**Enhancing *Scylla tranquebarica* (Fabricius 1798) crablet production in hatchery by addition of probiotics in their larvae culture.**

**Running Title: Enhancement of crablet production in mud crab larvae culture**

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**Novelty Statement**: 1). The application of erythromycin 15 mg L-1 to fight against bacterial pathogens from the first-day larvae stocking in the rearing tank can prevent mass mortality of mud crab, *Scylla* spp. larvae in the earlier days of rearing due to bacterial pathogenic. 2). Mass mortality of the larvae still often occurs after being reared for more than 10 days, or whenever larvae successfully develop into the consecutive stage, crablet production is still a low number. 3). Subsequent treatment is needed to enhance crablet production. 4). By the addition of probiotics, *Bacillus subtilis* (108 CFU g-1) at the doses 5 mg L-1 or 7.5 mg L-1 applied periodically during larvae rearing every three days starting at day eight of larvae rearing until the first instar of crablet was able to increase crablet production higher by 27.9-31.81% compared to 0 mg L-1 (added erythromycin 15 mg L-1 only).

**Abstract**

Mass mortalities of S*cylla* *tranquebarica* larvae often occurred even though erythromycin was added to the medium for larvae rearing. The study aims to obtain suitable doses of probiotics containing *Bacillus subtilis* (108 CFU g-1) as subsequent treatment added to *Scylla tranquebarica* larvae rearing, to increase crablet production. Larvae reared in 12 conical fiber tanks were filled with 200 L sterile seawater containing 15 mg L-1 erythromycin. Larvae fed rotifer, *Brachionus* sp. On the eighth day of larvae rearing, four different doses of probiotics (A= 2.5 mg L−1, B=5 mg L−1, C=7.5 mg L−1, and D=0 mg L−1) containing *Bacillus subtilis* were applied with intervals every three days until larvae develop into the crablet stage. Next-generation sequencing analysis to observe the abundance of microbiota including *Vibrio* spp. in the megalopa and the water media. Raw DNA samples were taken from each treatment for NGS analysis. Ammonia, nitrite, total organic matter (TOM), larvae, megalopa, and crablet production were monitored. Based on the NGS analysis, the *Vibrio* spp*.* decreased significantly (*p*<0.05) in megalopa treated with a dosage of 2.5 mg L-1 (A) and L-1 7.5 mg L-1 (C) and in the medium for larvae rearing treated probiotic dosage of 5.0 mg L-1 (F). Ammonia and TOM decreased significantly (*p*<0.05) in B and C treatments, resulting in a higher number of zoea survival, finally resulting in a significantly high crablet production in the dosage of 5 mg L-1 and 7.5 mg L-1, improving crablet production higher by 27.9-31.81% compared to 0 mg L-1.

**Keywords**: Crablet, probiotic, *Vibrio*, Water quality

**1. Introduction**

In the Asia Pacific region, mud crab (*Scylla* spp.) is highly valuable economically (Keenan & Blackshaw, 1999) especially in their bodyweight sizes of more than 200g/ind. and softshell crab (60-100g/ind.). Therefore, in Indonesia, mud crab culture in brackish water ponds has been developed in some places (Gunarto et al., 2020). Unfortunately, up to now mud crab seed for culturing is mostly caught in the wild, whereas mud crab seed from hatchery is still in low production. Thus, research to produce mass mud crab seeds from the hatchery is very important to fulfill the needs of the crab seeds for growth in brackish water ponds.

In Hatchery, the mass mortality of *Scylla* spp. larvae rearing frequently occurs after a few days of rearing, mostly due to pathogenic bacterial infections such as *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus* (Talib et al., 2017). Therefore, the application of erythromycin in the medium for larvae rearing to fight against bacterial pathogens was conducted in the first day before larvae stocked in the tanks can prevent mass larvae mortality due to bacterial pathogenic in the early rearing, and larvae can develop into subsequent stages. However, larvae often die after being reared for more than 10 days and crablet production is low in number. In contrast to the probiotics, *B. subtilis* application since the first-day larvae stocking, mostly the larvae fail to develop into the subsequent stage (Gunarto et al., 2021). Therefore, the probiotics application directed to the medium for larvae rearing as the subsequent erythromycin application conducted a week later after erythromycin application, may able to promote enhancing crablet production due to the capability of probiotics themselves to inhibit pathogenic bacteria development (Ayisi et al., 2017; Buruiană et al., 2014; Hlordzi et al., 2020).

Probiotic bacteria have already been widely applied in aquaculture practices (Kuebutornye et al., 2019). Beneficial probiotics application in the medium for larvae rearing are hoped to reduce the developing various diseases, including Vibriosis caused by *Vibrio* spp. infection and to optimize the larvae growth to the crablet stage. *Bacillus subtilis*, a beneficial bacterium, has been applied in shrimp and fish aquaculture, improving water quality (Hlordzi et al., 2020; Olmos, 2014). Earlier researchers reported that *B. subtilis* could improve disease resistance, minimizing larval mortality caused by *V. harveyi* infection (Zokaeifar et al., 2012). Thus, applying probiotics containing *B. subtilis* bacteria is essential to eliminate *V. harveyi* infection and support the successful rearing of larvae to develop into the crablet stage.

The ideal dose of *B. subtilis* as probiotics directed applied to the water as the medium for the mud crab *Scylla* spp. larval rearing in the hatchery, also microbiota abundance in water media for larva rearing after added erythromycin and probiotics, *B. subtilis* still not understood. Yang et al. (2021) reported that the addition of antibiotics will reduce microbiota diversity. Therefore, the study aims to obtain suitable dosages of probiotics containing *B. subtilis* applied to the rearing of the mud crab *S. tranquebarica* larvae, as subsequent erythromycin prevention to pathogenic bacteria develops in the larvae rearing tank, furthermore, impact to the enhancing crablet production. In addition, we also observed the population of microbiotas including *Vibrio* spp. in the megalopas and the water used for larvae rearing, water quality parameters, larvae development, and crablet production.

**2. Materials and Methods**

**Production of larvae, larvae rearing, and water quality monitoring.**

The mature gonadal of female *S. tranquebarica* brood stocks were collected from a middleman in Makassar, South Sulawesi Province, Indonesia. The brood stocks were stocked in the 500 L of recirculation conical fiber tanks in a crab hatchery belonging to the Research Institute for Brackish Water Aquaculture and Fisheries Extension (REBAFE) in Barru Regency, South Sulawesi, Indonesia. The spawned female crab was then incubated in a one-ton fiber tank filled with 700 L of sterile seawater salinity 30 ppt without given feed and aerated. Seawater at a salinity of 30 ppt prepared for the larvae rearing was sterilized using 20 mg L-1 chlorine overnight, then aerated and neutralized with 20 mg L-1 sodium thiosulfate the following night (Syafaat et al., 2019). Subsequently, the water was filtered using a filter membrane and through Ultraviolet light before being transferred to the 200 L volume of conical fiber tanks used for rearing the larvae. Before the larvae were stocked in the cone fiber tank, 15 mg L-1 of erythromycin was added, equal to three grams in each tank (Gunarto et al., 2021). Furthermore, the tanks were sufficiently aerated to supply oxygen to the larvae. The *S. tranquebarica* larvae seen swimming on the surface of the water when freshly hatched and in healthy condition were stocked into cone fiber tanks at a density of 80 individuals L-1. Larvae fed rotifer, *Brachionus* spp from the first day stocking at the density of 20 individuals, mL-1 enriched with highly unsaturated fatty acids/HUFA at concentrations of 100 mg L−1 (DHA Selco) (Kotani 2017). Moreover, starting on the seventh day of the rearing, the larvae were also fed with *Artemia* nauplii with a density of 0.5 individuals' mL-1 enriched with 500 mg L−1 HUFA for six hours. Meanwhile, on the 10th day, the larvae were only fed with Artemia nauplii at the density of 1 ind. mL-1. Finally, the number of *Artemia* nauplii increased to 1-2 individuals mL-1 in the megalopa stage until they developed to the crablet stage.

Each larvae-rearing tank measured water quality parameters such as ammonia, nitrites, and total organic matter by taking 300 mL of water respectively, then analyzing according to Clesceri et al (2005). Tryptic Soy Agar was employed for the observation of total bacteria. 50 mL sterile dark bottle used for taking water from every rearing tank. 9 mL of saline solution (NaCl, 0.85% (w/v) was used to dilute a 1 mL water sample. Serial dilutions, ten times (10-1, 10-2, 10-3, and 10-4) were made. Additionally, 100 µL water sample without dilution and with the first dilution (10-2) was swiped to a TSA late medium, then incubated for 48 h. The total bacteria calculate based on the formula:

P = Q /T x 1/S x 1/V

P = bacterial population (CFU mL-1). Q = total number of bacteria growing in one dilution level (colony). T = number of plates used. S = dilution rate. V = a plate sample volume (0.1 mL). Meanwhile, YSI Pro Plus multiparameter water quality is used to measure dissolved oxygen (D.O.), pH, and water temperature.

**Probiotic treatment**

The treatment tested in the mud crab larvae rearing medium was probiotics made in powder form with *B. subtilis* density of 108 CFU g-1. The probiotic administration began on the eighth day of larva rearing and continued with the interval of three days until the larvae reached the first instar crablet stage (about 24 – 28 days). The probiotic was administered at the following doses; A). 2.5 mg L−1, B). 5.0 mg L−1, C). 7.5 mg L−1, and D). 0 mg L−1 (control without probiotic application) by giving the probiotic powder at 0.5, 1.0, 1.5, and 0 g, respectively per 200 L of rearing water. The probiotics need to be activated before being added to the larvae-rearing tank. The probiotic powder was placed in a small basin with a volume of three liters, then filled with two liters of sterile seawater at a salinity of 30 ppt and aerated overnight. On the next day, the aeration stopped. The solution was settled for one hour to let the particle sink. Then only a clear solution is added to the mud crab larvae cultures.

**Monitoring of several biological and chemical parameters**

**Sample collection**

16S rRNA amplicon sequencing was carried out to elucidate microbial abundances in megalopa and media for larvae rearing treated with probiotics at different doses. The samples for the analysis were prepared as follows; first, megalopa extraction is conducted by taking ten individuals of megalopa from each replication of probiotic treatment and control tank. Then, the megalops from each treatment were put in each sterile microtube, crushed, and extracted using the cTAB dTAB DNA extraction kit (IQ2000, Taichung 407, Taiwan). Then the pelleted DNA dissolved in 200 mL T.E. buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.5). The sample of extract megalopa from each treatment was determined as A, B, C, and D. Each sample with two replications. Furthermore, the extract of water media samples was prepared through one liter of water sample taken from the larvae-rearing tank in each treatment. Afterward, the water was filtered using plankton net no.400 with mesh size 37 µm to eliminate larvae and rotifer from the rearing water. Furthermore, the water was filtered using 0.22 μm Whatman filter paper. The filter paper was extracted following the ZymoBiomics DNA miniprep kit extraction procedure. The sample of extract water media for rearing from each treatment was determined as E, F, G, and H. Each sample with two replications. Genomic DNA samples from megalopa and water media extracts were sent to the Genetics Science laboratory for 16S rRNA amplicon sequencing in an Illumina MiSeq.

**Monitoring of larvae population**

Larvae samples were taken on three distinct surfaces in each replication tank using a 100 mL bowl to monitor the larvae population. The larval population density per liter was then calculated from the mean larval number per 100 mL. In each replication tank, the Megalopa population was observed using a 1000 mL bowl placed on three different surfaces. The mean megalopa number per 1000 mL indicated the megalopa population per liter (Gunarto et al., 2021). The number of cablets produced per replication tank in each treatment was also monitored after the crab was harvested totally in each tank.

**3. Data analysis**

Data on the relative abundance of microbiota from the NGS analysis in each treatment were made graphically. The larval population and crablet production data in each treatment were subjected to logarithmic transformation. Furthermore, the data were compared and analyzed using analysis of variance for the complete randomized design patterns. The least significant difference test (LSD test) was applied if any difference between the treatments was tested. The statistical test was conducted using the SPSS (Statical Product Service Solution) program package 23. In comparison, the data on water quality (ammonia, nitrite, total organic matter) from each treatment were analyzed descriptively.

**4. Results**

**4. 1. Microbiota abundance**

The *Vibrio* spp. abundances decreased significantly (*p*<0.05) in the megalopa treated with probiotics at the dosage of 2.5 mg L-1 (A) and 7.5 mg L-1 (C) and in water for larvae rearing treated with probiotics at a concentration of 5.0 mg L-1 (F), compared to the control test (D). *Ralstonia* spp. abundance is more than 90% of total microbiota abundances in the megalopa sample from treatments A, C, and the control tank (D). In probiotics were added to the water for larvae rearing at a concentration of 7.5 mg L-1 (G2), it was found that *Acinetobacter* was extremely dominant (80%). In water media used to grow larvae in all probiotic treatments (E, F, G) and H (control), *Phaeodactylobacter* abundance ranged from 20 to 30%. However, it was not found in the whole body of the megalopa sample from all treatments (A, B, C, and D). *Incestsedis* was distributed in almost the same density in all treatments (20-30%) except in the treatment when *Ralstonia, Pseudomonas,* and *Acinetobacter* were dominant, the *incestsedis* show a low percentage (<5%) (Figure 1).

- Figure 1-

**4. 2. Cluster Sample**

Cluster samples based on the microbiota abundance are seen in Figure 2. There are four clusters obtained. The tank with the low relative abundance of the microbiota (value < 0.2) was observed on a sample of A1, C2, C1, and D2 was > 90% dominated by *Ralstonia*. The value in the range of 0.2-0.4 was observed on E, F, G1, and H (samples from water media for larvae rearing), which were dominated by *insertcedis* bacteria, *Phaeodactylibacter* and OM43\_Clade. While the value in the range of 0.4-0.6 was observed in samples B2, and D1, dominated by *insertcedis* bacteria, *Pseudoalteromonas*, *Enterobacter*, and *Vibrio*. Additionally, samples A2, B1, and G2 contained a sample with a high relative abundance of microbiota (>0.8), which was predominated by *Acinetobacter*, *insertcedis* bacteria, and *Pseudomonas*.

- Figure 2-

**4. 3. Water quality parameters**

The salinity for larval rearing was 30 ppt, dissolved oxygen in the range of 5.2 mg L-1 to 6.7 mg L-1, water pH: 8.1 to 8.4, and water temperature in the tank 27.5°C to 30.2°C. The ammonia concentration decreased at 10.3% (A), 15.23% (B), and 6.48% (C) compared to control (D) in the zoea stage. In contrast, the ammonia concentration significantly reduced to 3.45% (B) at the megalopa stage compared with the control treatment (D). All treatments' nitrite concentration at the zoea stage was relatively low, reaching 0.01-0.07 mg L−1. At the megalopa stage, all treatments' nitrite concentration was increased (Figure 3). The TOM in the zoea stage decreased to 23.63% and 19.40% in treatments B and C, while at the megalopa stage, the TOM decreased to 4.34% only in treatment B, compared with the D treatment (control) (Figure 4). Monitoring total bacteria by tryptic soil agar (TSA) showed treatments B and C were found higher (1.85x105 CFU mL-1 to 2.2x105 CFU mL-1) compared to 1.6x104 CFU mL-1 in the control tank (D treatment).

- Figure 3 and Figure 4 -

**4. 4. Larva and megalopa populations also crablet production**

The larvae population declined from zoea-1 to zoea-5. On the seventh day of rearing the highest larvae population was found in treatment C (69 ind. L-1) and on the seventeenth day of rearing the highest larvae population still occurred in treatment C (26 ind. L-1), the larvae in treatment B and C were significantly larger populations (*p*<0.05) than those in treatments A and D. On day 23, treatments C had more megalops than treatments A, B and D. However, finally in the harvested of crablet day-17, treatments B with highest crablet production (225 ind. tank-1) and treatment C (220 ind. tank-1) both of them yielded significantly (*p*<0.05) more crablets than treatments A (174 ind. tank-1) and D (172 ind. tank-1) (Table 1).

- Table 1 -

**5. Discussion**

Earlier research reported that the addition of probiotics, *B. subtilis* could increase the microbiota population and influence digestion, feed absorption, and assimilation (Ayisi et al., 2017). It may be the *B. subtilis* losses of the competition with other bacteria that grow rapidly in each treatment such as *Ralstonia* sp or *Acinetobacter* sp. In the present study, the microbiota such as *Ralstonia* highly increases population mostly at the megalopa reared with the addition of probiotics at 2.5 mg L-1 and 7.5 mg L-1. *Ralstonia* is a bacterium that can survive in highly purified water and disinfectant. It's able to be a used carbon source, thereby, bacteria can be a candidate for bioremediation (Adley et al., 2005). This activity may also cause improved water quality in larvae-rearing tanks as seen in the C treatment that ammonia and TOM decreased significantly compared to that in the control tank. Furthermore, *Acinetobacter* spp. also increased significantly in the water after being treated with probiotics at 7.5 mg L-1. *Phaeodactylibacter* distributed in all water media for larvae rearing is applied with different dosages of probiotics.

In the present research, the probiotic containing *B. subtilis* 108 CFU g-1 was applied at doses of 2.5 mg L-1 to 7.5 mg L-1. Based on the total count of bacteria soluble in the water by tryptic soil agar (TSA) media analysis (Buller N B, 2004), the density of total bacteria in treatments B and C were found to be 1.85x105 CFU mL-1 to 2.2x105 CFU mL-1 compared to 1.6x104 CFU mL-1 in the control tank. Therefore, concentrations of total bacteria at 5.0 mg L-1 to 7.5 mg L-1 are most effective in preventing the growth of the population of *Vibrio* spp. pathogenic in the larvae-rearing. *B. subtilis* found in the intestines of crabs and can be used as a probiotic. A density of 105 CFU g-1 will increase immunity against *V. parahaemolyticus* infection in *S. paramamosain* larvae (Wu et al., 2014). Treatments B and C produced crablets at the amount of 225±2.82 individuals' tank -1 and 220±16.97 individuals, while control (D treatment) produced crablets at an amount of 172.5±14.8 individuals. It was higher (27-30%) crablet production in the probiotic application of treatment B and treatment C (*p*<0.05) compared to D treatment with only added erythromycin 15 mg L-1. The previous study which was only 141.2 ± 34.1 individuals’ tank -1 used the application of erythromycin in their larvae rearing (Gunarto et al., 2021).

*Vibrio* spp. distributed in almost all treatments with different intensities. *V. harveyi*, is pathogenic to marine and inland fisheries biota (Alfiansah et al., 2018; Sahandi et al., 2019; Yilmaz et al., 2022), including larvae and adult mud crab (Aftabuddin et al., 2013; Poornima et al., 2014; Talib et al., 2017). Earlier researchers reported that *V. harveyi* attacks crab larvae by infecting the hemolymph and hepatopancreas (Poornima et al., 2014). Due to the open circulatory system in the crab, it was possible that all parts of the body of the crab as a place of attachment to pathogenic bacteria (Zhang et al., 2020). Therefore, in this study, all parts of the megalopa (the whole body of megalopa) were used to observe the microbiota including *Vibrio* spp. population by crushing to make raw DNA for sequencing analysis.

The accumulation of organic material in the larvae-rearing tanks will trigger the increasing *V. harveyi* population in the water. Therefore, the raw DNA from the water for larvae rearing applied with different doses of probiotics was also taken to observe the microbiota, including *Vibrio* spp. population in the present research. Bacteria such as *V. harveyi* can change from nonvirulent to virulent when they reach a certain density level or a dramatic environmental change (Zhou et al., 2012).

Adding probiotic bacteria such as *B. subtilis* to the larvae-rearing medium caused competition with pathogenic bacteria (Nagomi & Maeda, 2011). Additionally, these probiotics could complement the feed sources and prevent the proliferation of pathogenic bacteria in the larval intestine (Buruiană et al., 2014). The efficiency of these bacteria, not their concentration, determines the potency of probiotic bacteria against pathogenic bacteria (Sahandi et al., 2019). In contrast to the present study, dosages of 5 mg L-1 to 7.5 mg L-1 resulted in higher crablet production than dosages of 2.5 and 0 mg L-1. The higher concentration of probiotic bacteria applied in the larvae-rearing tanks in the present research affected the higher crablet production. In *Lactobacillus sakei*, the difference in secreted organic acid concentration determines the possible inhibitory level. The antibacterial activity of *B. subtilis* is mainly influenced by its ability to produce antibiotics, primarily peptides (Olmos, 2014; Stein, 2005).

Probiotics' effectiveness or efficacy in larvae could be due to probiotics' high intestine colonization capability, favorable development conditions, or larval resistance to foreign probiotics (Hlordzi et al., 2020). Therefore, *B. subtilis* supplementation in the current study may have an impact on mud crab larvae's improved immune response and disease resistance. Supplementation with *B. subtilis* will improve growth performance, immune response, and disease resistance to pathogenic bacterial infections (Buruiană et al., 2014). Probiotic bacteria have been proven to promote digestive function by generating digestive enzymes, resulting in better digestion and absorption of food and, as a result, increased feed utilization efficiency (Ghosh et al., 2016).

The probiotic containing *B. subtilis*, presumably at a dose of 5.0 and 7.5 mg L-1, produced the highest level capable of fighting harmful bacteria in mud crab larvae rearing, improving water quality for larvae rearing. Therefore, higher percentages of larvae successfully develop into the crablet stage.

The probiotics application in the present study directly also improved water quality. The ammonia concentration at the zoea was decreased by 6-15% compared to a control treatment (Figure 2), and the TOM of B and C probiotic treatments was also decreased by 19-23.6% compared to the control, D treatment (Figure 3). It was proven that probiotics containing *B. subtilis* added in larval rearing could improve water quality (Kuebutornye et al., 2019; Olmos, 2014) and its impact on crablet production in treatments B and C.

The LC-50 of unionized ammonia for zoea-1 and zoea-5 was at 4.05 mg L−1 to 6.64 mg L−1 after 24 hours (Neil et al., 2005). The nitrite in the zoea stage was in the range of 0.004 mg L−1 to 0.009 mg L−1 and increased in the megalopa stage, with the highest concentration at 0.21 mg L−1 to 0.35 mg L−1 in treatment D (control). The safe limits of nitrite in the larvae of Z1 to Z5 were in the range of 2.5 mg L−1 to 6.9 mg L−1 (Seneriches-Abiera et al., 2007). Thus, ammonia and nitrite concentration in the present study still support the larvae development, which means that the value was lower than the limits to support the survival of the larvae (Figure 3).

**6. Conclusions**

The treatment of probiotics containing *B. subtilis* added in the mud crab larvae rearing stimulate fast growing of microbiota population such as *Ralstonia* sp, incertsedis, *Pseudomonas* sp, *Acinetobacter* sp, furthermore impact suppressing *Vibrio* spp. population mostly in megalopa treated with probiotic doses of 2.5 mg L-1 (A) and 7.5 mg L-1 (C), improved water quality in the B (5.0 mg L-1 ) and C (7.5 mg L-1) treatments. Therefore, crablet production in the B and C treatments is higher by 27.91 to 30.81% compared to crablet production from control treatment (D) with added erythromycin only without probiotic application.

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**8. Ethics approval and consent to participate**

This research has followed the standard operating procedure of the mud crab, *Scylla* spp experimental of Research Institute for Brackish Water Aquaculture and Fisheries Extension, Marine Fisheries Research and Resources Agency Republic of Indonesia.

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100

Relative Sequence Abundance

80

60

40

20

0



Other

Vibrio spp.

uncultured

Ralstonia

Pseudomonas

Pseudoalteromonas

Phaeodactylibacter

OM43\_clade

incertsedis

Enterobacter

Alteromonas

Acinetobacter

Megalopa (D1) water media (H1) Megalopa (A1) Water media (E1)

Megalopa (B1) water media (F1)

Megalopa (C1) Water media (G1) Megalopa (D2)

Water media (H2) Megalopa (A2) Water media (E2)

Megalopa (B2) Water media (F2) Megalopa (C2)

Water media (G2)

Figure 1. Relative sequence abundance of *microbiota* in water media for larvae rearing and in megalopa after being treated with different doses of probiotics. (A=2.5 mg L−1, B=5 mg L−1, C =7.5 mg L−1 and D=0 mg L−1).

Cluster Dendrogram

0.0 0.4 0.8

B2

D2

E2

E1

F1

H1

H2

F2

G1

B2

D1

C2

A1

C1

A2

G2

Figure 2. Cluster dendrogram of the abundance of *microbiota* in water media for larvae rearing and in megalopa after being treated with different doses probiotics. (A=2.5 mg L−1, B=5 mg L−1, C =7.5 mg L−1 and D=0 mg L−1).

ab a bc c

a a a a

a a a a

a a a a

Figure 3. The concentration of ammonia (upper) and nitrite (below) in the zoea and megalopa stage after being treated with different doses of probiotics (A=2.5 mg L−1, B=5 mg L−1, C =7.5 mg L−1 and D=0 mg L-1 (Error bar = S.D.). Values on the same stage with different superscript letters differ significantly (p< 0.05).

ab a b b

a b b a

Figure 4. Total Organic Matter (TOM) concentration in zoea and megalopa stages after treatment with different doses of probiotics. (A=2.5 mg L−1, B=5 mg L−1, C =7.5 mg L−1 and D=0 mg L−1) (Error bar = SD). Values on the same stage with different superscript letters differ significantly (p< 0.05).

**Table 1**. Development of mud crab *S. tranquebarica* larvae cultured with the addition of different doses of probiotics.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments  (RICA-4  in mg L-1) | Larvae stocking density (individuals L-1) | | *Larvae population and crablet production*  \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | |
|  |  | | DOR7 | | DOR 17 | | DOR 23 | | Crablet D10 Prod./tank |
| A=2.5  B=5.0  C=7.5  D=0.0 | 80  80  80  80 | 46.67±8.02a  57.33±8,96ab  69.34±12.4b  46,33±5.51a | | 14.67±3.51a  25.67±4.93b  26.00±3.60b  14.00±2.64a | | Z5:8;M:2  Z5:8;M:2  Z5:6:M:4  Z5:9;M:1 | | 174±1.41a  225±2.82b  220±16.97b  172.5±14.8a | | |

DOR: Day of Rearing, Z5: zoea-5, M: megalopa

Values in the same column without the same superscript letters are significantly different (p < .05).