Effect of water salinity on survival and osmotic level of larval (Zoea Stage) of mud crabs Scylla tranquebarica

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Abstract. Mud crab of the genus Scylla are considered one of the most sought-after seafood today. This crab species has high quality and delicious aging growth rate and encourage expansion in the aquaculture sector especially in Southeast Asian Countries. However, salinity changes will cause changes in organisms osmotic pressure, and every aquatic biota has an optimal salinity range for survival. The study focuses on evaluating the effect of water salinity on the survival and osmotic levels of the purple mud crab, larvae of Scylla tranquebarica at the zoea stage. The LC50 assessment was performed in 10 different level of water salinity (0; 5; 10; 15; 20; 25; 30; 35, 40, and 50 ppt). Each treatment involved 20 ind./L of newly hatched crabs and being observed for 24 h in 10 different water salinity using 1 L volume glass container. The number of crab’s mortality were taken for each salinity regime. Larval behavior monitored during experiment. Meanwhile, the measurement of osmotic level was carried out at the salinity of 25, 30, and 35 ppt. The result shows that mud crab larvae exhibit any tolerance on the low salinity ranged from 0-10 ppt and the salinity of > 40 ppt. On the other hand, mud crab larvae were still able to survive at the salinity ranged from 20-40 ppt for more than 24 hours. The trend of the osmotic level of mud crab to survive is by hypo osmotic to iso osmotic.

1. Introduction
Mud crabs are a type of ten-legged animal whose natural habitat is in brackish watery coastal areas, especially in areas of thick muddy mangrove forests, channels and ponds, to reach the sea near the coast. Mud Crab with the scientific name Scylla sp. including the class Crustaceans, subclass Malacostraca, order Decapod, family Portunidae and genus Scylla. Currently, there are 4 (four) types of crabs from the genus Scylla, namely Scylla serrata, Scylla tranquebarica, Scylla paramamosain, and Scylla olivacea [1]. Mud crabs can be found in almost all coastal waters of Indonesia, especially in mangrove areas, brackish water ponds or river estuaries. Intensive exploitation of mud crabs which can threaten the availability of mud crabs in the wild has been observed recently in Indonesia. Based on [2] the Regulation of the Minister of Marine Affairs and Fisheries Number 12 of 2020 (Peraturan Menteri Kelautan dan Perikanan Nomor 12 Tahun 2020) that the legal size of mud crabs to catch is >12 cm in carapace length >150 grams in body weight. With this regulation, the government also expected that ready to spawn mud crabs broodstock will not be caught, so that they can reproduce to ensure the population sustainability in Indonesian waters.
*Scylla tranquebarica* is one of several crabs known as the mud crab, is found in mangrove, and popular known as purple mud crab or purple mud crab). This crab is an edible portunid crab with high commercial value. *S. tranquebarica* (Fabricius, 1798) is one of the candidate species that has widely been exploited and cultured. *S. tranquebarica* is easier to breed, larger in size and grows comparatively faster than *S. paramamosain* and *S. olivacea* [1]. Changes in salinity will cause changes in osmotic pressure of an organism through osmoregulation process, in which the lower salinity make the lower the osmotic pressure will be. Every aquatic biota has an optimal range of salinity to survive. Environmental conditions beyond the tolerable range of organisms may cause stress, growth and reproduction disruption, and even death [3]. Physiological mechanisms is used by fish (including crustacean) to cope with salinity stress. These mechanisms of dynamic control of osmoregulatory strategy include the ability to perceive salinity changes in the environment that disturb body water and salt homeostasis (osmosensing), signaling networks that encode information about the direction and magnitude of salinity change, and epithelial transport and permeability effectors [4].

The ongoing domestication of *S. tranquebarica's* mud crab activities that have been initiated by Research Institute for Brackish water Aquaculture and Fisheries extension Maros is expected to help preserve the genetic resources of native Indonesian mud crabs and increase the number of potential commodities for aquaculture businesses to increase fish production. The activity of testing the value of environmental tolerance and stress test carried out on water quality parameters, especially salinity, is one component of data collection needed in the context of domestication of mud crabs.

2. Materials and methods

2.1. Mud crab larvae

This study was conducted in the Mud crab hatchery of Research Institute for Brackish water Aquaculture and Fisheries Extension, at Barru Regency, South Sulawesi, Indonesia. The broodstock was collected from crab landings in Malili-East Luwu regency, South Sulawesi. It was kept in a 0.5-ton fiberglass tank and spawned in another 300-l tank at 30 ppt. At the zoea stage, larvae were randomly taken for the experiment.

2.2. LC50 assessment and larval behavior during experiment

The LC50 assessment was performed in 10 different level of water salinity (0; 5; 10; 15; 20; 25; 30; 35, 40, and 50 ppt). Each treatment involved 20 newly hatched crabs and being observed for 24 h. The number of crab’s die were recorded for each salinity regime. The statistical test for LC50 was done by using probit analysis Exel 2013. Observation of crab behavior in each treatment was carried out during the study.

2.3. Osmotic level of larvae (osmolality plasma and medium)

Based on the LC50 assessment, the observation was then continued with mass-scale testing with salinity ranges of 25, 30, and 35 ppt accompanied by measurement osmolality of larval plasma (hemolymph) and media. The osmolality was measured with Fiske-Osmometer [5].

The hemolymph sample of the zoea stage was obtain by grinding the larvae in 1 mL volume Eppendorf that has been pre-added anticoagulant (compound 2% NaCl, 0.1 M glucose, 30 mM Na citrate, 26 mM citrate acid, 10 mM EDTA) with the ratio of 4:1. One mL of the mixture was taken using a 1 mL disposable syringe, and then the sample was put into a 1.5 mL tube and centrifuged at 5000 rpm for 3 minutes. One mL supernatant was taken with a pipette and was transferred into a new tube. To analyze the osmolality rate, 20 µL supernatant was put into disposable tubes of an Osmometer, and the measurement was performed. Before doing the analysis for the next sample, the tube was cleaned using probe cleaner, and it was let to stay until dry [5], [6]. The larvae plasma is obtained by snarling filter larvae and inserted into the Eppendorf tube that has been added anticoagulants and subsequently crushed/grinding. *Osmotic Level of Larvae using two formula by* [5] and [7], [8].
2.4. Survival rate
The survival rate (SR) of larvae *S. tranquebarica* for both of the experiments were based on their initial and final number of zoea at each treatment calculated as follows SR (%) = the number of larva alive after exposure (Nt) / the initial number of the larva (No) x 100%.

3. Results and Discussion

3.1. The LC50
Refer to the concentration, which in this present study refers to the concentration of water salinity that causes 50% mortality of the crabs tested in a given of time. Twelve newly mature female purple mud crabs were tested in 10 different salinity for 24 h. Table 1 showed the result of different water salinity that cause mortality of zoea in 24 h.

Table 1. The Survival Rate (SR) of zoea stage of purple mud crab, *S. tranquebarica* at different water salinity regime within 24 h study period.

| Salinity (ppt) | Number of zoea die | SR (%) |
|----------------|--------------------|--------|
| 0              | 20                 | 0      |
| 5              | 20                 | 0      |
| 10             | 20                 | 0      |
| 15             | 20                 | 0      |
| 20             | 10                 | 50     |
| 25             | 8                  | 60     |
| 30             | 2                  | 90     |
| 35             | 17                 | 15     |
| 40             | 17                 | 15     |
| 50             | 20                 | 0      |

![Figure 1](image.png)

**Figure 1.** Concentration of lower salinity (0-20 ppt) that causes 50% mortality of *S. tranquebarica* (LC50) in 24 h and LC50 = 21.82 ppt
Figure 2. Concentration of higher salinity (25-50 ppt) that causes 50 % mortality of *S.tranquebarica* (LC50) in 24 h and LC50 = 38.50 ppt

Figure 1 showed the result of LC50 for low water salinity (0 until 20 ppt). The LC50 for low salinity for *S. tranquebarica* larvae in the present study is 21.82 ppt, meaning that the larvae can only survive in water salinity of more than 21.82 ppt. Figure 2 showed the result of LC 50 for higher water salinity (25 until 50 ppt). The LC50 for high water salinity for *S. tranquebarica* larvae in this study is 38.50 ppt, meaning that they can only survive in water salinity less than 38.50 ppt.

The LC50 point that had been determined for *S. tranquebarica* was 21.82 and 38.50 ppt. This result is the different range with the LC50 point that had been determined for *S. olivacea* (orange mud crab) was 5.14 and 39.82 ppt [9].

3.2. Larvae behaviour

The results of testing the salinity tolerance value of mud crab zoea larvae can be seen in Table 2 below. Salinity tolerance testing is performed on a test scale in the laboratory. Larvae and crablet of mud crabs are eurihaline animals, which are types of aquatic organisms that can adapt to the conditions of low to high maternity cultivation environment (5-35 ppt). The process of adaptation to salinity conditions is carried out through the osmoregulation process. For aquatic organisms, the process is used as a step to balance osmosis pressure between the substance in its body and the environment through permeable cells. Salinity that gives *S.olivacea* larvae the highest perform is 26 ppt [10] and for megalopa on salinity 28 ppt [11].
### Table 2. Stress test for mud crab larvae against salinity within 24 h study period.

| Salinity Test of Larval Stress | Larval Performance (number of individual) hours after spread | Initial | 1 hour | 7 hours | 17 hours | 24 hours |
|-------------------------------|-------------------------------------------------------------|---------|--------|---------|----------|----------|
| A (0 ppt) ~ 0,5               | 20 healthy larvae                                            |         | no longer active (25 % life in the bottom) | Larvae are no longer moving (completely dead) | -        | -        |
| B (5 ppt) ~ 5,5               | 20 healthy larvae                                            |         | Still moving but mostly at the bottom (50%) | Still moving at the bottom (20 %) | Still living in the base/bottom (10 %) | Larvae are no longer moving (completely dead) |
| C (10 ppt) ~ 11               | 20 healthy larvae                                            |         | Still moving but mostly at the bottom (50%) | Still moving at the bottom (20 %) | Still living in the base/bottom (10 %) | Larvae are no longer moving (completely dead) |
| D (15 ppt) ~ 14               | 20 healthy larvae                                            |         | Still actively hovering and partially at the bottom (80 %) | Still moving drifting and partially at the bottom (70 %) | Still moving and mostly at the bottom (50 %) | Larvae are no longer moving (completely dead) |
| E (20 ppt) ~ 21               | 20 healthy larvae                                            |         | 90% still actively hovering on the surface | Still moving drifting and partly at the bottom (75 %) | Still moving drifting and partly at the bottom (65 %) | Still moving drifting and partly at the bottom (50 %) |
| F (25 ppt) ~ 25               | 20 healthy larvae                                            |         | 90% still actively hovering on the surface | Still moving drifting and partly at the bottom (85 %) | Still moving drifting and partly at the bottom (65 %) | Still moving drifting and partly at the bottom (60 %) |
| G (30 ppt) ~ 32               | 20 healthy larvae                                            |         | 100% healthy drift | 100% healthy drift | 100% healthy drift | 90% of life |
| H (35 ppt) ~ 35               | 20 healthy larvae                                            |         | 100% healthy drift | 100% healthy drift | 100% healthy drift | 90% of life |
| I (40 ppt) ~ 40               | 20 healthy larvae                                            |         | 35 % still moving | Remaining 30% of the living | 20 % of life | 15 % of life but in the bottom |
| J (50 ppt) ~ 50               | 20 healthy larvae                                            |         | no longer actively moving | Larvae are no longer moving (completely dead) | -        | -        |

3.3. **Osmolality plasma of larvae and media**

The value of media osmolality was decrease when salinity decreases, and increases when salinity increases. Changes in media salinity cause fluctuations in larval plasma or hemolimph osmolality
which causes it to be in an osmotic imbalance condition, so it will try to make physiological adaptations [12], [5].

**Figure 3.** Osmolality of Plasma Larva *S.tranquebarica*

**Figure 4.** Osmolality of Medium (water)

Based on Figure 20, the average of Osmotic Level of larvae approaching 1 (iso osmotic) is larvae on a maintenance medium of 25 ppt which means that in such conditions the osmotic pressure of the larva is experienced/close to balance. It is supported by the production of megalopa and zoea 5 produced after 20-21 days of maintenance on 25 ppt media is the highest (287 and 216 indv), the 30 ppt media has not entered the megalopa phase (all still zoea), and the 35 ppt media produces 124 megalopa, 151 zoea 5 and 10 krablet. According to [13] the group of krustase (shrimp and crabs) is an isoosmotic group. The dominant ions in determining seawater osmolarity pressure are Na+ (450 mM) and Cl (560 mM). Na+ and Cl- portions were 30,61 and 55,04% of the total concentration of dissolved ions, respectively. In [4] explains that environments with varying salinity will be inhabited by organisms that have a wide range of salinity tolerances. Euryhaline fish have a control mechanism as an osmoregulatory strategy, from the active absorption and production of salt and water.

Based on the results of this study also found that the tendency of osmotic pressure for mud crab larvae is hypoosmotic to isoosmotic. So in hyperosmotic conditions are not able to be tolerated by larvae and cause death (other than due to differences in very extreme media conditions) as in salinity treatment 0-10 ppt. The Osmotic Level value in larvae in water media 0 (0.5), 5, and 11 ppt respectively is 10.26; 1.9 and 1.1 m Osm/kg.
Figure 5. The Osmotic Level between Plasma of Larva *S.tranquebarica* and Medium.  
A. Formula by [5]; B. Formula by [7], [8]

The larval Osmotic level approaching 1 (iso osmotic) condition reached when the medium salinity is 25 ppt, which means that under these conditions the osmotic pressure of the larvae approaches equilibrium. This is supported by the highest production of megalopa and zoea 5 after 20 days at 25 ppt.

3.4. Mortality and survival
The research was conducted in 2 stages of temperature testing, namely step 1 temperature testing with a wide range of 0-40 ppt and step 2 using temperature with narrow ranges (25-35 ppt). The test results are presented in Figure 6 and Table 3.

Step 1.

Figure 6. SR (%) of *S.tranquebarica* larvae in ten regime of salinity level (small scale)
Based on Fig.6, shows that salinity 0-10 ppt and salinity 50 ppt is extreme salinity for mud crab larvae indicated in the results of exposure to such salinity after 1 hour larvae that the SR were no more than 50 % (for salinity 5-10 ppt) and 0 % at salinity 0 and 50 ppt. For salinity 15 ppt still survives 80 % of life in the first 1 hour but after 24 hours is dead a total of 100 %. For salinity testing 20-40 ppt still survives up to 24 hours more. Information accompanied by the behavior of larvae during this salinity stress test as presented in Table 2.

**Step 2**

| Day | 35 ppt SR (%) | 30 ppt SR (%) | 25 ppt SR (%) |
|-----|---------------|---------------|---------------|
| 0   | 100           | 100           | 100           |
| 7   | 68            | 40            | 29            |
| 10  | 44            | 20            | 7             |
| 17  | 3             | 1             | 4             |
| 21  | 0.49a         | 0.23a         | 1.06b         |

Maintenance of larvae *S.tranquebarica* produced the highest survival rate presentation (1.06 %) at salinity 25 ppt where the results significantly differed (p<0.05) with survival of larvae at salinity maintenance 30 and 35 ppt. This result is approximately the same as [8] in *S. olivacea* larva with the highest survival at 26 ppt salinity. In contrast to [14] where *S. serrata* larvae rearing was carried out at 28-30 ppt salinity and resulted in a higher survival of 2.5%.

4. Conclusion

Larval of *S. tranquebarica* are intolerant to low salinity < 21 ppt, and high salinity > 38 ppt. The tendency of osmotic pressure for *S. tranquebarica* larvae to survive is from hypo-osmotic to iso-osmotic conditions.

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