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Effect of some minerals on shell hardening of mud crab, *Scylla serrata* (Forskal, 1775)

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Abstract
Study on optimum requirements of some minerals in shell hardening of mud crab, *Scylla serrata* was carried out during the present study. Juveniles of live mud crabs in the size range of 73 – 94 mm carapace width and weight range of 110-160 g were stocked in HDPE circular tanks of 125 L capacity. The experiment was carried out in two phases according to the completely randomized design having three replicates for each treatment. At first phase, the pre-moult crabs were subjected to water of various composition and concentrations of minerals such as $CaCO_3$ and KCl (each @ 500 to 800 mg L^{-1} at the interval of 100 mg L^{-1}) and $MgSO_4$ (1400 to 1700 mg L^{-1}). At second phase, the experiment was conducted with increased concentrations of minerals such as $CaCO_3$ and KCl (each @ 800 to 1100 mg L^{-1} at the interval of 100 mg L^{-1}) and $MgSO_4$ (1700 to 2000 mg L^{-1}). The results revealed minimum shell hardening period of 9 ± 0 days and 9 ± 0.58 days in the first and second phase in the water having mineral contents of $CaCO_3$ and KCl each at 800 mg L^{-1} and $MgSO_4$ at 1700 mg L^{-1} respectively.

Keywords: Mud crab, minerals, moulting, shell hardening

1. Introduction

Rearing of the soft crabs in captive conditions like pens, cages, etc is carried out in most parts of the country; however, the time taken for hardening of the shell varies from two weeks to four weeks incurring additional cost of maintenance. Thus, the present study is undertaken to evaluate probability of reducing the fattening period by triggering shell hardening process and to reduce the extra cost on maintenance of soft crabs by the farmers. As the crabs tend to absorb water during the moulting process, some major minerals were incorporated in the water medium to analyze the effect of incorporation of minerals in shell hardening. Although the diets play an important role, shrimps and fishes absorb some minerals from drinking, and by direct absorption via gills and epidermis (Deshimaru *et al*., 1978) [1]. Considering the importance of minerals in shell hardening process of the crabs, present study was attempted to find out the effect of some major mineral sources in hardening of the mud crab, *Scylla serrata.*

The review of literature shows that the fattening period of mud crab varies between 15 – 40 days (Ladra, 1992; Soundarapandian and Raja, 2008; Dat, 1999; Begum *et al*., 2009; Santhanakumar *et al*., 2010) [2, 3, 4, 5, 6]. De Silva (1992) [7] observed a mud crab fattening period of 62 days in cement tank. Anil and Suseelan (2001) [8] reported a fattening period of 45 days for mud crabs reared in brackish water ponds.

Mineral composition of mud crabs and marine crabs has been studied by numbers of researchers. The minerals were found in carapace, muscle, tissues exuvium, and haemolymph in hard shell and soft shell as shown by Salaenoi *et al*. (2006) [9], Benjakul and Suthipan (2009) [10], Sudhakar *et al*. (2009) [11], Rekha *et al*. (2014) [13]. However, the studies related to mineral uptake by the crustaceans are scanty. Roy *et al*. (2007) [14] studied the mineral requirements of *Litopenaeus vannamei* in inland low salinity water of approximately 4.0 ppt. In another study, Roy *et al*. (2010) used low salinity waters for *Litopenaeus vannamei* culture by adding potassium and magnesium fertilizers.

2. Materials and Methods

2.1 Experimental Design

The study was carried out in the Wet Laboratory of College of Fisheries, Ratnagiri, Maharashtra, India. The experiment was conducted as per completely randomized design in triplicates in high density polyethylene (HDPE) circular tanks of 125 L capacity. In experiment I, the pre-moult crabs were stocked in water of different compositions of minerals such as $CaCO_3$, KCl and $MgSO_4$ as shown in Table 1.

Table 1: Mineral media for experiment I

S. No	Mineral	Mineral Composition (mg L^{-1})																
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}
1.	$CaCO_3$	-	500	500	-	500	600	600	-	600	700	700	-	700	800	800	-	800
2.	$MgSO_4$	-	1400	1400	1400	-	1500	1500	1500	-	1600	1600	1600	-	1700	1700	1700	-
3.	KCl	-	500	-	500	500	600	-	600	600	700	-	700	700	800	-	800	800

On the basis of results obtained in experiment I, the minerals composition for experiment II was decided which is given in Table 2.

Table 2: Mineral media for experiment II

S. No.	Mineral	Mineral Composition (mg L^{-1})																
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}
1	$CaCO_3$	-	800	800	-	800	900	900	-	900	1000	1000	-	1000	1100	1100	-	1100
2	$MgSO_4$	-	1700	1700	1700	-	1800	1800	1800	-	1900	1900	1900	-	2000	2000	2000	-
3	KCl	-	800	-	800	800	90	-	900	900	1000	-	1000	1000	1100	-	1100	1100

2.2 Selection of pre-moult crabs

The juveniles of live mud crab, *Scylla serrata* in the size range of 73–94 mm carapace width and 110 – 160 g weight were procured from market and/or from crab collectors and acclimatized to 25 ppt salinity in 500 L capacity circular fibre glass tank. About 30 L of water volume with water depth of about 4–5 cm was maintained in acclimatization tank to facilitate respiration for the crabs, as the mouth parts remains under water. Pre-moult crabs were identified on the basis of separation of epicuticle from membranous layer of new cuticle (Drach, 1939) [15]. The occurrence of pre-moult stage of crabs was frequently observed during morning, afternoon and evening hours to undergo the experimental study.

2.3 Preparation of mineral media

The normal seawater of 35 ppt salinity contains Ca, Mg and K in the proportion of about 400 mg L^{-1}, 1300 mg L^{-1}, and 400 mg L^{-1} respectively (Harvey, 1957; Riley and Skirrow, 1965; Kennish, 1994) [16, 17, 18], Therefore, in the present study, the minimum level of 500 mgL^{-1}, 1400 mg L^{-1}, and 500 mg L^{-1} and the maximum of 1100 mg L^{-1}, 2000 mg L^{-1}, and 1100 mg L^{-1} of Ca, Mg and K minerals respectively were selected. The different composition of minerals media were prepared by dissolving required quantity of minerals in plastic containers. In the control treatment, 25 ppt salinity was maintained where the mineral composition of 308 mg L^{-1} Ca, 1207 mg L^{-1} of Mg, and 312 mg L^{-1} of K was observed.

2.4 Stocking and management

The healthy pre-moult juvenile crabs were carefully stocked in the treatment tanks at a density of 4 nos. m^{-2}. The juveniles were fed with chopped low cost trash fish at 10 % body weight day $^{-1}$ as suggested by Chandra *et al*. (2012) [19]. Mangalore tile pieces, PVC pipe pieces and asbestos sheets were used as hideouts for the crabs during the experiment. The water in the tanks was partially changed after every two days interval.

2.5 Duration of hardening

The crabs absorb water immediately after moulting. Therefore, the minerals dissolved in surrounding water in the treatment tank were supposed to be absorbed by the moulted crab. The day of moulting was considered as starting day of the experiment. The duration of hardening period was further counted on daily basis.

2.6 Determination of hard shelled crabs

Generally, hardening of the shell is observed on the basis of carapace texture. In the present study, in addition to the carapace hardening, state of crab hardening was assessed on basis of 2^{nd} thoracic appendage sternites (Gruber, 2012) [20]. This practice of assessing the hardening of the crab is also followed locally while purchasing the live crabs for export market. The hardening of the crabs was tested for the treatment crabs in the morning and evening hours.

2.7 Water parameters

Analyses of water quality parameters such as dissolved oxygen, total alkalinity, total hardness and free carbon dioxide was done every seven days during experiment using methods given by Boyd (1981) [21]. However, water temperature, salinity and pH were daily recorded by using thermometer, refractometer and universal indicator, respectively.

2.8 Statistical analysis

Standard error of hardening period of mud crab *Scylla serrata* with different mineral levels for each replicate was calculated. Data obtained from the experiment for hardening period was analyzed by one way ANOVA. Student-Newman-Keuls multiple range test was used to determine the significant difference between the treatments means.

3. Results

3.1 Hardening period

The average hardening period of *Scylla serrata* observed in the experiment I is depicted in Fig. 1. The minimum period for hardening of 9 ± 0 days was observed in T_{13} ($CaCO_3$ – 800 mg L^{-1} + $MgSO_4$ – 1700 mg L^{-1} + KCl – 800 mg L^{-1}) followed by 9.34 ± 0.13 days observed in T_{16} ($CaCO_3$ – 800 mg L^{-1} + KCl – 800 mg L^{-1}), 9.5 ± 0 days observed in T_{14} ($CaCO_3$ – 800 mg L^{-1} + $MgSO_4$ – 1700 mg L^{-1}) and 10 ± 0 days observed in T_9 ($CaCO_3$ – 700 mg L^{-1} + $MgSO_4$ – 1600 mg L^{-1}+ KCl – 700 mg L^{-1}). The control treatment (T_0) without any minerals showed the maximum period for hardening of 20.67 ± 0.84 days.

One way ANOVA evinced significant difference between the treatments. Student-Newman-Keuls test (SNK) showed that T_{13} recorded the lowest hardening period of 9 ± 0 days followed by T_{16}, T_{14} and T_9. The results of these treatments were significantly better ($p < 0.05$) than that of remaining treatments. The control treatment T_0 showed maximum hardening duration and the response was also statistically different ($p < 0.05$) when compared with all the remaining treatments.

Since, the highest mineral composition content in the experiment I evinced minimum period of shell hardening, the same level of minerals was used as the lowest treatment level in the experiment II. The average hardening period of *Scylla serrata* is depicted in Fig. 2. The minimum period for hardening of 9 ± 0.58 days was observed in T_1 ($CaCO_3$ – 800 mg L^{-1} + $MgSO_4$ – 1700 mg L^{-1} + KCl – 800 mg L^{-1}) and in T_{12} ($CaCO_3$ – 1000 mg L^{-1}+ KCl – 1000 mg L^{-1}). The hardening period in remaining treatments varied from 9.17 ± 0.31 days to 9.67 ± 0.83 days. T_0 as a control treatment without any minerals showed the maximum period for hardening of 22.16 ± 0.31 days.

One way ANOVA showed significant differences ($p < 0.05$) in the hardening period between the treatments of various mineral levels. Student-Newman-Keuls multiple range test (SNK) showed that treatment T_0 differed significantly ($p < 0.05$) from all treatments. It had the longest hardening period. Treatment T_1 and T_{12} differed significantly ($p < 0.05$) and evinced the least hardening period of 9 days.

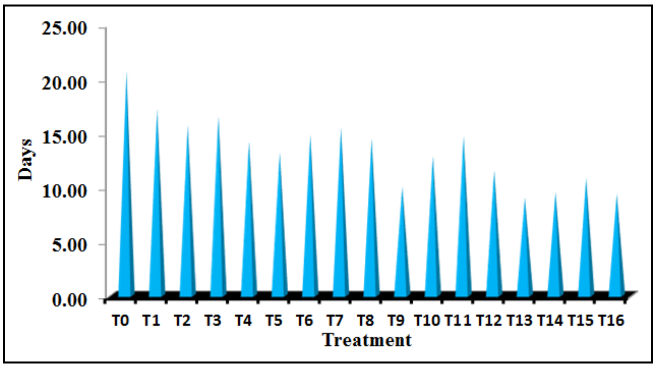

Fig 1: Hardening period of *Scylla serrata* (Experiment 1)

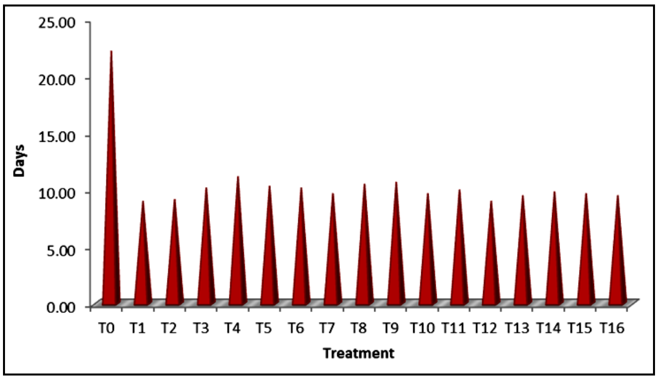

Fig 2: Hardening period of *Scylla serrata* (Experiment II)

3.2 Water quality parameters

During the experiment, the water parameters such as temperature, pH, total hardness, total alkalinity, dissolved oxygen, free carbon dioxide, and salinity were found to be in the range of 24 ˚C – 30 ˚C, 7 – 8.5, 1071.07 – 1141.14 mg L^{-1} as $CaCO_3$, 72 – 88 mg L^{-1} as $CaCO_3$, 5.4 – 8 mg L^{-1}, 0.9 – 3.9 mg L^{-1} and 23 – 28 ppt respectively.

4. Discussion

During the moulting process, crustaceans shed off epicuticle to grow them in size. Before the shedding of exoskeleton, a new soft exoskeleton is formed beneath the hard exoskeleton. This soft membranous cuticle tends to absorb water and minerals content required for shell hardening (Drach, 1939) [15]. Thus, the quality of absorbing minerals from surrounding water during the moulting process formed the basis of the present study. Crabs during pre-moult process, i.e. a stage when the epicuticle separates from the membranous new cuticle, do not feed for about a period of 12–15 hours. After moulting, they are unable to feed due to their soft feeding appendages. They generally start feeding after a period of 2.5–4 days after moulting (Drach, 1939) [15]. As such, during the present study, feed was put in the tanks at the end of 2 days post-moult period. The feed was given at the rate of 10 % of body weight of stocked crabs. The feeding regime was followed as per the practices generally adopted in crab fattening (Chandra *et al*., 2012) [19]. Feed was given in the form of chopped trash fishes. Same kind of feed has been used by Chang and Ikhwanuddin (1999) [22] and Soundarapandian *et al*. (2013) [23]. Generally, the animals derive the required mineral contents required for the body through their diets; however the crustaceans have the advantage of deriving required minerals from food as well as from surrounding environment during the moulting process.

Minerals are essential components for exoskeleton building and hard-tissue conditions. Among the most conspicuous events concerned with moulting in the calcified crustaceans is the resorption of minerals from the old exoskeleton to haemolymph in the pre-moult period and the subsequent calcification of the cuticle during post-moult. The net uptake of minerals is highest soon after the moult and decreases as the crustaceans advance towards intermoult stage (Roer, 1980) [24]. The post-moult crustaceans absorb minerals from the water at a higher rate as shown by the studies of Zilli *et al*. (2007) [25] and Shechter *et al*. (2008) [26].

The studies on influx and efflux of mineral contents of the crustaceans have shown the maximum rate of influx during pre-moult and post-moult stages. Thus, the pre-moult stage crabs were separated out in the present study and were facilitated to absorb minerals during those stages. Normal seawater mineral content of 35 ppt salinity showed the calcium content of 400 mg L^{-1}, magnesium – 1300 mg L^{-1} and potassium – 400 mg L^{-1} (Harvey, 1957; Riley and Skirrow, 1965; Kennish, 1994) [16, 17, 18]. Thus, in the present study, surplus amount of minerals than the normal seawater were put in the tanks in order to make minerals readily available to the crabs. The results showed that the composition of minerals above 800 mg L^{-1} of $CaCO_3$, 1700 mg L^{-1} of $MgSO_4$ and 800 mg L^{-1} of KCl evinced minimum period of hardening of 9 to 9.5 days. The mineral content to maximum dose of $CaCO_3$ – 1100 mg L^{-1}, $MgSO_4$ – 2000 mg L^{-1} and KCl – 1100 mg L^{-1} also reported the least hardening period of 9 ± 0.58 days. Since, addition of more minerals only add to extra cost of treatment, it is suggested to use mineral content of 800 mg L^{-1} of $CaCO_3$, 1700 mg L^{-1} of $MgSO_4$ and 800 mg L^{-1} of KCl in the rearing tanks.

5. Conclusion

Post-moult or soft crabs can be hardened at relatively shorter duration of up to 9 ± 0 days when they are reared in water of salinity between 23 – 28ppt with mineral contents of $CaCO_3$ – 800 mg L^{-1}, $MgSO_4$ – 1700 mg L^{-1} and KCl – 800 mg L^{-1} in rearing medium.

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