure of the test medium and the osmotic pressure of the hemolymph fluid determines the rate of osmotic action. The high density of tiger prawns can stress shrimp, disrupting body organs and osmoregulation activity. Table 2 shows the water media, hemolymph osmotic pressure, and osmoregulation capacity varied in each shrimp stocking density treatment. However, the highest osmoregulatory was A, followed by B and C. The high shrimp stocking density affected the osmoregulatory capacity due to differences in osmolarity in the external media and shrimp hemolymph.

The magnitude of the difference in osmolarity between external media and shrimp body fluids influences the level of osmotic action in tiger prawns. Shrimp with a low osmotic rate typically grow faster than shrimp with a high osmotic work rate. On the other hand, shrimps exposed to high osmoregulation capacity tend to use energy from feed to maintain their regulatory power; osmotic changes cause swelling or shrinkage in cells (Chaiyapechara et al., 2022). As a result, the energy obtained from feed may be minimal for the growth process. Although the density of shrimp in treatment A in this study was 2000 individuals/m<sup>3</sup>, tiger prawns in this treatment could adapt to the osmotic pressure of their environment. This adjustment still requires a significant amount of energy, so the energy from the feed is used partly for survival. Thus, if tiger shrimps are subjected to low osmotic pressure, the energy obtained from the feed is used for other physiological processes, including growth.

### 3.3 | Water quality

Water quality is critical as a medium in shrimp culture (Boyd et al., 2016; Boyd & Tucker, 1998). In shrimp farming, the remainder of the feed, excretion, and dead organisms will decompose into organic waste, potentially

contaminating the cultivated water (Iber & Kasan, 2021). Depicts data on water quality observations in tiger shrimp nurseries in fiber tanks with a recirculating culture system. The average dissolved oxygen concentration was more significant than  $5 \text{ mgL}^{-1}$  in all treatments, which was optimal for the growth and survival of tiger shrimps. The high oxygen concentration in the recirculation system during the study was because, in addition to providing oxygen through continuous aeration, the recirculation system provided good oxygen circulation for organisms and microorganisms (Boyd, 1998; Boyd & McNevin, 2021). In this study, the disposal of wastewater in the nursery container of the recirculation system every three days accounts for 5% of the total volume of water. The solubility of oxygen in water decreases as the water temperature rises. Conversely, the higher the level of oxygen consumption, the lower the dissolved oxygen level, affecting the life of tiger shrimps.

During the study, the water temperature ranged from  $27.7$  to  $29.8^\circ\text{C}$ , which was still within the tolerance range for tiger shrimps. Tiger shrimps thrive best at temperatures ranging from  $25.0$  to  $32.0^\circ\text{C}$ . The optimal water temperature for tiger shrimp growth and survival is  $28.0$ – $32.0^\circ\text{C}$ . This study released the dissolved oxygen concentrations in all treatments, which averaged more than  $5 \text{ mgL}^{-1}$ , and was within the tolerable limits for tiger shrimp survival (Table 3). In addition to continuous aeration, the RAS can reduce the use of oxygen by microorganisms by removing some organic waste particles from feed residues and excretions (Chen & Lei, 1990; Iber & Kasan, 2021; Sfez et al., 2015). Aeration in the recirculation system increases the oxygen concentration and stabilizes the water temperature. The water temperature rises, and its oxygen solubility decreases. If the temperature drops, oxygen consumption rises, threatening the life of tiger shrimps. The pH of the water in all treatments ranged from  $6.7$  to  $8.0$ , with an average pH of  $>7.0$ , which was still within a reasonable range for tiger shrimp survival. The ammonia concentration rises if the pH is too high ( $>9.0$ ), reducing shrimp survival.

**TABLE 3** The range and average of water quality (dissolved oxygen, temperature, pH, salinity, and alkalinity) during the study on rearing tiger shrimp fry in recirculation culture systems.

Treatment and water source	Range mean $\pm$ stdev	DO ( $\text{mgL}^{-1}$ )	Temperature ( $^\circ\text{C}$ )	pH	Salinity (ppt)	Alkalinity ( $\text{mgL}^{-1}$ )
A	Range	5.8–6.6	27.7–29.8	7.5–8.0	15.0–26.0	59.0–82.0
	Mean $\pm$ stdev	$6.4 \pm 0.2$	$28.9 \pm 0.6$	$7.7 \pm 0.2$	$20.1 \pm 1.1$	$58.3 \pm 18.2$
B	Range	5.6–6.7	27.7–29.7	7.5–8.1	15.0–29.0	57.4–82.0
	Mean $\pm$ stdev	$6.3 \pm 0.4$	$28.7 \pm 0.1$	$7.7 \pm 0.1$	$20.1 \pm 1.6$	$65.9 \pm 8.3$
C	Range	5.8–7.8	28.0–29.8	6.7–8.0	14.5–28.0	49.2–82.0
	Mean $\pm$ stdev	$6.4 \pm 0.5$	$28.9 \pm 0.6$	$7.6 \pm 0.5$	$20.9 \pm 1.4$	$67.0 \pm 9.4$
inlet 1	Range	5.7–6.3	30.9–31.8	7.5–8.0	14.5–28.0	57.4–82.0
	Mean $\pm$ stdev	$6.0 \pm 0.2$	$29.6 \pm 1.2$	$7.9 \pm 0.2$	$20.8 \pm 1.6$	$67.2 \pm 9.0$
inlet 2	Range	6.1–6.2	28.6–32.5	7.9–8.5	15.1–27.2	49.2–77.4
	Mean $\pm$ stdev	$5.9 \pm 0.4$	$29.6 \pm 1.6$	$8.1 \pm 0.2$	$22.2 \pm 3.5$	$67.0 \pm 11.6$
Outlet	Range	1.3–6.9	28.5–28.8	7.0–8.1	14.5–28.0	69.7–114.8
	Mean $\pm$ stdev	$4.1 \pm 2.9$	$27.6 \pm 1.1$	$7.7 \pm 0.5$	$21.0 \pm 2.5$	$90.1 \pm 20.5$

Note: Remark, shrimp density of  $2000 \text{ ind./m}^3$  (A);  $1500 \text{ ind./m}^3$  (B); and  $1000 \text{ ind./m}^3$  (C).



Water alkalinity acts as a buffer for water media, particularly in stabilizing pH changes, and it aids shrimp in their smooth adaptation to pH. The alkalinity of each treatment in this study was as follows: treatment A ( $58.3 \pm 18.2 \text{ mgL}^{-1}$ ), B ( $65.9 \pm 8.3 \text{ mgL}^{-1}$ ), and C ( $67.2 \pm 9.4 \text{ mgL}^{-1}$ ). High alkalinity was observed at the outlet container, namely  $90.1 \pm 20.5 \text{ mgL}^{-1}$ , and low at the inlet biofilter container and water storage container (inlet 1), with means of  $67.2 \pm 9.0 \text{ mgL}^{-1}$  and at the biofilter container (inlet 2)  $67.0 \pm 11.6 \text{ mgL}^{-1}$ . The ideal alkalinity range for aquatic organisms is  $20.0\text{--}500.0 \text{ mgL}^{-1}$ . The alkalinity range of  $60.0\text{--}140.0 \text{ mgL}^{-1}$  is suitable for aquaculture. According to Boyd et al. (2016), alkalinity and hardness in food fish ponds should be  $60.0 \text{ mg/L}$  or higher. Alkalinity should be greater than  $100.0 \text{ mg/L}$  in estuarine or marine species culture.

The content of organic matter in intensive shrimp farming, if not immediately appropriately handled properly will become a waste burden and pollute the aquatic environment. According to Martínez-Durazo et al. (2019), high pond organic content can have an impact on nitrogen (N) and phosphorus (P) loads in waters. Total organic carbon (TOC) fluctuated during shrimp rearing. However, in treatments A and B, the fourth week had a higher organic carbon content than treatment C. (Figure 2). The high TOC is due to increased feed waste and excretion at the bottom of the container, which is also partially soluble in water. Dissolved organic matter increased in the third week of observation, and the pattern was consistent across treatments; it appears that the organic carbon content increased with the length of maintenance. Feed residue and shrimp feces contribute to organic waste that can dissolve in water, thereby



**FIGURE 2** Observation of water quality in tiger shrimp aquaculture with different stocking densities and cultivation systems Simple recirculation. (A.  $2000 \text{ ind./m}^3$ ; B.  $1500 \text{ ind./m}^3$ , and C.  $1000 \text{ ind./m}^3$ ).

increasing the total organic carbon in aquaculture. Furthermore, by nitrifying bacteria, organic waste decomposes into ammonia, nitrite, nitrate, and carbon dioxide (Patil et al., 2021). The TOC ranges at the end of the study were A (33.6 mgL<sup>-1</sup>), B (33.7 mgL<sup>-1</sup>), and C (20.7 mgL<sup>-1</sup>). The presence of organic matter reduces the availability of dissolved oxygen, particularly during the four-week TOC observation. The dissolved organic carbon concentration in treatments A and B averaged >30 mgL<sup>-1</sup> and was relatively high compared to C; however, the TOC concentration was within tolerable limits for the survival of tiger shrimp. High concentrations of dissolved organic carbon (>32 mgL<sup>-1</sup>) can decrease the resistance of shrimp, and tiger shrimp are susceptible to disease.

Ammonia in water can affect shrimp survival because it causes stress and damage to the gills or tissues. The primary source of ammonia in juvenile cultivation is organic matter content. Because ammonia is a natural byproduct of fish metabolism, it is quickly accumulated. Some bacteria in the nitrification process can convert ammonia to nitrite. The concentration of ammonia in the water rises as the pH of the water rises. The ammonium form is not ionized at high pH and can be toxic to shrimp. Organic matter decomposes to produce enzymes, and organic acids and other acids contribute to lowering the pH of the water medium. Ammonia concentrations were relatively high in treatments B and C, reaching 0.88 and 0.92 mgL<sup>-1</sup>, respectively. However, the concentration was still within the tolerable limit for shrimp survival (Figure 2), indicating that treatment A was still relatively high at week four observations. The water quality, including ammonia levels, appears more stable in the recirculation system. The increased metabolic rate causes an increase in juvenile activity and feeding frequency, and the metabolic process produces ammonia.

Ammonia, nitrite, and nitrate are the most common forms of dissolved inorganic nitrogen ions essential in aquatic life, though ammonia may also be the most toxic (Martínez-Durazo et al., 2019). The direct effect of ammonia, which has not yet killed the shrimp, is the destruction of the gill tissue, particularly on the gill plates, resulting in swelling that interferes with breathing and, in chronic conditions, abnormal shrimp life and even death (AftabUddin et al., 2020; Chen & Lei, 1990; Sfez et al., 2015; Xu & Boyd, 2016). According to Boyd and Fast (1992), NH<sub>3</sub><sup>+</sup> concentrations greater than 1.0 mgL<sup>-1</sup> can impair shrimp growth, cause stress, and even death. The optimal ammonia content for tiger shrimp is 0.1 mgL<sup>-1</sup> (Boyd, 1998).

Various N compounds, including nitrite, were discovered in the accumulated organic waste. Nitrite is a partially oxidized form of nitrogen that lasts only briefly or is a by-product of the oxidation process between ammonia and nitrate. Excessive nitrites can poison shrimp and affect their growth and survival (Martínez-Durazo et al., 2019). Under aerobic conditions, however, nitrite is unstable because sufficient oxygen is readily oxidized to nitrate. Nitrite concentrations in wastewater rarely exceed 1.00 mgL<sup>-1</sup>, and in natural waters, they rarely exceed 0.10 mgL<sup>-1</sup>. However, nitrite is also unstable, particularly in the presence of sufficient oxygen, and nitrite is easily nitrated in aerobic conditions. In general, nitrite concentrations are low in water conditions that increase oxygen concentration. The observation findings are that the nitrate content tends to increase with maintenance time. At the end of the study, the nitrate concentration appeared to rise, and the pattern was nearly identical between treatments. According to Boyd and Fast (1992), the appropriate nitrate concentration in fish and shrimp culture media was 0.20–10.00 mgL<sup>-1</sup>. In each treatment at week four, the nitrate concentration are A. 0.37 mgL<sup>-1</sup>, B. 0.22 mgL<sup>-1</sup>, and C. 0.20 mgL<sup>-1</sup>. The concentration of water nitrates for the growth and survival of tiger shrimp juveniles in the RAS system in this study was still safe.

The concentration of phosphate in each observation varied across all treatments. However, the concentration is still within tolerance limits for tiger shrimp survival. Phosphate concentration in organic waste from feed residues and shrimp excretion is closely related to phosphate availability in aquaculture water media. The form of phosphorus in water is a result of orthophosphoric acid ionization. According to Lefrançois et al. (2010), its solubility in water bodies helps in the formation of proteins and the process of photosynthesis. Overall, phosphate concentration increased by the end of the study. The highest phosphate concentrations occurred in treatments B and C after two weeks of observation and four weeks compared to treatment A and at the outlet and inlet after four weeks of observation. Suspended solids and settled solids are two types of solid waste. Suspended solids in aquaculture come from excretory and feed waste, fine particles suspended in cultured water (Jasmin et al., 2020). TSS concentrations were around 0.02 mgL<sup>-1</sup> at the start of the study. However, in addition to treating shrimp fry in this study using the

pelleted feed, it produces organic waste in the bottom from feed residues and metabolites and is stirred by aeration. During the second to fourth weeks of observation, treatments A, B, and C contained an average TSS of 12.0 mgL<sup>-1</sup>. TSS in treatment A appears to be higher, reaching 26.0–109.0 mgL<sup>-1</sup> compared to treatments B (21.0–82.0 mgL<sup>-1</sup>) and C (20.0–63.0 mgL<sup>-1</sup>). Because the outlet was a reservoir for organic waste originating from the treatment container, the TSS observed at the outlet reached 204.0 mgL<sup>-1</sup> during the fourth week of observation. The alkalinity range of 60.0–140.0 mgL<sup>-1</sup> is favorable for shrimp survival. TSS at the biofilter tank was 115.0 mgL<sup>-1</sup>, while TSS at inlet 2 was 73.7 mgL<sup>-1</sup>. Although the water in the biofiltration container comes from the outlet, the TSS is lower. The low TSS in the biofilter tank container is caused by solid organic particles that settle in the container from cultivation waste disposal, and some of the solid waste undergoes the process of mineralization and nitrification at the outlet.

## 4 | CONCLUSION

In this study, the highest production of juvenile tiger shrimp was obtained in treatments B and C, with survival rates of 91.62% and 96.82%, respectively. Treatment A has the highest osmoregulation capacity of tiger shrimp, followed by B, and the water quality is still suitable for shrimp farming.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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