

## Effects of the Combination of Treflan and Furazolidone as Therapeutants on Molting, Survival, and Growth Performance of Blue Swimming Crab Instar *Portunus pelagicus*

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### Please cite this paper as follows:

Maribao, I. P., Alsim, A. M., Eldani, A. A., & Sarri, J. H. (2023). Effects of the Combination of Treflan and Furazolidone as Therapeutants on Molting, Survival, and Growth Performance of Blue Swimming Crab Instar *Portunus pelagicus*. *Acta Natura et Scientia*, 4(1), 17-26. <https://doi.org/10.29329/actanatsci.2023.353.03>

### ARTICLE INFO

#### Article History

Received: 18.11.2022

Revised: 27.12.2022

Accepted: 28.12.2022

Available online: 17.02.2023

#### Keywords:

Blue swimming crab

Furazolidone

Instar

*Portunus pelagicus*

Therapeutant

Treflan

### ABSTRACT

A variety of antibiotics and other chemicals are used in aquaculture for various purposes, including to promote growth and reduce mortality, as well as to treat and prevent disease. As part of the larval rearing of crustaceans such as prawns and shrimp, antibiotics such as Treflan and Furazolidone are used to increase the survival rate of the post-larvae. In this study, the combined effect of Treflan and Furazolidone on the molting frequency, growth performances, and survival of blue swimming crab (BSC, *Portunus pelagicus*) instar reared in fiberglass tanks was determined. Two treatments with three replicates each were tested. Twenty (n=20) individual crab instars of uniform size (average length  $1.5 \pm 0.00$  cm and average weight  $10.33 \pm 0.00$  g) were stocked per circular fiberglass tanks, each with a water capacity of 200 L for 37 days culture period starting from the crab instar up to the juvenile stage. Treatment 1 corresponds to the control without the application of Treflan and Furazolidone (NTF). Treatment 2 represents the use of Treflan and Furazolidone (TF) at the dosage of 0.2 ppm and 0.3 ppm, respectively. The results showed that the growth and molting frequency of BSC instar were not affected by TF application at the end of the culture period. In both treatments, survival rates declined due to the frequent and excessive use of TF, which might be the reason for descending rates. Hence, the combined effects of TF did not enhance the molting, growth, and survival performance of BSC instars when administered as therapeutics.

### INTRODUCTION

There are a number of economically important aquatic species in the Philippines, including the blue swimming crab (BSC, *Portunus pelagicus*), known locally as "alimasag" and providing family living opportunities (Camacho & Aypa, 2001; Efrizal, 2017;

BFAR, 2020; Toring Farquerabao & Tahiluddin, 2022). In addition to being sold as a processed product or in a live state, BSC is mostly exported to the USA, Japan, Hongkong, and Taiwan (Camacho & Aypa, 2001; Soegianto et al., 2022). It can also be used for preparing fishery meatballs as an alternative raw material (Ajik-Cerbas et al., 2022). Philippine fisheries are dominated

by BSCs, and they are a major component of the domestic seafood industry, representing more than 90% of the catch since crab fishing began in the 1950s (Ingles, 2004; BFAR, 2013; Mesa et al., 2018). A total of 5,924 metric tons of fisheries products were produced by the BSC, constituting 0.01% of total production in 2018 (PSA, 2018). As of 2020, the retail price of BSC was 294 pesos/kg (BFAR, 2020).

A variety of gear is used in the BSC fishery, including gill nets and crab pots (Germano & Melgo, 2003; Del Norte-Campos et al., 2004; Ingles, 2004), gleaning, crab lift nets, and bamboo traps (Romero, 2009; Gadhavi et al., 2013), all of which are used by crabbers. The challenges associated with increasing population pressure and the inaccessibility of wild catching have all contributed to aquaculture has become an important part of rural livelihoods (Carleton et al., 2013). It is the fastest-growing subsector of the food industry, outpacing natural terrestrial meat production and capture fisheries (Tacon, 2001). A growing number of countries in Asia and Africa have introduced aquaculture as a culture to help rural communities escape poverty and improve living standards (Edwards, 2000). As reported by the Food and Agriculture Organization (FAO), there has been a great deal of farming of crustaceans in aquatic environments (FAO, 2022).

In response to increasing demands, the aquaculture industry has become more interested in BSC farming (Andres et al., 2010). The aquaculture potential of BSC is high because it grows rapidly (Josileen & Menon, 2005), has a relatively short larval life cycle, and has high fecundity (Romano & Zeng, 2008). It is essential to understand basic cultural conditions to optimize the production of this species as aquaculture interest increases (Romano & Zeng, 2006). In a lying-in hatchery, the eggs of BSC are held in an enclosed container and monitored until hatching occurs (Andes et al., 2010). Furthermore, antibiotics and other chemical compounds are frequently used in aquaculture as the industry becomes more popular. The use of antibiotics, fungicides, and therapeutants for the rearing of crustaceans such as shrimps, prawns, and crabs has been documented among progressive fish farmers in developed and developing

countries (Uddin & Kader, 2006; Aftabuddin et al., 2009).

A wide variety of antibiotics have been successfully used in crab and shrimp hatcheries in order to prevent or treat disease, improve growth, and increase survival (Azam & Narayan, 2013). The most commonly used antibiotic in crustaceans' hatcheries such as shrimp and crabs are Treflan, Furazolidone, Formalin, Methylene Blue, and Malachite Green (Taufik, 1996; Uddin & Kader, 2006). The control of bacterial and fungal growth in crustacean hatcheries was achieved by using antibiotics such as Treflan and Furazolidone (Tareen, 1982; Baticados & Cecilia, 1988; Wijegoonawardena & Siriwardena, 2000). The antibiotic Treflan was found to kill fungi when applied to shrimp (Tareen, 1982). In addition, preventing the transmission of cholera by shrimp requires treating them with Furazolidone (Baticados & Cecilia, 1988). A number of researchers have considered the use of Treflan and Furazolidone as means for increasing the growth and survival as well as for treating bacteria, fungi, and other pathogenic bacteria which may endanger crustaceans, including shrimp and prawns (Tareen, 1982; Lio-Po & Sanvictores, 1986; Baticados & Cecilia, 1988; Chen, 1992; Nakamura et al., 1994). However, no information regarding the use of antibiotics, Treflan, and Furazolidone in crustacean crabs, especially in young BSCs. Thus, this study evaluates the combined effects of Treflan and Furazolidone on the molting, growth, and survival of BSC instar *P. pelagicus*.

## MATERIALS AND METHODS

### Study Site and Duration

The study was conducted at Multi-species Hatchery, College of Fisheries, Mindanao State University Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Sanga-Sanga, Bongao, Tawi-Tawi, Philippines. The duration of the study was the 37-day culture period from crab instar up to the juvenile stage.

### Source and Transport of Berried Crab

Berried BSCs *P. pelagicus* were purchased from the local fishers in the coastal area of Bongao, Tawi-Tawi.

Two berried crabs having yellow eggs on its belly were selected for this study. The berried crabs were transported for about 30 minutes from the source to the hatchery using a plastic pale with sea water inside and each crab was placed in it.

### **Berried Crab Conditioning**

As the crabs reached the hatchery, each was placed in a plastic basin for initial conditioning. After one hour in the basin provided with seawater (with salinity ranging from 26–30‰ and temperature ranging from 27–31°C) and aeration, each berried crab was treated with a formalin bath to disinfect the presence of fungi and bacteria on the belly. A 10% formalin solution was used for bathing at the rate of 150 ppm in 10 L seawater in the basin for 30 minutes (Quinitio & Parado-Esteva, 2008).

### **Hatching**

A 200 L capacity circular fiberglass tank equipped with an aeration system was used for hatching the crabs. Sand-filtered seawater was used after treating it with 15 ppm chlorine and neutralized with sodium thiosulfate at the rate of 80% of the amount of chlorine applied and allowed aeration to take place for a 24-hour period prior to stocking the berried crab (Quinitio & Parado-Esteva, 2008). The berried crabs were stocked into the hatching fiberglass tank and took observation on the behavior of the crab and monitored the water parameter during the period of hatching. The berried crabs having yellow eggs on its belly are usually hatched three to five days in the hatchery (Allan & Fielder, 2003). As hatching happened, the mother crab was removed from the hatching tank. The newly-hatched larvae were transferred into a previously prepared larval rearing tank with a water capacity of 5 tons

### **Larval Rearing**

The method of Quinitio & Parado-Esteva (2008) was followed for the larval rearing of BSC. A 5-ton capacity rectangular tank was used for the rearing of hatched zoea with a stocking density of 80,000 per ton. The cleaned tank was filled with 5 tons of treated seawater with subsequent aeration was fitted into the larval rearing tank at 1-meter intervals to uniformly

aerate the tank. Feeding with the use of a rotifer at the rate of 30 individuals per liter of water was done twice a day. The supplementary artificial feed of # 0 feed for *Penaeus japonicus* (Grobest Feeds Philippines Inc.) was also added at a rate of 2 g/ton given twice a day. After a week of larval culture, *Artemia* sp. nauplii were introduced as live feed at the rate of 1 L per 5 tons. Harvest of crab instars was done at the end of 23 days period. Active and uniform-size (average length  $1.5\pm 0.00$  cm and average weight  $10.33\pm 0.00$  g) instars were selected for this study. Length-weight measurement as well as the width of the instars, was taken to determine the initial biomass for stocking.

### **Tank and Aeration Preparation**

Calcium hypochlorite at a rate of 30 mg/L was used to clean and disinfect the inside surfaces of the tanks (Quinitio & Parado-Esteva, 2008). The hypochlorite mixture was allowed to stay for at least overnight. After rinsing, the tanks were sun-dried for at least 2 days. The aeration system was set up by connecting the tank's aeration through plastic hoses to the air pipes above the tanks. Each tank was provided with one aeration hose with an air stone attached to the submerged end of the air hose.

### **Seawater Treatment**

Following the method of Quinitio & Parado-Esteva (2008), sand-filtered seawater was used as a culture medium and placed in the six tanks (200 L capacity each). Calcium hypochlorite was applied at a rate of 15 mg/L and allowed the mixture to stay overnight. Sodium thiosulfate was also added at the rate of 80% of the amount of hypochlorite applied to neutralize the residual content of chlorine in the water. Thereafter, continuous aeration of the tanks for 24 hours was employed. Each tank of water was ready to receive the seed stocks.

### **Installation of Shelter and Stocking**

Artificial shelters made of black net material with a 1cm mesh size were installed in each tank. Ten net shelters with a dimension of 30×30 cm each with attached sinker were submerged into each tank to minimize cannibalism and to provide additional surface area for the crabs to attach and hide, especially

during molting. Stocking was done in the early morning with twenty active and uniform-sized BSC instars for each tank.

### Experimental Design

The set-up was arranged using a completely randomized design (CRD). A total of six circular fiberglass tanks (200 L water capacity) were used. Two treatments with three replicates each were tested and evaluated. Treatment I corresponds to the control with no application of Treflan and Furazolidone. Treatment II represents the use of Treflan and Furazolidone at the dosage of 0.2 ppm and 0.3 ppm (Tareen, 1982; Baticados & Cecilia, 1988), respectively. Application of these antibiotics was scheduled after every partial water exchange, which was also done every three-day interval (Quinitio & Parado-Estepa, 2008). Fifty percent (50%) of tank water was uniformly drained from each tank and replaced with new filtered seawater to the desired original level of 200 L.

### Feeding

Instars of BSC were fed twice daily at a five percent (5%) feeding rate of their total body weight. The rations were divided into two equal portions and given in the morning and afternoon, respectively. Spider conch shell meat (*Lambis lambis*) was chopped and blended into very small pieces before being fed to young crabs.

### Sampling

The molting of the crab instars was monitored weekly, starting when they were stocked. This was observed by physically examining each net shelter and counting how many times they molted by looking at their appearance and by observing molted exoskeletons on the shelters. The bottom of the tank was also observed for the presence of molted crabs and their exoskeletons. A weekly sample was taken to determine the growth rate and survival rate of the young crabs. For each tank, 25% of the BSCs were measured for length and weight. Afterward, the samples were returned to their respective tanks. In

addition, crab populations were counted in each tank to determine how many crabs survived. The following formulas were used to determine a specific growth rate (SGR) and survival rate (Kader et al., 2017):

$$SGR = \frac{\ln(W_f) - \ln(W_i)}{\text{Days of culture}} \times 100 \quad (1)$$

Where:

$W_f$  = final weight

$W_i$  = initial weight

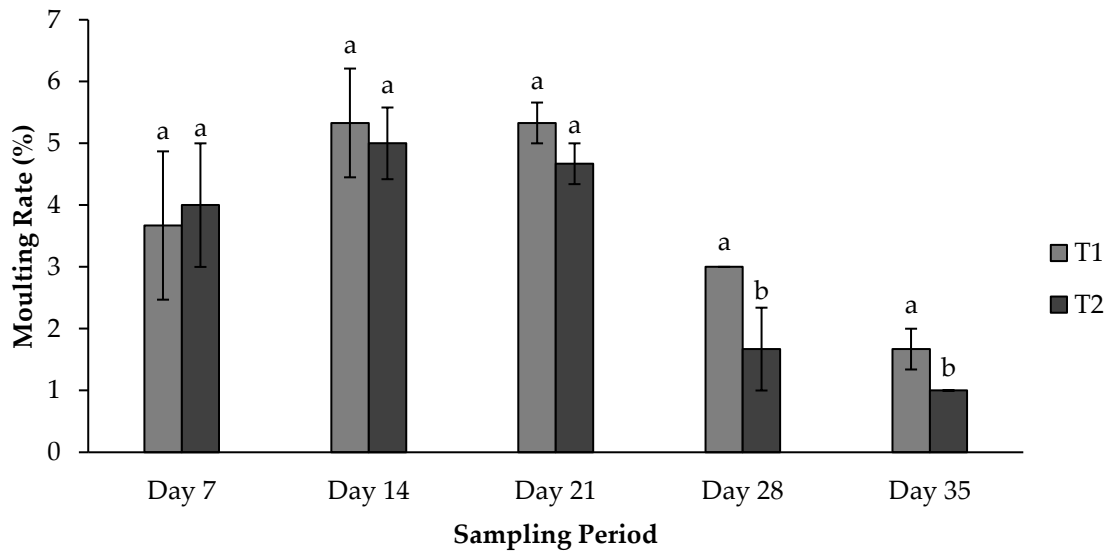
$$\text{Survival rate} = \frac{\text{Final number of stocks}}{\text{Initial number of stocks}} \times 100 \quad (2)$$

### Statistical Analysis

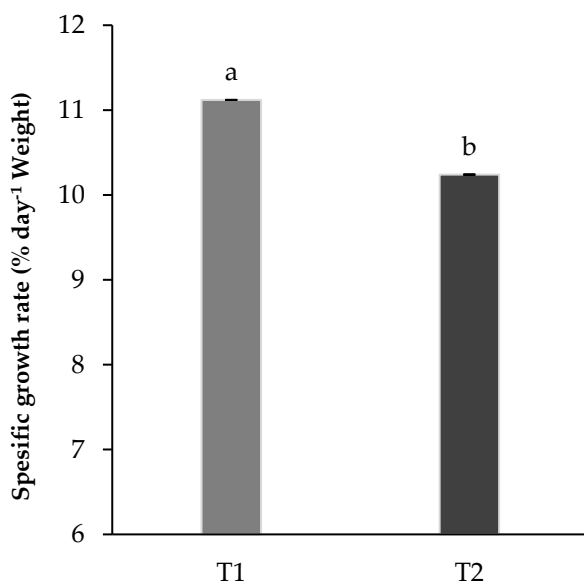
IBM SPSS software version 20 was used to analyze the significance of differences between the molting, growth, and survival of the two treatments using an independent sample *t*-test. This study used a 0.05 significance level. Data were presented as mean±SE (standard error).

## RESULTS

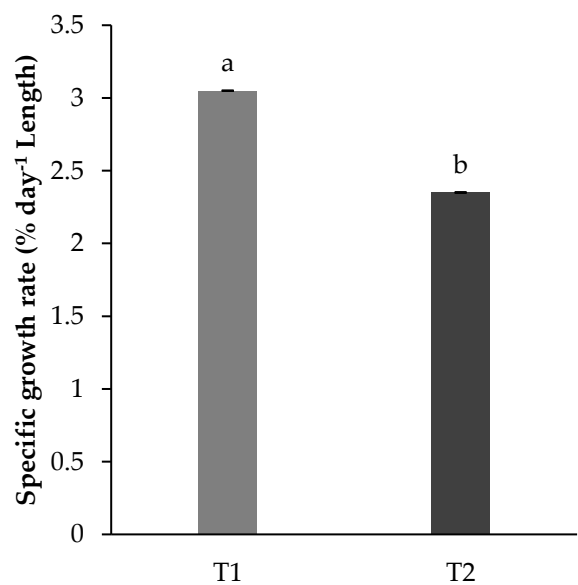
Figure 1 shows the molting rate of the BSC instar. In both experimental groups, the rate of molting increased on Days 7, 14, and 21, with T1 slightly higher than T2, although there was no significant difference ( $p > 0.05$ ) in the rates between the two groups. Moreover, T1 ( $3.00 \pm 0.00\%$ ) was significantly higher ( $p < 0.05$ ) than T2 ( $1.67 \pm 0.67\%$ ) on Day 28. In addition, on Day 35, T1 ( $1.67 \pm 0.33\%$ ) was significantly different ( $p < 0.05$ ) than T2 ( $1.00 \pm 0.00\%$ ) in terms of molting rate. The specific growth rate (SGR) of the BSC instar in terms of weight is shown in Figure 2. SGR of T1 and T2 were  $11.12 \pm 0.00\%$  day<sup>-1</sup> and  $10.24 \pm 0.00\%$  day<sup>-1</sup> respectively. T-test showed that T1 was significantly higher ( $p < 0.05$ ) than T2. In addition, the SGR of the BSC instar in terms of length revealed that T1 ( $3.05 \pm 0.00\%$  day<sup>-1</sup>) significantly improved than T2 ( $2.35 \pm 0.00\%$  day<sup>-1</sup>) (Figure 3). Figure 4 shows the survival rate of BSC instar. The survival rate of T1 and T2 was  $61.67 \pm 6.01\%$  and  $48.33 \pm 6.01\%$ , respectively, indicating no significant difference ( $p > 0.05$ ).



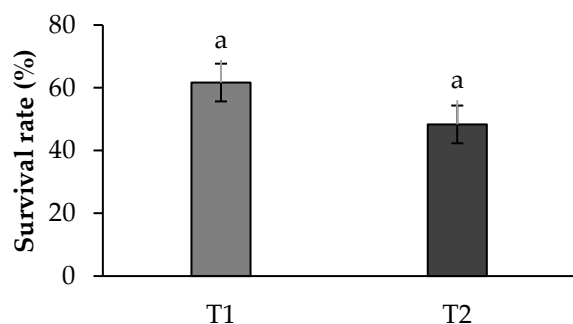
**Figure 1.** Molting rate of BSC instar every sampling period. T1 (No application of Treflan and Furazolidone) and T2 (combination of Treflan and Furazolidone). The bar with the different letters is significantly different ( $p < 0.05$ ). Error bars in SEM (standard error mean),  $n=20$ .



**Figure 2.** Specific growth rate of BSC instars in terms of weight. T1 (No application of Treflan and Furazolidone) and T2 (combination of Treflan and Furazolidone). The bar with the different letters is significantly different ( $p < 0.05$ ). Error bars in SEM (standard error mean),  $n=20$ .



**Figure 3.** Specific growth rate of BSC instars in terms of length. T1 (No application of Treflan and Furazolidone) and T2 (combination of Treflan and Furazolidone). The bar with the different letters is significantly different ( $p < 0.05$ ). Error bars in SEM (standard error mean),  $n=20$ .



**Figure 4.** Survival rate of BSC instars. T1 (No application of Treflan and Furazolidone) and T2 (combination of Treflan and Furazolidone). The bar with the same different letter is not significantly different ( $p > 0.05$ ). Error bars in SEM (standard error mean),  $n=20$ .

## DISCUSSION

As aquaculture has become increasingly popular, the use of antibiotics and other chemicals has become widespread. It has been found that these antibiotics, fungicides, and therapeutants are used to some extent by progressive fish farmers in both developed and developing countries for larval rearing of fin fish and crustaceans such as prawns/shrimps and crabs (Uddin & Kader, 2006; Aftabuddin et al., 2009; Tahiluddin & Terzi, 2021). In the present study, we examined the effects of combined antibiotics such as Treflan (0.2 ppm) and Furazolidone (0.3 ppm) on the growth, molting frequency, and survival rate of BSC instar. According to the results of our study, these antibiotics had no significant impact when administered as therapeutants. Other researchers have stated that the application of 0.1 ppm Treflan has a mild impact on farmed shrimp larvae growth (Chen, 1992; Nakamura et al., 1994). It has been found that Furazolidone (2 ppm) was less effective in terms of survival of the farmed shrimp; however, it was more effective against bacteria such as *Vibrio* sp. (Tareen, 1982). Comparatively, eggs, larvae, and post-larvae of crustaceans such as shrimp could be disinfected with Treflan at therapeutic levels up to 0.2 ppm, followed by complete water changes just before hatching if the water is changed completely before hatching (Lio-Po & Sanvictores, 1986; Baticados & Cecilia, 1988). The use of Treflan as an antifungal agent has been previously studied (Tareen, 1982; Wijegoonawardena

& Siriwardena, 2000). Tareen (1982) found that the application of the antibiotic Treflan to shrimp killed fungi's biflagellated zoospores at 0.1 ppm. Disinfecting shrimp spawners with high concentrations of Treflan (5 ppm) followed by thorough rinsing may be effective (Gacutan, 1979; Baticados & Cecilia, 1988). Furthermore, the bacterium *Vibrio* sp., which is considered to be an essential part of shrimp microbiology, caused high mortality within postlarval and juvenile shrimp, resulting in redly pereopods and pleopods and opaque abdominal muscles (Vanderzant et al., 1971). Using Furazolidone (2 ppm) with Terramycin increased the effectiveness of the treatment against bacteria such as *Vibrio* sp. (Tareen, 1982). Imported shrimp spawners should be treated with Furazolidone to prevent cholera transmission (Baticados & Cecilia, 1988). Wijegoonawardena & Siriwardena (2000) stated that correct diagnosis and treatment are essential to an effective therapeutic treatment. Despite the fact that antibiotics enhance growth, survival, and molting, their excessive and frequent use creates and spreads antibiotic-resistant bacteria, which in turn reduces the growth rate of crab larvae and results in mass mortality of prawns (Karunasagar et al., 1994; Defoirdt et al., 2011; Azam & Narayan, 2013). In the present study, antibiotics (Treflan and Furazolidone) were combined with a high dosage, which may have adverse effects on BSCs instar. Furthermore, antibiotic treatment can lead to morphological changes in crab *Scylla serrata* larvae and juveniles, including deformities in their dorsal, lateral spines, abdominal, and rostral (Pates et al., 2017). Giant crab *Pseudocarcinus gigas* morphology has also been observed to change as a result of excessive antibiotic treatment (Gardner & Northam, 1997). In aquaculture, antibiotic residues may also be found in products. These residues might affect microbe communities, deteriorate the quality of water, and affect human health (Gräslund & Bengtsson, 2001; FAO, 2002). Although crab larvae may survive and grow better with chemicals when compared with controls, however, can suffer adverse effects with prolonged usage at high concentrations (De Pedro et al., 2007). Therefore, crab hatcheries should be careful when using chemicals. In addition to their adverse effects on

the environment and the host, antibiotics and chemicals may pose a serious threat to health risks (Gräslund & Bengtsson, 2001). Hence, the use of alternative biocontrol techniques is recommended as an alternative to eliminating these substances (De Pedro et al., 2007; Pates et al., 2017). In particular, due to their beneficial effect on the crab larvae, probiotics are recommended as a preferred prophylactic approach in instar BSC rearing.

## CONCLUSION

Due to the prevalence of diseases in aquaculture food commodities, antibiotics are now used in aquaculture for a variety of reasons, including promoting growth and reducing mortality, as well as treating and preventing diseases. Among the antibiotics used in the larval rearing of tiger prawns and other shrimp are Treflan and Furazolidone. As a result of the combination of antibiotics such as Treflan and Furazolidone, Blue swimming crab *Portunus pelagicus* instars are adversely affected in terms of their growth, survival, and molting frequency. On the other hand, it may also be due to the immoderate and persistent use of antibiotics that crab weights have declined over time, resulting in descending rates. The use of specific treatments, such as Treflan and Furazolidone, and their positive reactions on other crustaceans, specifically prawns and crabs, needs to be confirmed in more similar studies.

## ACKNOWLEDGEMENT

The authors would like to thank Cherry T. Nian and Ainulyakin H. Imlani. The abstract of this study was presented at the International Symposium on Fisheries and Aquatic Sciences (SOFAS 2022) in Trabzon, Türkiye, on October 25-27, 2022.

## Compliance with Ethical Standards

### Authors' Contributions

IPM: Manuscript design, laboratory experiment, draft checking, statistical analyses, writing, reading and editing.

AMA & AAE: Manuscript design, laboratory experiment, draft checking.

JHS: Statistical analyses, writing, draft checking, reading, and editing.

All authors read and approved the final manuscript.

### Conflict of Interest

The authors declare that there is no conflict of interest.

### Ethical Approval

For this type of study, formal consent is not required.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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