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Research Article
Effect of Water Salinity on the External Morphology of Ovarian Maturation Stages of Orange Mud Crab, *Scylla olivacea* (Herbst, 1796) in Captivity

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Abstract

Background and Objective: Mud crab from the genus *Scylla* are considered as one of the most demanded seafood items nowadays as their flesh has high quality, tasty and higher growth rate thus support and boosted expansion in aquaculture sector especially in Malaysia. Present study was designed to focus on the effect of water salinity on the ovarian maturation of orange mud crab, *Scylla olivacea* based on morphological characteristics. **Methodology:** Samples were collected from Setiu wetlands, Terengganu, Malaysia from July-September, 2015. Ovarian maturation of *S. olivacea* was classified into four stages based on previous study which were: Immature (Stage 1), early mature (Stage 2), late mature (Stage 3) and fully mature (Stage 4). **Results:** Morphologically as the ovary develop the colouration start to change from translucent or whitish in colour and sometimes creamy to pale yellow, follow by light orange and lastly reddish orange. Stage 1 ovary was translucent and whitish in colour, stage 2 ovary was pale yellow in colour, stage 3 was light orange and stage 4 ovary was reddish orange in colour. Gonad Somatic Index (GSI) of *S. olivacea* remained low at stage 1 and 2 and began to increase started at stage 3. This present study involved three different salinities treatments, which treatment 1 (10 ppt), treatment 2 (20 ppt) and treatment 3 (30 ppt). Treatment 2 produce the highest number of stage 4 ovarian maturation based on colouration and the highest GSI recorded, follow by treatment 1 and lastly treatment 3. **Conclusion:** This present study proved that salinity does affected the ovarian maturation of *S. olivacea* in captivity and provides important information regarding the effect of water salinity on ovarian maturation for further studies on reproductive biology of this species.

Key words: Ovarian maturation stages, *Scylla olivacea*, salinity, LC_{50}, captivity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mud crabs, genus *Scylla* is also known as mangrove crab[1] are associated naturally in the estuary zone and mangrove areas[2-4], where the salinity varies over time[5]. Nowadays, mud crabs are increasingly known to be part of aquaculture cultivated species due to high demanded as their possessed impressive size, high meat yield, delicious taste[6], rapid growth during culture[7] and sweetness and delicate taste of its meat[8]. However, due to lack of knowledge on ovarian maturation in captive hatchery, mud crab production in aquaculture for commercialization is still not well develop. Reproductive biology of fish or crustacean is important for the sustainable and successful production of seeds or offspring in aquaculture[9]. Based on Azra and Ikhwanuddin[10] the lack of information during reproductive cycle on the changes in the ovary had limited the expansion and development of the mud crab seed hatchery technology nowadays. By focusing on the understanding about reproductive biology, proper management practices can be plotted in the aquaculture industry. Even though orange mud crab, *Scylla olivacea* is well known and common mud crab species[2], there are still lack of information about the reproductive biological information especially on the ovarian maturation for this species. There are not much studies have been done so far on the reproductive biology of *S. olivacea* except for present studies by Azmie *et al*.[9], Ikhwanuddin *et al*.[11] and Muhd-Farouk *et al*.[12,13]. The reproductive biological information from this present study is important to understand the characteristics of *S. olivacea* that can be used for management purposes and also to support the expansion in aquaculture sector.

Based on Lesser[14], salinity plays important factor for physiological regulation of marine organism. Besides that, based on Talpur and Ikhwanuddin[15] water salinity does affected the habitat preferences and many functional responses of organisms including reproduction and survivality. Due to the facts that mud crabs inhibit mostly in mangrove and coastal area, the salinities changes and fluctuation of water surely influences the ovarian maturation of mud crab. Hence, optimum water salinity preferences for development of ovarian maturation of mud crabs can be established. Therefore, this study was design to study the effect of water salinity on the external morphology of ovarian maturation of *S. olivacea* in captivity.

MATERIALS AND METHODS

Sampling: A total of 150 immature females *S. olivacea* were collected from Setiu wetlands Mangrove forest, Terengganu, Malaysia (5°31'23.1"N 102°55'56.1"E). The identification of *S. olivacea* species was based on morphological description provided by Keenan *et al*.[16]. The crabs were brought back to the Marine Hatchery of Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu and were measured for its Carapace Width (CW) and Body Weight (BW) using Vernier caliper and digital electronic balance and each crab individuals were labelled with cable tie tag. The CW is the distance between the tips of 9th antero-lateral spines of the carapace.

Newly matured female *S. olivacea* samples: In order to obtain newly matured female *S. olivacea* samples, limb autotomy technique[17] was executed on 150 crabs by cutting off the chelipeds and walking legs and only pleopods (known as swimming legs) were left. A sharp cutter was used to cut a joint between the carpus and merus of the pincers and subsequently, three pairs of legs were casted off by cutting the periopod merus using the cutter. The pleopods were left to allow crab to swim. Next, all autotomized crabs were cultured in tanks for observation of moulting events. The moulted crabs were then isolated to other tanks to let their shell harden prior to the experimental treatment.

LC_{50} assessment of different water salinity: Prior to the treatment, an LC_{50} assessment have been done in 9 different water salinity (2, 5, 10, 15, 20, 25, 30, 35 and 40 ppt). This assessment were done in order to determine the level of water salinity that *S. olivacea* can withstand and survive. Each treatment involved 10 newly matured crabs and being observed for 72 h in 9 different water salinity. The number of crab's mortality were taken for each salinity regime.

Experimental design: The experimental design consist of three treatments. The first treatment (T1) comprised of 20 newly mature female crabs of gonad development stage 1 in 10 ppt, 2nd treatment (T2) comprised of 20 newly mature female crabs of gonad development stage 1 in 20 ppt and 3rd treatment (T3) comprised of 20 newly mature female crabs of gonad development stage 1 in 30 ppt water salinity. Each crabs were culture in 5 L aquarium individually and the culture period was 60 days fed with chopped fish at 20% of crab biomass as their diet. Five crabs from each treatments were randomly chosen at every each 15 days (15, 30, 45 and 60 days) to undergo dissection for ovary external morphology assessment.

Ovary external morphology assessment: Ovarian maturation stages was distinguished by coloration of the ovary based on

previous study observation[9-13], stage-1: Translucent to creamy white, stage-2: Yellowish, stage-3: Pale to dark orange and stage-4: Dark orange to red-orange. Gonadal Somatic Index (GSI) was also recorded for each treatments.

The calculation of GSI is as below:

$$\text{Gonad somatic index} = \frac{\text{Ovary weight (g)}}{\text{Body weight (g)}} \times 100\ (\%)$$

Data analysis: The coloration and the GSI of the ovarian maturation were recorded and measured. Collected data were analyzed by using microsoft office Excel 2013 and one-way ANOVA through the application of IBM SPSS statistics version 22.

RESULTS

LC_{50} data collection: The LC_{50} refer to the concentration, which in this present study refer to the concentration of water salinity that causes 50% mortality of the crabs tested in a given of time. Ten newly mature female orange mud crab, *S. olivacea* each were tested in 9 different salinity for 72 h. Table 1 showed the result of different water salinity that cause mortality of crabs in 72 h.

Figure 1 showed the result of LC_{50} for low water salinity (2 until 15 ppt). The LC_{50} for low water salinity for *S. olivacea* in this present study is 5.14 ppt, so, LC_{50} showed that *S. olivacea* can only survive in water salinity more than 5.14 ppt.

Figure 2 showed the result of LC_{50} for higher water salinity (15 until 40 ppt). The LC_{50} for high water salinity for *S. olivacea* in this present study is 39.82 ppt, so, LC_{50} showed that *S. olivacea* can only survive in water salinity less than 39.82 ppt. The LC_{50} point that had been determine for *S. olivacea* was 5.14 and 39.82 ppt. The statistical test for LC_{50} was done by using probit analysis through the application of IBM SPSS statistics version 22. The result showed there was significant difference as $p = 0.02$ ($p<0.05$) for the percentage of crab mortality versus the concentration of salinity toward days culture.

External morphology of ovarian maturation with different salinity: The external morphological characteristics in this present study referring the colouration from the previous research done by Azmie *et al.*[9], Ikhwanuddin *et al.*[11] and Muhd-Farouk *et al.*[12,13].

Table 2 and Fig. 3 showed the external morphological characteristics of *S. olivacea* ovarian maturation stages. Figure 3a showed stage 1 ovarian maturation, which is translucent or whitish colour of ovaries. The ovaries was small and hard to separate from the digestive gland. Figure 3b showed stage 2, which is pale yellow in colour. The volume of ovary increase compared to stage 1. Figure 3c showed stage 3 ovarian maturation with orange in colour and occupies approximately 50-60% of cavity. Figure 3d showed stage 4 ovarian maturation, which is fully matured stage. Volume of ovary increase and occupies 70% of the ovary and eventually covered the hepatopancreas.

Figure 4 showed results of external morphology of ovarian maturation of *S. olivacea* based on colouration in

Table 1: Number and percentage of orange mud crab, *S. olivacea* mortality in different water salinity regime within 72 h study period

Salinity regime (ppt)	No. of crab mortality (10 crabs/salinity regime)	Percentage of crab mortality (%)
2	7	70
5	6	60
10	0	0
15	0	0
20	0	0
25	0	0
30	0	0
35	5	50
40	6	60
Total sample	90	

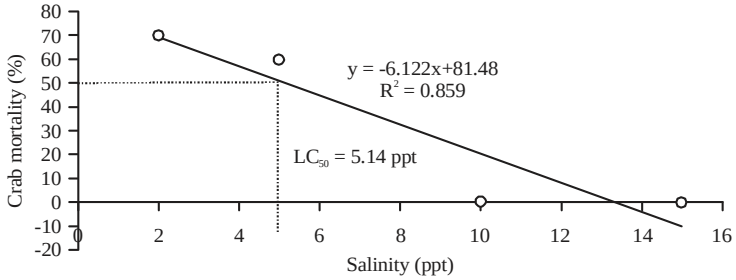

Fig. 1: Concentration of lower salinity (2-15 ppt) that causes 50% mortality of orange mud crabs, *S. olivacea* (LC_{50}) in 72 h and LC_{50} = 5.14 ppt

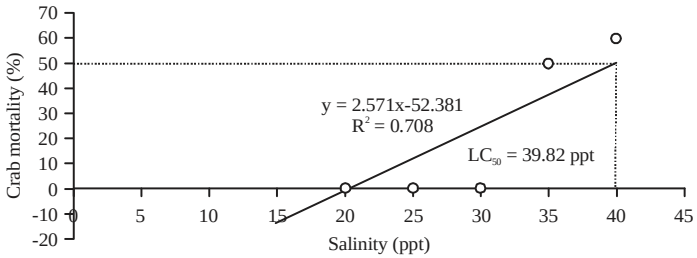

Fig. 2: Concentration of higher (15-40 ppt) salinity that causes 50% mortality of orange mud crabs, *S. olivacea* (LC_{50}) in 72 h and LC_{50} = 39.82 ppt

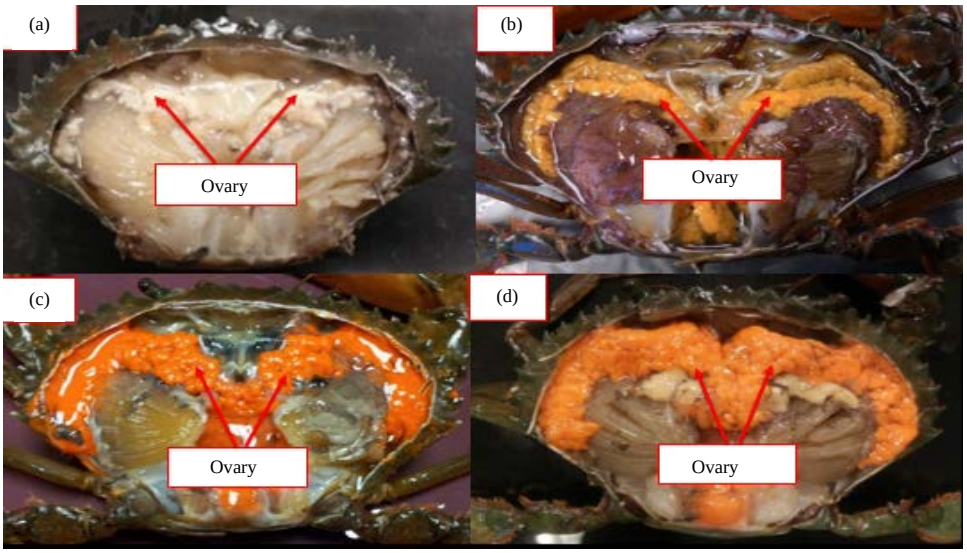

Fig. 3(a-d): External morphological view of ovary at various ovarian maturation stages in the present study of orange mud crab, *S. olivacea*, (a) Immature (stage 1), (b) Early maturing (stage 2), (c) Pre-maturing (stage 3) and (d) Fully matured (stage 4)

Table 2: External morphological characteristics of ovary at various ovarian maturation stages in the present study of orange mud crab, *S. olivacea*

Ovarian maturation stages	External morphological characteristics
Immature (1)	Translucent jelly like to pale white ovary and sometimes creamy white in colour
Early maturing (2)	Ovary pale or light yellow in colour and increase in size
Pre-maturing (3)	Ovarian size increase and colouration change from yellow to orange
Fully matured (4)	Ovary increase in size, occupying available space in body cavity and colouration change from orange to reddish orange

three different treatment which are 10, 20 and 30 ppt. Figure 4a showed the result for day 0 as control. On day 0, all treatments showed stage 1 ovarian maturation, which is translucent and whitish in colour, respectively. Figure 4b showed the result for day 15. On day 15, all treatment showed stage 1 ovarian maturation based on ovaries colour, which is translucent and whitish in colour respectively. Figure 4c showed result for day 30. Beginning from day 30, ovaries colouration started to differ between each treatment. The T1 (10 ppt) showed all stage 1 ovarian maturation based on ovaries colour. The T2 (20 ppt) showed ovarian development with three stage 3, which is orange in colour and two stage 1 ovarian maturation based on ovaries colour. The T3 (30 ppt) also showed ovaries development with the present of one stage 3, one stage 2 and three stage 1 ovarian maturation based on colouration. Figure 4d showed result for day 45. On day 45, T1 started to showed ovarian development. Both T1 and T2 showed three stage 3 and two stage 1 ovarian maturation based on ovaries colour. The T3 showed only one stage 3 and 4 stage 1 ovarian maturation based on colouration. Figure 4e showed result for day 60. On day 60, each treatment managed to produce stage 4 ovarian

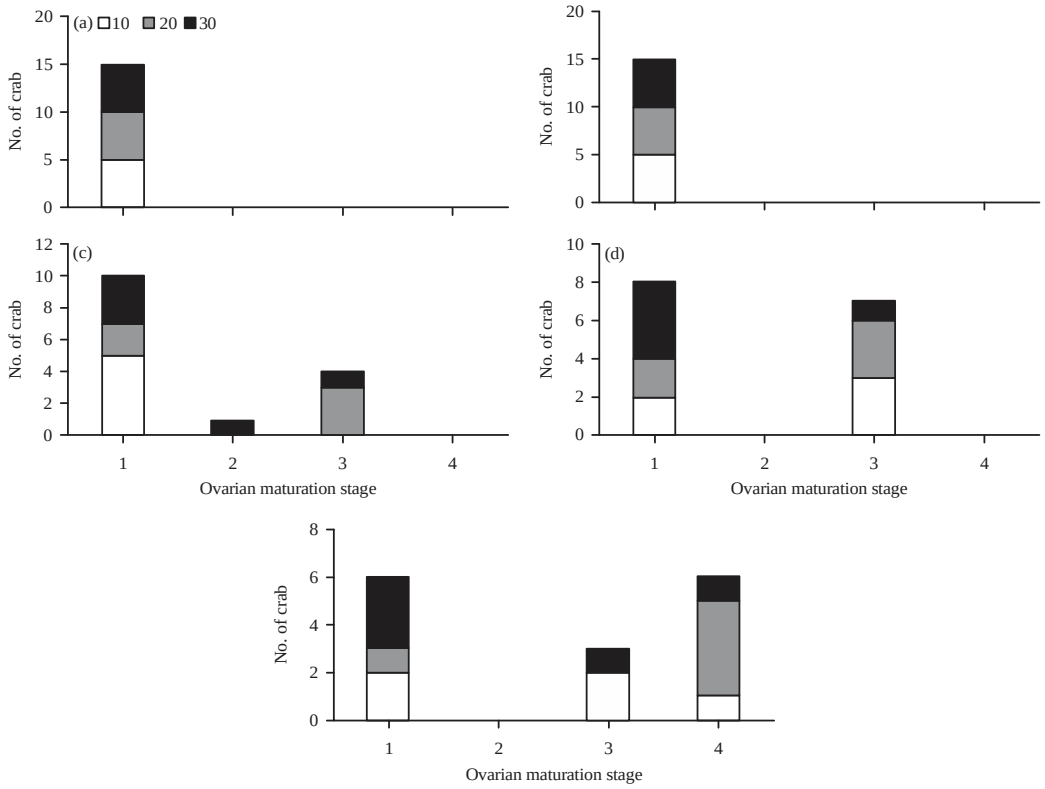

Fig. 4(a-e): Colouration of ovary at various ovarian maturation stages in the present study of orange mud crab, *S. olivacea*, (a) Day 0 (control), (b) Day 15, (c) Day 30, (d) Day 45 and (e) Day 60

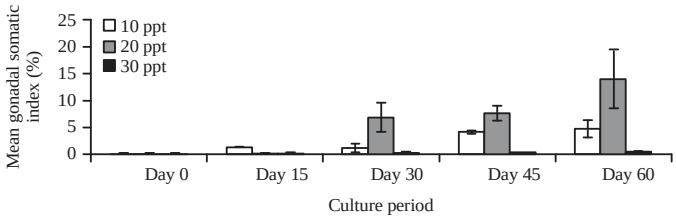

Fig. 5: Mean Gonadal Somatic Index (GSI) of orange mud crab, *S. olivacea* in different salinity regimes (10, 20 and 30 ppt) within 60 days culture period

maturation. The T1 showed one stage 4, two stage 3 and two stage 1 ovarian maturation. The T2 showed four stage 4 and one stage 1 ovarian maturation and T3 showed one stage 4, one stage 3 and three stage 1 ovarian maturation based on ovaries colouration.

Gonadal Somatic Index (GSI) in different salinity: Gonad Somatic Index (GSI) refers to gonad mass over total body mass. For day 0 as control, mean GSI was 0.19±0.04%. For day 15, mean GSI for each treatment was T1 (1.37±0.04%), T2 (0.16±0.03%) and T3 (0.26±0.09%). For day 30, mean GSI for each treatment was T1 (1.12±0.82%), T2 (6.86±2.74%) and T3 (0.34±0.04%). For day 45, mean GSI for each treatment was T1 (4.17±0.29%), T2 (7.68±1.40%) and T3 (0.36±0.03%). On day 60, mean GSI for each treatment was T1 (4.86±1.70%), T2 (14.10±5.50%) and T3 (0.45±0.17%) (Fig. 5). The statistical test for GSI in different salinity was done by using one-way ANOVA analysis through the application of IBM SPSS statistics version 22. The result showed there was significant difference as $p = 0.01$ ($p<0.05$) between GSI and the salinity concentration towards day culture.

This present study showed that all treatment managed to produce stage 4 ovarian maturation in 60 days. Based on colouration of ovarian stage, T2 managed to produce highest

number of stage 4 ovarian maturation, followed by T1 and lastly T3, respectively. Besides that, based on GSI also, T2 showed the highest GSI, followed by T1 and lastly T3 respectively. On day 15, T1 has the highest mean GSI with 1.37±0.04% but starting from day 30 until day 60, T2 have the highest GSI compared to other treatments. The highest GSI recorded in this present study was 14.10±5.50% on day 60 T2, while the lowest GSI recorded was 0.16±0.03% on day 15 T2. The T2 showed the highest increase of GSI by day, followed by T1 and T3 showed only slightly increased of GSI by day in 60 days treatment been done.

DISCUSSION

The present study is the first report of the effect of different water salinity (10, 20 and 30 ppt) on the external morphology of ovarian maturation of *S. olivacea* in captivity. Mud crab genus *Scylla* live in the muddy area, which is influence by tidal. In order to live in such condition, they are well known to tolerate with a wide range of salinity[5,18].

Based on previous study done by Ikhwanuddin *et al.*[11] and Muhd-Farouk *et al.*[12,13] ovarian m aturation of *S. olivacea* was classified to have four stages (stage 1-4) based on their external morphological characteristics and histological assessment characteristics. The volume of ovary increased as the stages increased from stage 1-4 ovarian maturation and the classification also related closely with the increase of GSI volume. Mean percentage GSI according to previous study done by Azmie *et al.*[9] showed that stage 1 with 0.66±0.98%, stage 2 with 2.22±1.68%, stage 3 with 7.96±3.48% and stage 4 with 10.20±3.74%. This present study showed higher mean GSI in T2 compared to previous study done by Azmie *et al.*[9] as water salinity does affected the reproduction of *S. olivacea*[19] and that salinity could become the optimum salinity for selected species in this present study.

The T2 and T1 showed higher development of ovarian maturation stages and higher increase of GSI value by days compared to T3. This may due to the osmosis stress that crabs need to handle on that salinity. Besides that, crab samples on T3 only relying energy in order for their survival and not for their growth development thus reduce the ovarian maturation growth performance on that salinity. Salinity does influence the reproductive biology of crabs. Based on previous study by Ikhwanuddin *et al.*[2] and Teshima and Kanazawa[20] factors such as diets, salinity and temperature does influenced the reproductive biology of crabs. Besides that, previous study by Rasheed and Mustaquim[21] states that environmental factors such as salinity and temperature does affected the sexual maturity of mud crab. Salinity and temperature also affected CW_{50} of female blue crab, *Callinectes sapidus* from nine Texas coast as temperature and salinity that vary from one bay to another[22].

It was observed in this present study that the colour of stage 1 ovaries, which is immature is translucent to yellow and become orange and reddish orange in mature ovaries as similar with previous study done by Azmie *et al.*[9], Ikhwanuddin *et al.*[11] and Muhd-Farouk *et al.*[12,13] for *S. olivacea* and by Quinitio *et al.*[23] for *S. serrata*. Based on Quinitio *et al.*[23] this variability in colour may also due to the diet intake by the crab. Previous study by Ikhwanuddin *et al.*[11] stated that, lipid accumulation in the form of yolk in the oocytes which serves as the nutrition source for the developing embryo had changed the colouration in ovaries.

This present study have proved that salinity does affected the ovarian maturation of *S. olivacea*. Even though external morphology such as ovarian colouration and GSI can be taken as factors to determine the stages of ovarian maturation, histological assessment need to be taken as it give more accurate results[24]. Salinity 20 ppt showed the best result in this present study followed by 10 ppt and lastly 30 ppt, thus can be used as important information in culturing mud crabs in captivity for hatchery broodstock maturation.

CONCLUSION

The result of this experiment indicated that water salinity does affected the ovarian maturation of female *S. olivacea* in captivity and the best treatment is treatment 2 (20 ppt) as it produced the highest GSI percentage and most stage 4 ovarian maturation stages based on colouration. In addition, results also showed that all treatments were able to produce stage 4 ovarian maturation of *S. olivacea* in captivity. However, there are still lack of in-depth study about water salinity that can be emphasized to increase the production of matured female broodstock for hatchery crab seed production.

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SIGNIFICANCE STATEMENT

This article is suitable with Pakistan Journal of Biological Sciences because the findings will give a big contribution in the understanding of the mud crab reproductive biology:

- Mud crab seedling especially *Scylla olivacea* is getting less landing every year, new approach needed in ensuring enough supplies of *S. olivacea* to be available
- Approaching method to induce the ovarian maturation of mud crab without using hormones ensured the safety of the consumers
- Ensuring the longevity of *S. olivacea* species by restocking program through the finding of this research article

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