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Effect of sediment depth, calcium hardness of water and feeding ration on nacre formation in freshwater mussel *Lamellidens corrianus* (Lea, 1834)

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ABSTRACT

Survival and nacre formation was evaluated in freshwater mussel *Lamellidens corrianus* by varying sediment depth, calcium hardness of water and feeding ration. Different sediment depths (0, 3 and 6 cm), calcium hardness levels of water [(220.47 (T_1), 282.63 (T_2), 314.17 (T_3) and 338.18 mg l^{-1} (T_4)] and feeding rations [(1.5 x 10^6 (T_1), 3.0 x 10^6 (T_2) and (T_3) 4.5 x 10^6 algal cells ml^{-1}] were evaluated. Survival did not show significant difference while nacre formation was found to vary significantly ($p<0.05$) between different treatments. In the present study, sediment depth of 6 cm, calcium hardness of 338.18 mg l^{-1} and feeding ration of 3.0 x 10^6 algal cells ml^{-1} resulted in significantly ($p<0.05$) better nacre deposition.

Keywords: Calcium hardness, Feeding ration, Freshwater mussel, Nacre formation, Sediment depth, Survival

Introduction

Freshwater cultured pearls occupy a major chunk in the world trade of cultured pearls (Janaki Ram *et al.,* 1998). The addition of a substrate promotes enhanced growth and survival of freshwater mussels in laboratory studies (Hudson and Isom, 1984; Gatenby *et al.,* 1996; O' Beirn *et al.,* 1998). This could be attributed to support pedal feeding activity (Yeager *et al.*, 1994), mechanical digestion of feed (Beck, 2001) and supply of minerals and organic food material in addition to algae. Therefore, identifying the optimum sediment depth for freshwater pearl culture may be a critical factor. Calcium being the basic component of the pearl as well as shell of the mussels, it is considered important in the process of formation of pearl (Janaki Ram and Tripathi, 1992). Majority of freshwater mussels are mucoid filter feeders and are known to subsist on natural phytoplankton, suspended particulate, organic detritus and minute zooplankters (Mishra *et al.,* 1998). The present study was undertaken to examine the effect of sediment depth, calcium hardness of water and feed ration on survival and nacre formation in the freshwater mussel *Lamellidens corrianus*.

Materials and methods

Test animals

L. corrianus were procured from a reservoir in Maharashtra, India. The species was identified following the key as per Sakpal and Singh (2000) and confirmed by experts from Zoological Survey of India, Kolkata. Cleaned mussels in wet gunny bags (42.5 cm x 62.5 cm) with 50 mussels per bag were transported to the wet laboratory. Water was sprinkled over the bags at 4 h interval during transportation. Plastic pools (4'x2') containing aged (15 days) aerated freshwater (300 l) was used for acclimatisation. The acclimaised mussels were stocked in plastic pools at a density of 100 mussels per pool. Animals were fed with mixed algae.

Mixed algal culture

Mixed algal culture was developed in outdoor plastic pools (4' x 2'; 350 l), disinfected with 20 ppm $KMn0_4$ solution and dried under sunlight. Soaked groundnut oil cake powder (140 g) was added to 300 l of water in plastic pools. On the 3rd day, 50 l of stock solution containing mixed algae (2.0 x 10^6 cells ml^{-1}, *Chlorella* 90% and other algal cells 10%) was added to the plastic pool. Algal culture developed within three days. Soaked groundnut oil cake, at the rate of 140 g per pool was added every third day to maintain the mixed algal culture. This mixed algal culture was sufficient for feeding the mussels for 15 days. Subsequently fresh culture was developed in the same plastic pool and used as feed for the mussels.

Mabe nucleus preparation

Acrylic powder and Acrayln 'R' liquid were used for preparation of mabe nucleus. Mabe nucleus (each of 145 mg) was prepared by mixing 220 mg Acrylic powder and 0.15 ml of Acrayln 'R' liquid in a petri dish to form the paste, which was filled in a brass flower mould. After 10 min nucleus was removed, kept in cooled freshwater for 30 minand then stored in polythene bags until implanted.

Mabe nucleus implantation

Healthy mussels acclimatised for 30 days (size range 7-10 cm; weighing 23-90 g) were used for implantation. Implantation was carried out by the mantle cavity insertion method (Sakpal, 1999). Post-operative care was given as recommended by Janaki Ram and Tripathi (1992).

Experimental design

Glass aquaria of 36 l capacity (2' x 1' x 1') holding 30 l freshwater were used for the experiments. In experiment I, sediment layers of different depths *viz.*, 0 (T_1), 3 (T_2) and 6 cm (T_3) were prepared using river sand. The rearing duration of mussels was six months. The initial mean shell length (SL) of mussels were 84.53 ± 19.42 mm, 78.68 ± 18.10 mm and 83.00 ± 19.08 mm for the mussels reared in T_1, T_2 and T_3 respectively, and corresponding mean weights were 48.99±11.39g, 44.40±10.82 and 47.46±10.99 g respectively. Mixed algal culture at a concentration of 4.0 x 10^5 cells ml^{-1} was used to feed mussels.

In experiment II, different levels of algal concentration such as 1.5 x 10^6, 3.0 x 10^6 and 4.5 x 10^6 algal cells ml^{-1} were used for feeding the implanted mussels in T_1, T_2 and T_3 respectively. Initial mean SL of the experimental mussels used were 84.53±19.42, 78.68±18.10 and 83.00±19.08 mm for the mussels reared in T_1, T_2 and T_3 respectively, and corresponding mean weight were 48.99±11.39, 44.40±10.82 and 47.46 ±10.99 g.

For evaluating the effect of calcium hardness (experiment III), water calcium hardness was adjusted using $CaCO_3$ to 220.47 (T_1), 282.63 (T_2), 314.17 (T_3) and 338.18 mgl^{-1} (T_4) for rearing implanted mussels. Initial mean SL of the mussels were 82.38±18.95, 77.45 ± 17.80, 76.48 ± 17.60 and 78.83 ± 18.14 mm in T_1, T_2, T_3 and T_4 respectively. Corresponding mean weights were 45.41±10.73, 40.03 ± 9.38, 37.04 ± 8.77 and 40.73 ± 9.54 g respectively. Feeding ration was maintained at 4.0 x 10^5 algal cells ml^{-1}.

In all experiments, 20 mussels were used per treatment. The pseudofaeces were removed daily before feeding the animals. Ten percent water was exchanged daily with same quantity of mixed algal culture.

Water parameters

Temperature and pH of water in the experimental tanks were monitored daily while dissolved oxygen, carbon di oxide, total alkalinity and total hardness were recorded fortnightly according to standard methods (APHA, 1998).

Mabe harvest

Three implanted mussels were opened every 30th day to record observations on nacre coating on mabe. On termination of the experiment, weight of the mabe was recorded using monopan balance with 0.1 mg accuracy.

Survival

Survival rate (%) of the mussels in the experimental tanks was calculated as:

$$\text{Survival (\%)} = \frac{(\text{Initial no. of mussels} - \text{Final no. of mussels})}{\text{Initial no. of mussels}} \text{ X } 100$$

Statistical analysis

Statistical analyses of the data were done by one-way ANOVA and Least significant difference (LSD) test was used to determine significant difference between the treatments, whenever ANOVA was significant. The rate of nacre deposition on mabe after rearing period was estimated by linear equation and it was compared by ANCOVA (Snedecor and Cochran, 1967; Zar, 1974).

Results

Sediment depth (*Experiment I*)

Survival of implanted mussels was 65, 60 and 65% in T_1, T_2 and T_3 respectively after 180 days of rearing. There was no significant difference in survival among different treatments. Mean initial weights of mabe nuclei used were 0.138 ± 0.032, 0.136±0.031 and 0.137±0.031 g in T_1, T_2 and T_3 respectively and the corresponding mean final weights were 0.156±0.045, 0.161±0.049 and 0.167±0.048 g. Maximum nacre coating of 0.047 g was recorded for mussels reared in T_3, followed by 0.032 g and 0.023 g in T_2 and T_1 respectively (Fig. 1.). Significant difference ($p<0.05$) was observed in nacre deposition between different sediment depths. The least significant difference (LSD) revealed significant difference between the weights of mabe grown in T_3 and was significantly higher ($p<0.05$) than T_1 and T_2. However, there was no significant ($p>0.05$) difference observed between T_2 and T_1. Thus, 6 cm sediment depth was found to be better for nacre formation.

Feeding ration (*Experiment II*)

Survival of implanted mussels was 65, 60 and 60% in T_1, T_2 and T_3 respectively after 180 days of rearing. There was no significant difference in survival between the treatments. The results of nacre coating are given in Fig. 2. Mean initial weights of mabe nuclei were 0.137±0.031, 0.131±0.030 and 0.132±0.030 g in T_1, T_2 and T_3 respectively and the corresponding mean final weights recorded were 0.163±0.047, 0.164±0.049 and 0.155±0.047 g. Maximum coating of 0.048 g was

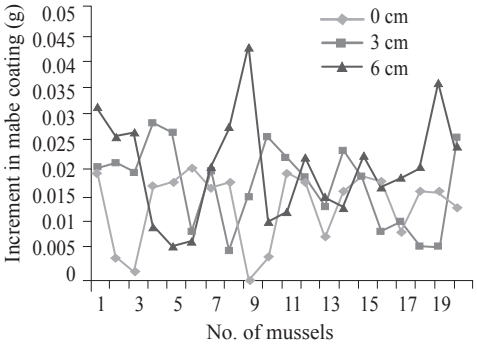

Fig. 1. Increment in nacre coating in *L. corrianus* reared for 180 days in tanks with different sediment depths (Experiment I)

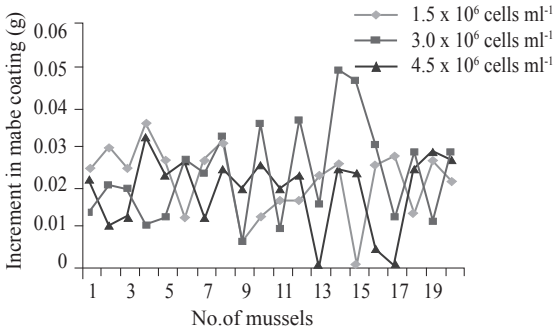

Fig. 2. Increment in nacre coating in *L. corrianus* reared for 180 days fed on varying concentrations of mixed algal culture (Experiment II)

observed in T_2 followed by 0.035 and 0.031 g in T_1 and T_3 respectively. Significant difference ($p<0.05$) in nacre deposition was observed among different feeding rations. LSD test revealed significanty higher mabe weight increment in T_2 ($p< 0.05$) than T_1 and T_3. However, there was no significant difference between T_1 and T_3 with respect to weight of mabe. Thus feeding mussels at the rate of 3.0 x 10^6 cells m l^{-1} resulted in better nacre formation.

Calcium hardness of water (*Experiment III*)

Survival of implanted mussels after 180 days of rearing was, 65, 60, 50 and 55% in T_1, T_2, T_3 and T_4 respectively. There was no significant difference in survival among the treatments. Mean initial weights of nuclei were 0.138 ± 0.032, 0.134±0.031, 0.137±0.031 and 0.131±0.030 g in T_1, T_2, T_3 and T_4 respectively and the corresponding mean final weights obtained were 0.156±0.045, 0.156±0.047, 0.163±0.054 and 0.171±0.054 g. The maximum nacre coating of 0.073 g was observed in T_4 followed by 0.037, 0.031 and 0.023 g in T_3, T_2 and T_1 respectively (Fig. 3). Significant difference ($p< 0.05$) was observed between nacre deposition in different treatments.

LSD test revealed significant difference between the weights of mabe grown in T_4 which was significantly better ($p< 0.05$) than rest of the treatments. However, there was no significant difference ($p>0.05$) observed between T_1, T_2 and T_3. Water calcium hardness of 338.18 mg l^{-1} was found to be better for nacre formation.

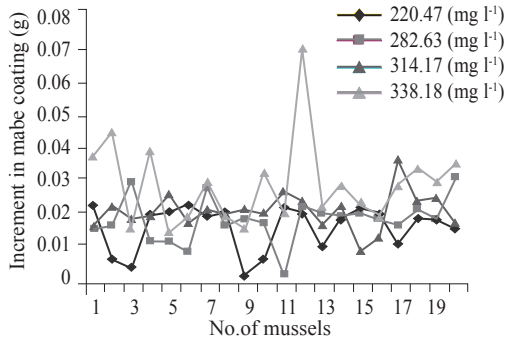

Fig. 3. Increment in nacre coating in *L. corrianus* reared for 180 days in tanks with different levels of water calcium hardness (Experiment III)

Water parameters

The data on water parameters recorded during the experiments are presented in Table 1, 2 and 3.

Table 1. Water parameters recorded during rearing of mabe implanted *L. corrianus* in different sediment depths for 180 days in Experiment I

Water parameters		Mean ± SE	Range
Temperature (°C)	T_0	27.83 ± 8.39	26.4-29.8
	T_1	27.74 ± 8.37	26.1-30.4
	T_2	27.46 ± 8.28	25.8-28.9
pH	T_0	7.17 ± 2.16	6.85-7.38
	T_1	7.25 ± 2.18	6.88-7.46
	T_2	7.25 ± 2.19	6.94-7.51
Dissolve oxygen (mg l^{-1})	T_0	6.17 ± 1.86	5.8-6.4
	T_1	6.30 ± 1.90	6.0-6.8
	T_2	6.30 ± 1.90	5.8-6.8
Total alkalinity (mg l^{-1})	T_0	33.67 ± 10.18	5.8-6.4
	T_1	34.50 ± 10.18	6.0-6.8
	T_2	35.08 ± 10.60	5.8-6.8
Total hardness (mg l^{-1})	T_0	83.83 ± 25.29	80-86
	T_1	83.00 ± 25.03	82-88
	T_2	84.83 ± 25.59	80-88

Table 2. Water parameters recorded during rearing of mabe implanted *L. corrianus* by feeding different algal concentration for 180 days in Experiment II

Water parameters		Mean ± SE	Range
Temperature (°C)	T_0	28.18 ±8.50	26.8-30.2
	T_1	27.98 ± 8.07	26.0-29.6
	T_2	27.83 ± 8.40	26.0-29.4
pH	T_0	7.22 ± 2.17	7.05-7.44
	T_1	7.22 ± 2.18	7.10-7.36
	T_2	7.36 ± 2.18	7.17-7.53
Dissolve oxygen (mg l^{-1})	T_0	6.27 ± 1.89	6.0-6.6
	T_1	6.43 ± 1.98	6.0-7.4
	T_2	6.62 ± 1.91	6.4-7.6
Total alkalinity (mg l^{-1})	T_0	31.58 ± 9.54	28-34
	T_1	33.17 ± 10.07	28-42
	T_2	32.17 ± 9.75	26-38
Total hardness (mg l^{-1})	T_0	67.00 ± 20.24	60-74
	T_1	66.17 ± 19.99	62-72
	T_2	66.00 ± 19.93	62-74

Table 3. Water parameters recorded during rearing of mabe implanted *L. corrianus* at different concentration of calcium hardness of water for 180 days in Experiment III

Water parameters		Mean ± SE	Range
Temperature (°C)	T_0	27.83 ± 8.39	26.4-29.8
	T_1	27.80 ± 8.31	25.4-29.6
	T_2	27.51 ± 8.32	25.6-29.8
	T_3	27.58 ± 8.32	26.2-29.2
pH	T_0	7.17 ± 2.16	6.85-7.38
	T_1	7.48 ± 2.25	7.38-7.58
	T_2	7.70 ± 2.22	7.54-7.82
	T_3	7.83 ± 2.36	7.69-7.94
Dissolve oxygen (mg l^{-1})	T_0	6.17 ± 1.86	5.8-6.4
	T_1	6.20 ± 1.87	5.6-6.6
	T_2	6.17 ± 1.86	5.8-6.6
	T_3	6.23 ± 1.88	6.0-6.6
Total alkalinity (mg l^{-1})	T_0	83.83 ± 25.29	80-86
	T_1	93.58 ± 28.26	83-102
	T_2	94.83 ± 28.66	86-108
	T_3	99.00 ± 29.91	92-112
Total hardness (mg l^{-1})	T_0	220.47 ± 66.49	214.68-228.46
	T_1	282.63 ± 84.79	272.45-302.84
	T_2	314.17 ± 94.73	308.52-320.84
	T_3	338.18 ±101.98	330.82-346.64

Discussion

Sediment depth

L. corrianus are found buried in mud and are sedentary in habits but ploughs slowly in the mud with its muscular foot Addition of substrate promotes enhanced growth and survival of freshwater mussels (Hudson and Isom, 1984; Gatenby *et al.,* 1996; O'Beirn *et al.,* 1998). Substrate is essential for holding the mussel because it facilitates pedal-feeding activity of freshwater mussels by supplying a medium to burrow (Yeager *et al.,* 1994). Beck (2001) reported that mussels ingest fine substrate to aid mechanical digestion of food. Buddensiek (1995) found that moderate levels of sedimentation were beneficial for holding the mussels *Margaritifera margaritifera* and reported that sediment depth <6 mm resulted in higher survival.

In the present study, rejection of mabe nucleus was not observed in any of the implanted mussels except for an individual in T_1, which died 6 days after implantation. Survival rate of implanted mussels was 65, 60 and 65% in T_1, T_2 and T_3 respectively after 180 days. The results of the present study indicated sediment depth of 6 cm is beneficial to freshwater mussels which is in agreement with the results of Hudson and Isom (1984) and Yeager *et al.* (1994). During the present study, significantly better ($p< 0.05$) deposition of the nacre layer was recorded at sediment depth of 6 cm. The study in general indicated enhanced nacre deposition with increase in sediment depth.

Feeding ration

Nutritional requirements for captive holding of Unionids remain undetermined (Beck, 2001). Algae are the most popular food source for captive juvenile mussels. Various combinations of algal species have been used in rearing freshwater mussels (Hudson and Isom, 1984; Gatenby *et al.,* 1996; Gatenby *et al.,* 1997). Several workers (O'Beirn *et al.,* 1998; Tankersley and Butz, 2000; Henley *et al.,* 2001) have incorporated algal species as the primary food source in captive rearing systems. Beck (2001) reported that the algal feeding ration is critical for mussels in captive conditions.

Algal concentration of 10^6 cells ml^{-1} have been shown to be good for rearing adult *Amblema plicata* and *Quadrula pustulosa* in the laboratory condition (Patterson *et al.,* 1999). Gatenby *et al.* (1997) fed algae at the rate of 3.0 x 10^5 to 5.0 x 10^5 cells ml^{-1} to achieve excellent survival rates of 66.5% for rearing juveniles of *Villosa iris* in captive conditions for a period of 45 days (O'Beirn *et al.,* 1998; Tankersley and Butz, 2000; Henley *et al.,* 2001).

In the present study, the maximum nacre layer deposited was of 0.048 g in the mabe nucleus at the feeding ration of 3.0 x 10^6 cells ml^{-1}. There is no scientific information available on the effect of algal concentration on the nacre deposition of freshwater mussels. However, Sakpal (1999) recorded average survival of 75% in *L. marginalis* fed with mixed algal culture suspended in plastic pool, during 180 days of rearing. During the present study, the survival was 65, 60 and 60% in T_1, T_2 and T_3 respectively. With this study, it is not possible to draw firm conclusion regarding effect of feeding ration on release of nacre layer. However, the results are in agreement with Patterson *et al.* (1999) on maintaining the mussel, *Amblema plicata* in laboratory condition.

Calcium hardness

Calcium is the basic element required for formation of shell in freshwater mussels (John and Raghavan, 2002). Calcium and magnesium salts are important factors in pearl formation. The optimum level of calcium reported was 16 ppm for rearing freshwater mussels (Das, 1995).

Janaki Ram and Tripathi (1992) reported that in freshwater pearl culture ponds, calcium salts concentration should be in the range of 20 to 54 ppm. However, Sengupta *et al.* (2000) opined that pearl culture pond water should have calcium concentration of 10 to 25 ppm.

In the present study, after 180 days of rearing, maximum nacre coating of 0.073 g was obtained in 338.18 mg l^{-1} (T_4). However, there is no published information available on the effect of calcium hardness

on nacre formation in freshwater mussels. Survival rate recorded in the present study was 65, 60, 50 and 55% in T_1, T_2, T_3 and T_4 respectively and is similar to that reported in the study of Bhowmick (1996). The author observed an average survival of 51.6% in *L. marginalis* reared under different calcium concentrations.

Water parameters

Water quality is considered to be important for better growth of pearl in freshwater mussels (Fassler, 1991; Liu, 1993). Liu (1993) reported 15-28ºC as the ideal temperature for freshwater pearl culture. Temperature lower than 10ºC can prevent mussels from secreting nacre. Too high temperature on the other hand can affect the physiological functions of animal. Researchers have noted that freshwater mussels should be released during late spring when temperatures exceed 15ºC (Hanlon, 2000). In the present study, the average water temperature ranged between 25.8 to 30.4ºC, 26.0 to 30.2ºC and 25.4 to 29.8ºC in experiment I, II and III respectively (Table 1, 2 and 3). Janaki Ram and Tripathi (1992), Bhowmick (1996) and Sakpal (1999) have reported 20 to 30ºC, 21.1 to 27.2ºC and 24 to 33ºC respectively as the ideal water temperature. Beck (2001) recommended 24ºC as ideal water temperature for rearing of freshwater mussels. The water temperature range observed in the present study was found similar to the reports of Janaki Ram and Tripathi (1992), Bhowmick (1996) and Sakpal (1999).

Janaki Ram and Tripathi (1992), Sengupta (1994), Das (1995), Bhowmick (1996) and Sakpal (1999) reported pH values of 7.0 to 8.3, 7.0 to 8.0, 7.0 to 8.0, 7.1 to 8.6 and 7.47 to 7.73 respectively during rearing of implanted *L. marginalis*. Beck (2001) reported pH of 8.4 to 8.8 as ideal for rearing the freshwater mussel *V. iris*. In the present study, pH ranged between 6.85 to 7.51, 7.05 to 7.53 and 6.85 to 7.94 in experiment I, II and III respectively (Table 1, 2 and 3 respectively). These ranges observed during 180 days of rearing of *L. corrianus* are in agreement with the above observations.

Many researchers (Janaki Ram and Tripathi, 1992); Das, 1995; Sakpal, 1999) have reported ideal dissolved oxygen levels of 5.4 to 7.2 mg l^{-1}, 5 to 10 mg l^{-1} and 2.9 to 4.8 mg l^{-1} respectively for rearing the implanted mussels. In the present study, the dissolved oxygen ranged between 5.8 to 6.8 mg l^{-1}, 6.0 to 7.6 mg l^{-1} and 5.6 to 6.6 mg l^{-1} during the 180 days of rearing in the laboratory conditions. The results are in agreement with that of earlier works.

Different scientists working on freshwater pearl culture reported broad range of pond water alkalinity. Sengupta *et al.* (2000) reported total alkalinity of rearing tank of pearl mussel to be 70 to 150 ppm. In the water bodies, where natural pearls were collected, the total alkalinity observed was 88 ppm in the ponds of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha and 80 ppm in ponds in Agartala, Tripura (Janaki Ram and Tripathi 1992). Sengupta (1994), Bhowmick (1996) and Sakpal (1999) have reported the total alkalinity in the range of 75 to 150 mg l^{-1}, 90 to 195 mg l^{-1} and 53 to 72 mg l^{-1} respectively. During the present study, lower values of total alkalinity in 180 days were recorded in experiment I and II respectively. According to Janaki Ram and Tripathi (1992), in freshwater pearl culture ponds, total hardness of the water should be in the range, 40 to 110 ppm. In experiment III, hardness was manipulated by adding $CaCO_3$ in rearing water. Results of the present study, clearly indicated that mabe nucleus implanted *L. corrianus* reared in water having 338.18 mg l^{-1} calcium hardness and fed on mixed algae at the rate of 3.0 x 10^6 cells ml^{-1} can lead to better nacre deposition. However, further studies with respect to physiological changes related to different sediment depths and calcium hardness would help to improve our understanding of pearl formation in freshwater mussel.

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