

Effect of different salinity levels on megalopa *Scylla tranquebarica* (Fabricius, 1798) rearing to the crablet production

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Abstract. The salinity level for the culture of megalopae affects crablet production. The research aimed to determine the optimum salinity level for the megalopa of *S. tranquebarica* rearing. Nine aquariums randomly selected and filled with sterile seawater, have different salinity levels (A. 29-30 ppt, B. 27-28 ppt, and C. 25-26 ppt) used for megalopae rearing. Each aquarium is stocked with 30 individuals of megalopa. Each treatment with three replications. Crumble pellets and *Artemia* nauplii are given as feed for megalopae. Monitoring in each treatment consists of total hemocytes, the osmolality of megalopae and water media, osmotic level, *Vibrio* spp, total bacteria, and crablet production. The osmolality rate of megalopa hemolymph was highest at the salinity of 25-26 ppt. The lowest osmotic level at the salinity of 29-30 ppt. The total bacteria population at salinity 29-30 ppt was lower ($p < 0.05$) than at salinity 27-28 ppt and 25-26 ppt. Consequently, the *Vibrio* spp population was higher at 29-30 ppt salinity. The total hemocytes of megalopae at a salinity of 27-28 ppt were higher ($p < 0.05$) than the total hemocytes at a salinity of 25-26 ppt and 29-30 ppt. Therefore, the most increased crablet production in the megalopa reared at the salinity of 27 28 ppt.

1 Introduction

The mud crab genus *Scylla* spp. is highly valuable economically both locally and globally [1,2] and market demand is increasing every year, therefore, crab fishing in nature is intensifying. Moreover, hatchery technology could immediately successfully produce crablet in large quantities. However, there are several obstacles in the hatchery of *Scylla* spp. including the low percentage of megalopae that successfully become crablet is only about 10%.

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Scylla spp. is a euryhaline organism that lives in a mangrove ecosystem with a salinity range of 5-40 ppt. However, when viewed in detail on their life cycle, each stage has an optimal salinity range. The optimal salinity for zoea stadia cultured was 30 ppt [3,4], although larvae can live in the salinity range of 20-32 ppt [5]. The juvenile level of mangrove crab *S. olivacea* grows optimally at a salinity of 5-20 ppt, compared to a salinity of 30 ppt [6]. The megalopa stage means the larvae have already begun to be not planktonic but are beginning to be sedentary in the substrate [7] and their life will migrate from marine waters as the larvae stage to mangrove areas at a megalopa stage and continue until they reach young crabs and even adults [8]. Therefore, the salinity required for megalopa life is not the salinity of seawater (32-33 ppt), but the probability has decreased at salinity below 30 ppt. Megalopa stage is a crucial stadium because soon it will become a crablet. The success of megalopae metamorphoses in the crablets stage is critical to improving crablet production for cultured purposes or restocking in nature.

Research on the effect of the environment on the growth and development of larva, megalopa, and mud crab juvenile *Scylla* spp. has been done for a few years [9–11]. Larvae *S. serrata* most develop at salinity 25–35 ppt and water temperature 26 and 32°C, while megalopa developed in juveniles at 15–45 ppt at 20, 26, and 32°C. Interaction between salinity and temperature influences the larvae development. However, for the megalopa and juvenile, no interaction between salinity and temperature influences the megalopa and juvenile development [12]. The salinity for maintaining larvae up to megalopae is 25-30 ppt [13]. Although the optimum salinity for the larvae developed to the megalopa stage at 32 ppt [5]. However, of course, there are still other factors, such as the total content of cellular defense that involves the crustacean blood cells, called, hemocytes [14]. Total hemocyte level will decrease correlated with decreasing salinity for acclimation in *Litopenaeus vannamei* postlarvae-12 [15]. Furthermore, hemolymph, and osmotic levels due to changes in different ambient salinity levels, the development of *Vibrio* spp. bacteria, and total bacteria when megalopae are reared in each salinity level from 25 ppt to 30 ppt influence the megalopae successfully metamorphose to the crablet stage. A crucial part of the host immune response occurs in the hemolymph [16]. These factors are also likely to have a natural effect on crablet production. Therefore, research on megalopae cultured at different salinity levels, namely 25-26 ppt; 27-28 ppt, and 29-30 ppt, must be done. The study aims to obtain the optimum salinity level for the rearing of megalopae until they become the crablet stage. Its indication is the highest crablet production obtained from the salinity level tested.

2 Materials and methods

a. Megalopa production

Mature gonad females broodstock of mud crab, *S. tranquebarica*, with carapace width >10 cm from the middleman in Malili, East Luwu, South Sulawesi, Indonesia. The broodstock reared in the cone-shaped fiber tank volume of 500 L until spawned. The female spawned and then incubated for approximately ten days without being fed until the larvae hatched. Hatched larvae are immediately stocked in a conical fiber tank volume of 200 L salinity 30 ppt and fed rotifer and nauplius artemia. Once the larvae develop to the megalopa stage, a part of the megalopa is taken for a megalopa test at different salinity regimes. Nine aquariums, each size 60x30x34 cm, were used for this study and were filled with 30 L of sterilized seawater. Megalopa day first is stocked with a 30 ind./aquarium density.

b. Treatment test and parameters monitored.

The salinity treatment tested is A). 29-30 ppt, B). 27-28 ppt and C). 26-27 ppt. Before being stocked to the salinity of the treatment, the megalopa adapted to the salinity specified in each treatment. Each treatment with three repetitions. Feed was given daily as nauplius artemia at one ind./mL density, and artificial feed crumble pellets of as much as 5-7.5 mg / L/day. The production of crablet also monitors water quality by taking water samples then taken to the water quality laboratory to analyze ammonia, nitrite, and Total Organic Matter (TOM) based on the method of [17]. In addition, 50 mL of water samples from each treatment were taken to the Pathology laboratory to monitor *Vibrio* bacterial populations and total bacteria based on the methods [18]. Total hemocytes of megalopa from each treatment were also calculated based on the methods of [19]. At the same time, the measurement of the osmolality of megalopae hemolymph and water media for megalopae rearing is based on the procedure of [11]. The Osmotic level of megalopa in each treatment was also calculated based on the method of [20]. Crablet production, hemocytes, osmolality, and osmotic level data from each treatment are compared and analyzed using analysis of variance for the completely randomized design patterns. The least significant difference test (LSD test) was applied if any difference between the treatments was tested. The statistical test was carried out using the SPSS (Statistical Product Service Solution) program package 23.

3 Results

a. Water quality in megalopae rearing

Based on Table 1, ammonia, nitrite, TOM, and water temperature conditions are still within acceptable limits for the water quality criteria for megalopae to develop into the crablet stage. The ammonia concentration in the megalopae rearing tank ranges from 0.20-0.23 mg L⁻¹. In comparison, nitrite concentration is in the 0.01-0.12 mg L⁻¹. Total organic matter (TOM) ranged from 45-55 mg L⁻¹. It's relatively high in all treatments. The water temperature in the megalopa rearing tank was 27-28°C in all treatments.

Table 1. Ammonia, nitrite, and TOM concentrations in megalopa reared at the different salinity regimes.

Treatments	Ammonia (mg L ⁻¹)	Nitrite (mg L ⁻¹)	TOM (mg L ⁻¹)	Water temperature (°C)
A	0.10-0.20	0.01-0.12	45.22-55.99	27.6-28.1
B	0.11-0.23	0.01-0.12	43.23-55.05	27.5-28.2
C	0.13-0.21	0.02-0.12	43.36-50.92	27.6-28.3

A). 29-30 ppt, B). 27-28 ppt and C). 25-26 ppt.

b. Total *Vibrio* spp. and Total Bacteria in Megalopa Rearing

The highest *Vibrio* spp. population (3.69×10^3 CFU mL⁻¹) is in treatment A (salinity of 29-30 ppt) and the lowest (2.83×10^3 CFU mL⁻¹) in treatment C (salinity of 25-26 ppt). While in treatment B (salinity 27-28 ppt) with a *Vibrio* spp. population of 3.45×10^3 CFU mL⁻¹. The total bacteria population is high in treatment B (salinity 27-28 ppt) and C (salinity 25-26 ppt), namely, 3.475×10^4 CFU mL⁻¹ and 3.462×10^4 CFU mL⁻¹, compared to treatment A, with the lowest of total bacteria population ($1,825 \times 10^4$ CFU mL⁻¹) (Figure 1).

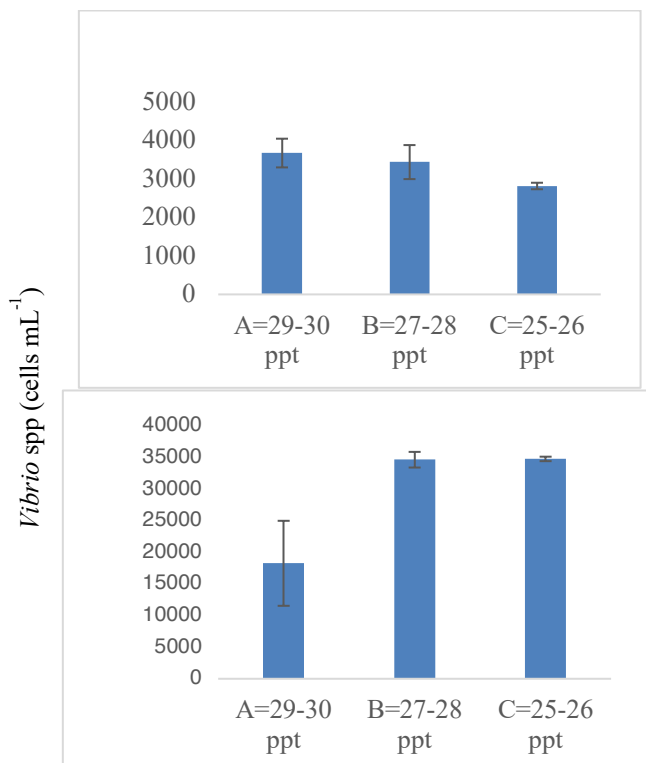


Fig. 1. The *Vibrio* spp. (left) and the total bacteria population (right) in the water media for megalopa rearing with different salinity levels.

After nine days of megalopa culturing, crablet production in treatment A = 4±1.5 ind. / aquarium (13-17%); B=7±2.5 ind./aquarium (23-30.3%); C=6±3.2 ind./aquarium (20-30%). The total hemocytes in megalopae cultured at a salinity of 27-28 ppt are the highest (600.3±26 x 10⁴ cells mL⁻¹) and significantly different (P<0.05) from total hemocytes in the megalopae cultured at the salinity of 25-26 ppt (344±43.3x10⁴ cells mL⁻¹) and salinity of 29-30 ppt (369±43.3x10⁴ cells mL⁻¹). The highest osmotic level (670.3±120.9 mOsm/L H₂O) in megalopae cultured in the treatment C (salinity of 25-26 ppt) and is significantly different (P<0.05) from the osmotic level of megalopae cultured in treatments A (salinity of 29-30 ppt) was 391.6±84.60 mOsm /L H₂O. However, there was no significant difference (P>0.05) with the osmotic level of megalopae cultured in treatment B (625.0±201.8 mOsm/L H₂O).

Table 2. Total hemocytes, hemolymph and water media osmolality, osmotic level, and crablet production in the megalopae-rearing at the different salinity levels

Treatment	Total haemocytes (x 10 ⁴ cells mL ⁻¹)	Osmolality hemolymph (mOsm kg ⁻¹)	Osmolality media (mOsm kg ⁻¹)	Osmotic level (mOsm kg ⁻¹)	Crablet Production Aq ⁻¹ (ind.)	Percentage (%)
Density Megalopa (ind./Aq)						

A	369±43.3 ^a	1109±84.28 ^a	717.3±2.88 ^a	391.6±84.60 ^a	4±1.5 ^a	13.3-17.3 ^a
30	600.3±26 ^b	1257.3±179.86 ^a	632.3±27.5 ^b	625.0±201.8 ^b	7±2.5 ^b	23.3-30.3 ^b
B	344±43.3 ^a	1237.3±105.45 ^a	566.3±70.2 ^b	670.3±120.9 ^b	6±3.2 ^b	20.0-30.0 ^b
C						

A). 29-30 ppt, B). 27-28 ppt and C). 25-26 ppt.

Numbers in the same column followed by different letters indicate a significantly different (P<0.05)

4 Discussions

Based on Table 1, the water quality parameters such as ammonia nitrite, TOM, and water temperature condition are still tolerable by megalopae to develop into the crablet stage. The LC-50 of unionized ammonia for zoea-5 was at a concentration of 4.05 mg L⁻¹ to 6.64 mg L⁻¹ after 24 hours [21]. The nitrite concentration in the megalopa rearing at the range of 0.01-0.12 mg L⁻¹. The safe limits of nitrite concentration for the larvae of Z5 were in the range of 2.5 mg L⁻¹ to 6.9 mg L⁻¹ [22]. In the present research, we used megalopa, which could have a higher value of these saves limit concentrations of ammonia and nitrite than the safe limits to support the survival of the larvae zoea-5. Total organic matter (TOM) ranged from 45-55 mg L⁻¹. It's relatively high in all treatments. The organic material in the larvae-rearing tanks originally comes from excessive feed. The water temperature in the megalopa rearing tank was 27-28°C in all treatments. The ideal water temperature for larvae rearing is 29°C[23], while [10] found that the ideal temperature is between 28 and 30°C. That means the water temperature in the present research is close to the optimum water temperature for larvae rearing.

Observations of the *Vibrio* spp. population shows that the highest *Vibrio* spp. population (3.69x10³ CFU mL⁻¹) is at a salinity of 29-30 ppt (treatment A). The *Vibrio* population's lowest (2.83x10³ CFU mL⁻¹) is in treatment C, with a salinity of 25-26 ppt. While in treatment B with a *Vibrio* spp. population of 3.45x10³ CFU mL⁻¹. The total bacteria population is relatively high in treatment B (3.475x10⁴ CFU mL⁻¹) (salinity 27-28 ppt) and C (salinity 25-26 ppt) (3.462x10⁴ CFU mL⁻¹), compared to treatment A, 1,825x10⁴ CFU mL⁻¹ (Figure 1). This shows that at high salinity (29-30 ppt) the population of *Vibrio* spp. is highest. It was caused by the total bacteria population at a salinity of 29-30 ppt being lower than that at a salinity of 27-28 ppt and 25-26 ppt. Thus, at salinity 25-26 ppt and 27-28 ppt, the *Vibrio* spp. development is more inhibited than in salinity 29-30 ppt. In nature, megalopae can adapt to drastic changes in salinity by regulating the rate of metabolism and response to immunity and by osmoregulation [8]. Therefore, this study analyzed the total hemocytes, osmolality, and osmotic levels in each salinity level treatment.

The rearing of megalopae with a low salinity of 12 ppt or a high of more than 40 ppt will affect the megalopa production [3]. This is because too much energy comes out to regulate the balance of body fluids with the body's outside environment, which has too low or too high salinity. If the salinity is too low, the crab will experience a mineral deficiency in its body.

The crablet production in treatment A = 4±1.5 ind. / aquarium (13-17%); B=7±2.5 ind./aquarium (23-30%); C=6±3.2 ind./aquarium (20-30%). Based on these results show that the salinity of 27-28 ppt (treatment B) is the most suitable (P<0.05) for the cultured megalopa *S. tranquebarica* until it develops into the crablet stage when compared to the production of crablet from the cultured megalopa in treatment A (salinity 29-30 ppt) and C (salinity 25-26 ppt). An analysis of the total hemocyte megalopa at each salinity level supports these results. The total hemocytes in megalopae cultured at a salinity of 27-28 ppt are the highest (600.3±26 x 10⁴ cells/mL) and significantly different (P<0.05) from total hemocytes in the megalopae cultured at a salinity of 25-26 ppt (344±43.3x10⁴ cells/mL) and salinity of 29-30 ppt

($369 \pm 43.3 \times 10^4$ cells/mL). Hemocyte cells in crustaceans are important in the host immune response, including recognition, phagocytosis, melanization, cytotoxicity, and cell-cell communication [24]. Hemocytes will differentiate, and mature hemocytes contain prophenoloxidase (proPO) in hemolymph [25]. Hemocyte cells' function is phagocytosis, coagulation processes, and release of prophenoloxidase, synthesis of alpha-2 macroglobulin with glutenin, and anti-bacterial peptides. The spread and increase in the number of hemocytes is a form of cellular immune response in the body of crustaceans [14]. The body of crustaceans, including mud crabs, does not have immunoglobulins that play a role in immunity but only have hemocyte cells which are cellular and humoral defense factors that are important in the body's defense against the attack of pathogenic organisms. Previous research on stadia zoea larvae with erythromycin applications whose larvae are more successful in becoming crablet with a total hemocyte value of $391.2 \pm 100.8 \times 10^4$ cells mL⁻¹ [26] This means that the total number of hemocytes in the current study is substantially higher. Especially in treatment B, then, its impact on the production of crablet is also a higher number compared to other treatments, which reach 30% from megalopae stocked in the aquarium.

Based on the number of crablets produced in this study, there is still too low, the highest is only 30% of the number of megalopae cultured success develop into crablet stage. Not synchronizing larvae development is the most important factor that affects lower crablet production. Besides, pathogenic bacteria often attack megalopa during metamorphoses to the crablet stage, and cannibalism of the crablet causes low crablet production. The higher crablet production ($67.0 \pm 14.95\%$) was obtained [27] and megalopae *S. paramamosain* was cultured using a plastic-coated earth pond, plus aeration, and covered with a greenhouse to stabilize the room temperature. Differences in species may also affect the success of megalopae rearing into the crablet stage. Based on the experiences, crablet production of *S. paramamosain* is more manageable than other species, such as *S. tranquebarica*, *S. olivacea*, and *S. serrata*.

Knowledge of the behavior of megalopae before metamorphosis to the first crablet instar is also essential, for example, whether it needs a sand substrate first or shelters such as seaweed that could add to the megalopa cultured. This needs more detailed observation because megalopae require a ride /stopover after the megalopa becomes the first instar crablet. Thus, in the first instar crablet stadia, there is no behavior to immerse yourself into the sand substrate. Research in the fiscal year 2020 on the use of mangrove leaves as a shelter in the megalopae of *S. paramamosain* and *S. olivacea* rearing in aquariums also obtained 20% of the number of megalopae was successful in being a crablet. This study gave aquarium containers sand substrate at all salinity treatments. At salinity, 27-28 ppt produces a more significant amount of crablet, about 23-30% of the total megalopae cultured. However, the combination of the two kinds of shelter (mangrove leaf shelter and the addition of sand substrate) to megalopa culture has never been tried in rearing megalopae. Previous research reports that from megalopa stadia to crablet instar first requires a sticking place, clinging before finally getting into the substrate to immerse oneself in sand or soil as its place of life on the third and fourth crablet instar [28]. However, for mud crabs, *S. serrata* larvae were constantly reared at a salinity of 32 ppt, which resulted in more megalopa production than if the larvae were kept on fluctuated salinity [5]. In contrast, the highest osmotic level (670.3 ± 120.9 mOsm/L H₂O) was obtained at the 25-26 ppt (treatment C) in the present study, is significantly differs from the osmotic level in treatment A (391.6 ± 84.60 mOsm /L H₂O). The salinity of 25-26 ppt is the lowest compared with the other treatments, it must be a lot of energy expended by megalopae for osmoregulation balance, therefore, the osmotic level value becomes high. While the lowest osmotic level was obtained at a salinity of 29-30 ppt. In treatment C the osmotic level is also high at 625.0 ± 201 . mOsm /L H₂O. However, crablet production is high in treatment B, therefore, total hemocytes and total *Vibrio* spp. may have

an important role in determining whether more megalopa successfully developed into the crablet stage.

5 Conclusion

The optimum salinity level for megalopa *S. tranquebarica* rearing is at 27-28 ppt, with the highest crablet production compared to the salinity of 25-26 ppt and 29-30 ppt. Total hemocytes of megalopae are significantly higher at salinity 27-28 ppt than at salinity 29-30 ppt and 25-26 ppt.

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