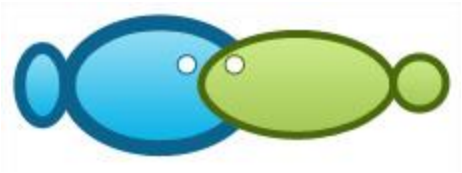

Effects of β-glucan enrichment in *Artemia* on growth performance and survival of mud crab (*Scylla paramamosain*) larvae

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Abstract. The low survival rate during hatchery stages is a major bottleneck in the seed production of mud crab (*Scylla paramamosain*). This study investigated the effects of enriching *Artemia* nauplii with varying doses of β-glucan on the growth performance, metamorphosis, digestive enzyme activities, and survival rates of mud crab larvae. The experiment was structured into five distinct dietary treatments containing different β-glucan concentrations including T1: 0 g L^{-1} (control), T2: 0.25 g L^{-1}, T3: 0.5 g L^{-1}, T4: 0.75 g L^{-1}, and T5: 1 g L^{-1}, administered during the zoea 3 to zoea 5 stages. The experimental results revealed that environmental water quality parameters, total bacterial counts, and *Vibrio* densities remained stable and within optimal thresholds across all treatment groups. While β-glucan enrichment did not significantly impact larval metamorphosis rates, developmental stage indexes, or the total length growth of the crab 1 stage, it noticeably influenced larval digestive physiology. Dietary supplementation enhanced the specific activities of proteolytic enzymes, specifically trypsin and pepsin, as well as lipase, while significantly decreasing amylase activity. Crucially, larval survival rate *megalopa* from the zoea 4 stage onward and overall crablet productivity were significantly enhanced in the 0.5 g L^{-1} and 0.75 g L^{-1} treatment groups compared to the control. The highest survival rate at crab 1 (6.8%) and peak crablet productivity (27,200 individuals m^{-3}) were achieved in the group enriched with 0.5 g L^{-1} of β-glucan. These findings suggest that incorporating β-glucan as a nutritional supplement into standard larviculture protocols optimizes nutrient utilization and boosts physiological resilience.
Keywords: digestive enzymes, immunostimulant, larviculture, nauplii, *Scylla paramamosain*.

Introduction. Mud crab (*Scylla paramamosain*) aquaculture has expanded rapidly in the Mekong Delta, Vietnam, due to the species' high economic value, rapid growth, and adaptability to diverse environments (Ut & Khoa, 2025). Despite mastering hatchery technologies, regional seed production continues to face challenges in meeting commercial demand, primarily due to low survival rates during the larval stages. Currently, larval survival from zoea 1 to crablet remains suboptimal, yielding only 5-15% (Ut & Khoa, 2025). These low survival rates are often exacerbated by infections from filamentous bacteria, pathogenic fungi, and protozoa, which can originate from both the broodstock and the rearing environment (Hudson & Lester, 1994; Lavilla-Pitogo & de la Peña, 2004; Jithendran et al., 2010; Wu et al., 2016). Nutritional protocols are equally critical, as larvae exhibit strict carnivorous feeding patterns that necessitate frequent administration of live feeds such as rotifers and newly hatched *Artemia* during the early zoeal stages (Sanudin et al., 2022). As development progresses, the transition to *Artemia* enriched with high unsaturated fatty acids is essential for successful metamorphosis from the late zoeal stages to megalopa (Suprayudi et al., 2004). Therefore, research is needed to improve the seed production protocols to enhance the quality and survival rate of larvae during rearing, contributing to an increase in both the quantity and quality of juvenile crabs.

In studies on improving the nutritional characteristics of aquatic animals, β-glucan has been shown to play an important role in many physiological and immune functions, increasing survival rates in fish and crustaceans. Accordingly, the use of β-glucan increases survival rates, growth, and immune parameters in whiteleg shrimp *Litopenaeus vannamei* (Yeh et al., 2006; Wongsasak et al., 2015), tiger shrimp *Penaeus monodon* (Chang et al., 2003; Uengwetwanit et al., 2025), and Japanese prawn *Marsupenaeus japonicus* (Adachi

et al., 1999). The application of *β*-glucan as an immunostimulant has demonstrated significant potential in enhancing the physiological resilience and survival of mud crab larvae in hatcheries. Previous studies indicate that β-glucan immersion can substantially influence the survival rates of *Scylla serrata* larvae, particularly during critical developmental transitions from the zoea to megalopa stages (Alaban et al., 2014). This physiological improvement is primarily attributed to the modulation of non-specific immune responses, including the upregulation of phenoloxidase activity and enhanced phagocytic capacity (Meena et al., 2013). Furthermore, β-glucans function as essential feed additives that improve growth performance and bolster resistance against opportunistic pathogens, such as *Vibrio* species, which are known to cause high mortality in hatchery environments (Zhao et al., 2012; Tkaczenko & Kurhaluk, 2025). By stimulating key immune genes and mitigating environmental stressors, these polysaccharides provide a robust mechanism for supporting larval health and productivity (Kristiansen & Amen, 2022). This investigation is an important consideration for diet optimization, provides information on the appropriate dose of β-glucan supplementation to improve the survival rate and growth of larvae in mud crab breeding.

Material and Method. The study was conducted at College of Aquaculture and Fisheries, Can Tho University, from December 2024 to May 2025.

Experimental design. The study used marine water with a salinity of 30 ppt, prepared from brine (100-120 ppt) mixed with tap water, then treated with 50 ppm chlorine and strong aeration. EDTA (10 g m^{-3}) was added to precipitate heavy metals and filtered through a filter bag before being supplied to the rearing tanks. Alkalinity in the culture tanks was maintained at 140-150 mg $CaCO_3$ L^{-1} using NH_4CO_3.

Female crabs with full ovary used in the experiment were sourced from Ca Mau. The broodstock crabs were subjected to eyestalk ablation and reared in a recirculating system with blood cockles as feed. After laying eggs, the berried crabs were transferred to a 100 L incubation tank and 100% of the water was changed daily until the eggs hatched. Highly phototactic crab larvae were collected and used for the experiment. Larvae were stocked in 250 L composite tanks in a sheltered enclosure (light intensity 6,000-10,000 lux) at a stocking density of 400 larvae L^{-1} (Khoa, 2018), with continuous and even aeration. When the larvae reached the zoae 5 stage, the density was reduced to 50 larvae L^{-1}.

The enrichment experiment used powdered β-glucan (98%, Vemedim), which had been used in the experiment. The experiment consisted of five treatments (T1 - 0 mg L^{-1}; T2 - 0.25 g L^{-1}; T3 - 0.5 g L^{-1}; T4 - 0.75 g L^{-1}; and T5 - 1 g L^{-1}), with treatment 1 being the control without β-glucan. Each treatment was conducted in triplicate. In which, *Artemia* cysts were incubated for 36 hours, and the shells were removed before enrichment. The amount of β-glucan was prepared and adjusted according to the different treatments and added to a blender along with Selco DHA, using amount specified by the manufacturer (4 g / 3 L of water). Then, water was added and blended into a solution. After blending, the solution was filtered through a zoea mesh (50 µm) to remove large particles. Next, the solution was added to *Artemia* enrichment tanks for 12 hours at a salinity of 30 ppt. After enrichment, the *Artemia* were collected and washed before being fed to the crabs. Crab larvae are fed 4 times a day. During the zoea 1-2 stage, they were fed with bloomed *Artemia* from Vinh Chau (Vietnam) at a rate of 1-2 larvae/mL/feeding. In the final zoea 3-5 stage, they were fed *Artemia* enriched with selco DHA and β-glucan, at a dosage of 2-4 larvae/mL/feeding time. During the megalopa stage, feed was given *Artemia* biomass and Lansy PL commercial feed at dosages as studied by Khoa, (2018).

During the rearing period, a 30% water exchange was carried out every 3 days. After the larvae metamorphosed to the megalopa stage, nylon substrates were placed helping to limit cannibalism during the rearing period.

Sample collection. The treatments were observed, monitored, and samples were collected periodically.

Water quality. Temperature, dissolved oxygen (DO), and pH were measured daily using YSI (Fisher Scientific) at 7:30 AM and 2:00 PM. Alkalinity, total ammonia nitrogen (TAN), and nitrite concentration were measured every 3 days using the Sera test kit. Total bacteria and *Vibrio* were sampled for analysis every 7 days. Total bacterial density was determined by dilution and counting on nutrient agar plates supplemented with 1.5% NaCl (NA) and total *Vibrio* density was determined by dilution and counting on thiosulfate citrate bile salt sucrose (TCBS) agar plates (Huys, 2002).

Digestive enzyme activities in mud crab larvae. They were evaluated 2h after feeding. Samples were collected at the crab 1 stages and stored at −80°C until analysis. Larval tissues were homogenized in a cold Tris-HCl buffer containing EDTA and $CaCl_2$ following the method described by Bolasina et al., (2006), and the homogenates were centrifuged to obtain enzyme extracts. Trypsin activity was determined using CBZ-LArg-MCA as a fluorogenic substrate and measured by fluorescence spectrophotometry (Bolasina et al., 2006). Chymotrypsin activity was analyzed using SAPFNA as the substrate, with absorbance measured at 405 nm. Pepsin activity was assessed using hemoglobin as the substrate according to the procedures of Worthington et al., (2016) and Suzer et al., (2007). Lipase activity was measured using 4-methylumbelliferyl butyrate (4-MUB) and calculated from the difference in fluorescence between incubations at 37°C and 4°C (Bolasina et al., 2006). Amylase activity was determined using soluble starch as the substrate and the dinitrosalicylic acid (DNS) method. Soluble protein concentration in the enzyme extracts was quantified using the Bradford assay with Coomassie Brilliant Blue reagent (Bradford, 1976). All enzyme activities were standardized to protein content and expressed as units per milligram of protein (U mg^{-1} protein).

Larval survival rate and metamorphosis. Determined every 3 days, larval survival rate was monitored using a 500 mL glass beaker, collecting and counting the number of larvae three times per tank, and calculating using the following formula:

$$\text{Survival rate (\%)} = (\text{Live larvae} / \text{Initial reared larvae}) \times 100$$

Larval stage index (LSI). Every 3 days, 20 larvae per tank were randomly collected and observed under a digital microscope (Nikon ECLIPSE Ti2 microscope with a DS-Qi2 camera - Nikon Corporation, Tokyo, Japan; 4x) to determine the larval stage and calculated using the formula:

$$LSI = [(N_1 \times n_1) + (N_2 \times n_2) + (N_i \times n_i)] / (n_1 + n_2 + n_i)$$

where: N_1, N_2, N_i = larval stage ;
n_1, n_2 , n_i = number of larvae at the corresponding stage.

Total body length was measured for 20 specimens per tank at the zoea 1, zoea 3, zoea 5, megalopa, and crab 1 stages. Samples were fixed in 10% formalin and measured using a computer-connected magnifying microscope.

The productivity was calculated using the formula:

$$\text{Productivity (crablet } m^{-3}) = \text{Total count of crablet} / \text{Volume}$$

Data statistical analyses. The data presented as mean±standard deviation were measured using Excel 2016. SPSS version 24.0 software was used with ANOVA analysis to find differences between treatment means using the Tukey test at the significance level ($p < 0.05$). Percent data (suvival) were normalised using the arcsin transformation before analysis.

Results

Water quality. Physicochemical water quality parameters remained stable and within optimal ranges across all experimental groups throughout the study period. Temperature fluctuations were observed in all treatments, with morning (AM) values ranging narrowly between 28.5 and 28.6°C and afternoon (PM) values increasing to 29.9-30.1°C. pH

exhibited remarkable stability, maintaining a baseline of 8.2 to 8.3. DO levels showed a slight fluctuation, moving from 7.6 to 7.4 mg L^{-1}.

Total alkalinity was stably maintained across all five experimental configurations, maintaining an identical mean value of 143-147 m $CaCO_3$ L^{-1}. Nitrogenous waste metrics showed minor fluctuations among the treatments. Nitrite levels ranged from 1.3 to 1.6 mg L^{-1}. Total ammonia nitrogen (TAN) concentrations peaked in T4 at 1.5±0.44 mg L^{-1}, compared to the minimum baseline of 1.1±0.61 mg L^{-1} recorded in the control and T2. Overall, these results showed that no significant difference in water quality was recorded among treatments.

Table 1

Water quality parameters among treatments during the rearing period

Parameters		*Treatment*				
		1	*2*	*3*	*4*	*5*
Temp. (°C)	AM	28.5±0.1	2 8.5±0.1	28.6±0.1	2 8.5±0.1	28.6±0.1
	PM	30.1±0.1	30.1±0.3	30.1±0.2	29.9±0.2	30.1±0.2
pH	AM	8.2±0.1	8.2±0.1	8.2±0.1	8.2±0.1	8.2±0.1
	PM	8.2±0.1	8.2±0.1	8.2±0.1	8.2±0.1	8.3±0.1
DO (mg L^{-1})		7.6±0.2	7.5±0.1	7.5±0.1	7.5±0.2	7.4±0.2
Alkalinity (mg $CaCO_3$ L^{-1})		145.2±17.2	143.5±15.5	145.4±15.2	147.3±14.9	145.2±17.9
Nitrite (mg L^{-1})		1.4±0.3	1.3±0.4	1.4±0.3	1.5±0.3	1.6±0.4
TAN (mg L^{-1})		1.1±0.6	1.1±0.5	1.2±0.4	1.5±0.4	1.2±0.3

Total bacterial density and Vibrio density in water. According to the results in Table 2, the total bacterial density and *Vibrio* density in the water did not differ significantly between the treatments ($p > 0.05$). With total bacterial density ranging from 1.42 x 10^4 to 1.61 x 10^4 CFU mL^{-1} and *Vibrio* density ranging from 0.82 x 10^2 to 1.13 x 10^2 CFU mL^{-1}, the total bacterial and *Vibrio* density in the water of the treatments were within acceptable levels.

Table 2

Total bacterial density and *Vibrio* density in rearing water among treatments

Target	*Treatment*				
	1	*2*	*3*	*4*	*5*
Total bacteria (10^4 CFU mL^{-1})	1.53±0.25^a	1.45±0.32^a	1.56±0.58^a	1.42±0.25^a	1.61±0.52^a
Vibrio spp. (10^2 CFU mL^{-1})	0.94±0.51^a	0.88±0.35^a	0.82±0.45^a	1.13±0.55^a	0.84±0.51^a

Values within the same row that have different letters are statistically significantly different ($p < 0.05$).

Digestive enzymes. The digestive enzyme activities in mud crab larvae were affected by dietary supplementation (Table 3).

Table 3

Digestive enzymes of mud crab larvae

Enzyme	*Treatment*				
	1	*2*	*3*	*4*	*5*
T (U mg^{-1} protein $\times 10^{-4}$)	4.68±0.78^a	5.42±1.29^a	9.48±1.42^b	8.79±1.47^b	8.21±1.38^b
C (U mg^{-1} protein)	187±69^a	198±81^a	172±61^a	178±57^a	181±62^a
P (U mg^{-1} protein)	264±35^a	403±76^b	511±182^b	468±127^b	446±119^b
L (U mg^{-1} protein)	66.3±11.2^a	95.8±18.8^{ab}	141.2±13.1^b	146.8±18.4^b	149.7±19.1^b
A (U mg^{-1} protein)	31.7±7.9^b	19.4±7.1^a	15.9±2.7^a	14.8±3.0^a	14.1±2.8^a

Note: T = trypsin; C = chymotrypsin; P = pepsin; L = lipase; A = amylase. Values within the same row that have different letters are statistically significantly different ($p < 0.05$).

Trypsin activity increased significantly with supplementation, reaching the highest value at T3 (9.48±1.42 U mg^{-1} protein $\times 10^{-4}$) and remaining significantly higher in T4 and T5 compared with the control and T2 ($p < 0.05$). In contrast, chymotrypsin activity did not differ significantly among treatments, with values ranging from 172 to 198 U mg^{-1} protein

($p > 0.05$). Pepsin activity was markedly enhanced by supplementation, increasing from 264±35 U mg^{-1} protein in the control group to 403-511 U mg^{-1} protein in the supplemented groups, all of which were significantly higher than the control ($p < 0.05$). Similarly, lipase activity showed a dose-dependent increase, rising from 66.3±11.2 U mg^{-1} protein in the control to approximately 141-150 U mg^{-1} protein in T3-T5, indicating improved lipid digestion. Conversely, amylase activity decreased significantly in all supplemented treatments, from 31.7±7.9 U mg^{-1} protein in the control to 14.1-19.4 U mg^{-1} protein in the treated groups ($p < 0.05$). Overall, dietary supplementation enhanced the activities of proteolytic enzymes (trypsin and pepsin) and lipase, while reducing amylase activity, suggesting a shift in digestive physiology toward greater protein and lipid utilization in mud crab larvae.

Larval stage index (LSI) of mud crab larvae. The metamorphosis index of mud crab larvae is shown in Table 4. The results show that the metamorphosis rate of larvae in the treatments supplemented with β-glucan and the control treatment did not differ significantly ($p > 0.05$). In all treatments, the metamorphosis rate of larvae ranged from 1.8 to 6.8, with the highest in treatment 3 (ranging from 2.0 to 6.8) and the lowest in the control treatment (ranging from 1.8 to 6.6). Furthermore, Table 4 also shows that megalopa larvae appeared after 18 days and molted into crab 1 from day 24, which was more prevalent in β-glucan supplemented treatments.

Table 4

LSI (larval stage index) of mud crab larvae among treatments

Day	*Treatment*				
	1	*2*	*3*	*4*	*5*
3	1.8±0.1[a]	1.9±0.1[a]	2.0±0.1[a]	1.9±0.1[a]	1.9±0.1[a]
6	2.0±0.0[a]	2.0±0.0[a]	2.0±0.0[a]	2.0±0.0[a]	2.0±0.0[a]
9	2.9±0.1[a]	2.9±0.1[a]	2.8±0.1[a]	2.9±0.1[a]	2.8±0.1[a]
12	3.1±0.1[a]	3.1±0.1[a]	3.1±0.2[a]	3.1±0.2[a]	3.0±0.1[a]
15	4.2±0.3[a]	4.5±0.1[a]	4.3±0.1[a]	4.2±0.1[a]	4.4±0.1[a]
18	4.8±0.1[a]	5.0±0.2[a]	4.9±0.2[a]	4.9±0.2[a]	5.0±0.2[a]
21	5.6±0.1[a]	5.6±0.1[a]	5.6±0.1[a]	5.7±0.1[a]	5.6±0.1[a]
24	6.6±0.1[a]	6.7±0.1[a]	6.8±0.1[a]	6.7±0.2[a]	6.7±0.1[a]

Values within the same row that have different letters are statistically significantly different ($p < 0.05$).

Length of crab larvae through different stages. The results in Table 5 show that the larval length at the zoea 1, zoea 3, zoea 5, megalopa, and crab 1 stages did not differ significantly ($p > 0.05$) when supplemented with β-glucan. After 9 days of rearing at the zoea 3 stage, the length of crab larvae ranged from 2.9 to 3.0 mm, and after 24 days of rearing, the average length of crab 1 was 3.2-3.3 mm. Therefore, supplementing β-glucan in the diet during the rearing of marine crab larvae at different amounts did not affect the growth of carapace width (CW) of crab 1.

Table 5

Length of mud crab larvae at different stages among treatments

Length (mm)	*Treatment*				
	1	*2*	*3*	*4*	*5*
Zoea 1	1.5±0.1[a]	1.5±0.1[a]	1.5±0.1[a]	1.5±0.1[a]	1.5±0.1[a]
Zoea 3	2.9±0.1[a]	3.0±0.2[a]	3.0±0.2[a]	3.0±0.2[a]	2.9±0.1[a]
Zoea 5	4.3±0.1[a]	4.3±0.1[a]	4.5±0.2[a]	4.2±0.1[a]	4.1±0.1[a]
Megalopa	4.0±0.0[a]	4.1±0.2[a]	4.2±0.0[a]	4.1±0.0[a]	4.1±0.0[a]
Crab 1 (CW)	3.3±0.1[a]	3.2±0.1[a]	3.3±0.1[a]	3.2±0.1[a]	3.2±0.1[a]

Values within the same row that have different letters are statistically significantly different ($p < 0.05$).

Larval survival rate and productivity. Table 6 presents the survival rates of mud crab larvae across six developmental stages subjected to five different treatment concentrations (0 to 1 g L^{-1}). In the early developmental phases (zoea 2 and zoea 3), survival rates

remained statistically uniform across all experimental groups ($p > 0.05$), with zoea 2 survival exceeding 80% and zoea 3 survival ranging between 61.8% and 64.8%. However, significant β-glucan treatment effects emerged starting at the zoea 4 stage, where T3 achieved the highest survival rate (49.1 %), which was not statistically higher than T4 (48.1 %) but significantly higher than the control (37 %). The survival advantage conferred by the T3 treatment remained statistically superior during the megalopa and crab 1 stages (29.1% and 6.8%, respectively) compared to both the control and T5.

Table 6

Survival rate of mud crab larvae in different treatments

Survival rate (%)	*Treatment*				
	1	*2*	*3*	*4*	*5*
Zoea 2	80.6±0.7^{a}	80.5±5.4^{a}	81.6±1.1^{a}	83.7±2.2^{a}	83.0±1.6^{a}
Zoea 3	63.5±2.4^{a}	62.8±1.5^{a}	61.8±0.3^{a}	64.8±2.8^{a}	62.5±2.1^{a}
Zoea 4	37.0±1.6^{a}	42.8±1.9^{b}	49.1±3.3^{c}	48.1±0.5^{c}	46.3±1.8^{bc}
Zoea 5	39.6±5.0^{ab}	46.9±2.4^{b}	48.4±3.2^{bc}	45.1±4.9^{b}	39.1±3.2^{ab}
Megalopa	20.3±1.7^{a}	19.5±2.4^{a}	29.1±1.4^{b}	20.3±2.0^{a}	22.2±1.1^{a}
Crab 1	5.3±0.7^{a}	5.7±0.5^{a}	6.8±0.5^{b}	5.9±0.8^{ab}	4.6±0.7^{a}

Values within the same row that have different letters are statistically significantly different ($p < 0.05$).

As illustrated in Figure 1, significant differences in crablet productivity were observed among the experimental treatments ($p < 0.05$). While the minimum productivity was recorded in T5 and T1 (18,400 and 21,200 crablet m^{-3}, respectively), the maximum yield was produced under T3 (27,200 crablet m^{-3}), which lacked a statistically significant variance from T4 (23,600 crablet m^{-3}).

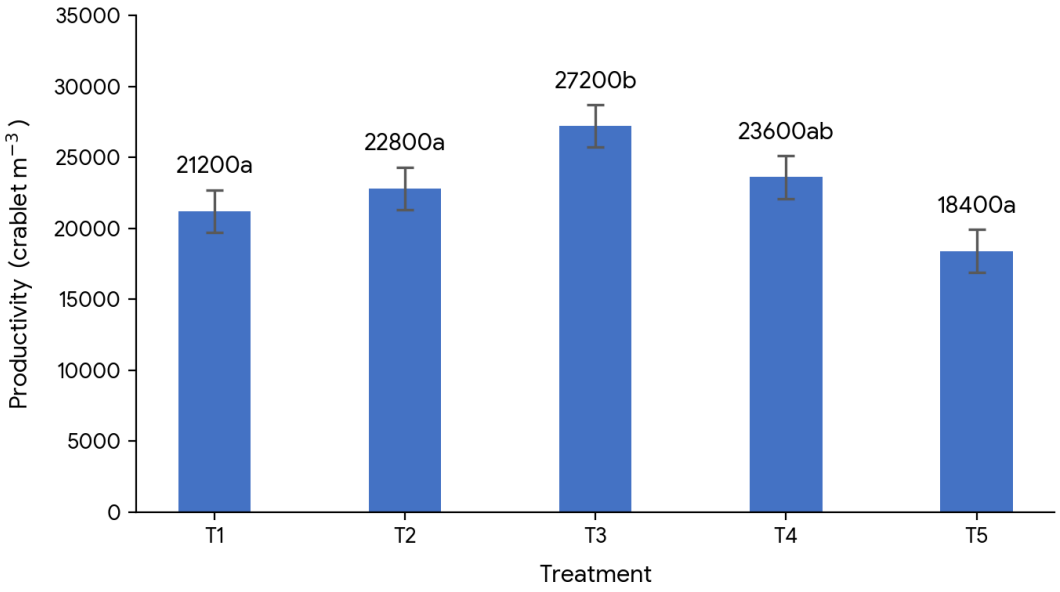

Figure 1. Crablet productivity obtained from different treatments (Values that have different letters are statistically significantly different ($p < 0.05$)).

Discussion. The monitored water quality parameters remained stable and within acceptable physiological ranges for mud crab larviculture across all experimental groups, indicating that the treatments did not adversely alter the rearing environment. The temperature range (28.5-30.1°C) and consistent pH values (8.2-8.3) closely align with the optimal thresholds recommended by Waiho et al., (2018) for the genus *Scylla*, which minimizes ionization and thermal stress. Furthermore, DO levels remained robust (7.4-7.6 mg L^{-1}), safely exceeding critical limits, while the shared total alkalinity baseline (143.2 mg $CaCO_3$ L^{-1}) provided a strong buffering capacity against unexpected acidic shifts, surpassing the standard pond baseline of 120 mg L^{-1} documented by Shelley & Lovatelli, (2011). Although minor elevations in nitrite (1.3-1.6 mg L^{-1}) and TAN (1.1-1.5 mg L^{-1}) were

observed in the higher-dose treatments (T4 and T5), these levels are typical for intensive closed-system larviculture before water exchange. This overall stability ensures that any observed variations in larval survival or productivity can be confidently attributed to the direct biological effects of the treatments rather than confounding water quality degradation.

Total bacterial and *Vibrio* spp. densities showed no statistically significant variations across all treatments ($p > 0.05$), remaining within acceptable safety thresholds for mud crab larviculture. Total bacterial counts (1.42×10^4 to 1.61×10^4 CFU mL^{-1}) remained safely below the 10^5 CFU mL^{-1} maximum recommended by Pinoargote et al., (2018). Crucially, *Vibrio* spp. densities 0.82×10^2 to 1.13×10^2 CFU mL^{-1} were kept well below the critical pathogen threshold of 10^3-10^4 CFU mL^{-1} associated with hatchery vibriosis outbreaks (de Souza Valente & Wan, 2021). These results indicate that the treatment concentrations did not disrupt the microbial equilibrium or promote pathogenic proliferation in the rearing water.

The beneficial effects of β-glucan on digestive enzymes are likely mediated through several mechanisms. β-glucan can improve the structural and functional integrity of the hepatopancreas, the primary organ responsible for digestive enzyme synthesis in decapod crustaceans (Vogt, 2021). β-glucan also stimulates innate immune responses and reduces physiological stress, allowing more energy to be allocated toward growth and digestive processes (Meena et al., 2013; Guluarte et al., 2023). Moreover, β-glucan may modulate gut microbial communities, thereby enhancing digestive efficiency and nutrient assimilation (Doan et al., 2024). Overall, current evidence indicates that β-glucan supplementation can improve digestive enzyme activities, particularly trypsin, pepsin, and lipase in mud crab larvae. In other crustaceans, β-glucan has been associated with increased activity of enzymes such as esterase, aminopeptidase, trypsin, and chymotrypsin, though results can vary by species and concentration (Tian et al., 2023). These improvements contribute to enhanced nutrient utilization, growth performance, disease resistance, and overall health. However, further research is needed to elucidate the optimal dosage, duration of administration, and species-specific responses of digestive enzymes to β-glucan supplementation in mud crab hatcheries.

In crustacean aquaculture, β-glucan supplementation has been widely applied to improve larval and post-larval quality. Studies on black tiger shrimp (*P. monodon*) and Pacific white shrimp (*L. vannamei*) have reported significant improvements in growth performance, feed utilization efficiency, survival rate, and disease resistance following dietary β-glucan administration (Luan et al., 2021; Qiao et al., 2022). The enhanced immune competence provided by β-glucan contributes to reduced mortality during critical developmental stages and increases resistance against common pathogens such as *Vibrio* spp. The survival rate of crab 1 obtained from this study ranged from 5.3 - 6.8%, which was comparable to the actual rate in mud crab seed production (5-15%) (Ut & Khoa, 2025). In which, the lowest survival rate was in T1 and T5 (4.6-5.3%). The reason may be due to the uneven molting rate in this treatment, leading to a high mortality rate due to cannibalism. In addition, high levels of β-glucan compared to the required level also affected the absorption of the crab larvae, and may become ineffective, potentially due to immune fatigue (Alaban et al., 2014). The highest survival rate in the crab 1 stage was in T3 (6.8%), significantly different from the other treatments. The survival rate of larvae in T3 and T4 was significantly improved compared to T1 ($p < 0.05$). The survival rate of crab larvae in this study was higher than that of the study by Viet & Hai, (2019) when rearing crab larvae with different feeding regimes but without supplementation, the survival rate ranged from 2.6 to 3.0% (zoea 1-crab 1). Chang et al., (2003) used β-1,3-glucan extracted from the fungus *Schizophyllum commune* to enhance immunity in broodstock black tiger shrimp (*P. monodon*), the results showed that it could help to increase shrimp resistance against WSSV (White Spot Syndrome Virus). Recent studies have demonstrated that β-glucan is an effective immunostimulant in aquatic animals and can be administered through multiple routes, including immersion, dietary supplementation, and injection (Rodrigues et al., 2020). Numerous investigations have shown that β-glucan enhances both innate and adaptive immune responses by stimulating the activity of hemocytes, phagocytosis, respiratory burst, and the production of antimicrobial compounds. These

immunomodulatory effects improve the ability of aquatic organisms to resist pathogenic bacteria, viruses, and environmental stressors, ultimately leading to increased survival, better health status, and enhanced production performance (Vetvicka, 2011; Meena et al., 2013). Consequently, the use of β-glucan in hatchery and grow-out systems has become an important strategy for improving crab productivity, sustainability, and economic profitability (Chang et al., 2003; Meena et al., 2013).

Conclusions. Dietary enrichment of *Artemia* nauplii with β-glucan does not affect the growth performance, body length, or metamorphosis rate of mud crab larvae. However, it effectively improves larval survival rates and final crablet productivity by enhancing digestive enzyme activities, specifically upgrading trypsin, pepsin, and lipase production for better protein and lipid utilization. Based on the experimental findings, an enrichment dose of 0.5 g L^{-1} of β-glucan combined with DHA Selco (4g/3L) for 12 hours is recommended as an optimal and practical feeding protocol to improve seed production efficiency in mud crab hatcheries.

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