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Assessing the numbers of mantle tissues in non-nuclei pearl production in freshwater mussel, *Lamellidens marginalis*

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Abstract

In order to determine the suitable number of mantle tissues for non-nuclei (rice) pearl production, a research was conducted at Freshwater station, Bangladesh Fisheries Research Institute (BFRI) from July 2014 to June 2017 by inserting the numbers of mantle tissues 2 (t_1), 4 (t_2), 6 (t_3), 8 (t_4) and 10 (t_5) in freshwater mussel *Lamellidens marginalis*. Earthen pond was used for stocking operated mussels (80 mussels/dec). Different water quality parameters *viz.*, Temperature, Dissolve oxygen, pH, Ammonia, Alkalinity, Calcium and Phytoplankton were monitored fortnightly and found within normal range. After three years of culturing, survival rate of operated mussel was found highest (77%) in t_2 followed by t_1 (76%), t_3 (73%), t_4 (72%) and t_5 (62%). Pearl production rate was highest in t_3 (34%) followed by t_4 (33%), t_5 (30%), t_2 (18%) and t_1 (10%). Highest nacre layer was observed as 4.85±0.21 mm in t_3 while, 4.7±0.27 mm in t_1, 4.6±0.32 mm in t_2, 2.3±0.28 mm in t_4 and 2.12±0.30 mm in t_5. From the current study, considering the pearl production rate, nacre layer, luster and shape of produced pearl implantation of 6 numbers of mantle tissues showed the best performance than others.

Key words: Non-nuclei pearl, *Lamellidens marginalis*, Mantle tissue, Survival, Pearl production, Pearl quality

Introduction

Pearl is the precious and wondrous gem which is biologically produced in the living animal (Rathor, 2017; Tanu *et al.*, 2019b). Pearl's jewelry is one of the most attractive objects considered as symbol of beauty, love, purity and aristocracy; it added unique levels of all style and fashion (Dirlam *et al.*, 1985; Pandey and Singh, 2015). Not only for jewelry but also it has

other uses like, raw materials of medicine, cosmetics etc. (Li and Li, 2009; Misra and Mukhapadhyay, 2008). Non-nuclei pearl is a special kind of pearl which is full of pearly layer (Li and Li, 2009). Different color and shape of pearl like oval, round, rectangular can be produced after insertion of mantle tissue which looks like the grain or rice and that`s why it is called rice pearl. A piece of mussel epithelial membrane located at the outer edge of mantle tissue (mantle tissue block) is inserted into a living mussel's mantle. The inserted mantle tissue slice starts cell division, and then forms a pearl sac and secretes nacre, layer by layer, then accumulates as rice pearl (Dan *et al.,* 2001).

Freshwater pearl culture is growing as a source of employment and income in many South-East Asian countries (Ram, 1997). Chinese freshwater cultured pearls have high demand throughout the world. Twenty percent of total pearl production in the world market comes from Chinese freshwater pearl having the price of 150 million US$ (Anon, 2006). Pearl culture has a great potentiality but due to lack of technical knowledge, it has not yet been commercially developed in Bangladesh (Sarker, 1994). The prospect of rice pearl culture is bright and promising in the country due to the warm weather which is favorable for the growth of pearl producing mussel and pearl. Pearl culture might provide more employment opportunity to the rural women and can play a vital role for women empowerment. The native mussel *Lamellidens marginalis* and *L. corrianus* are suitable for producing rice pearl which is available in the country (Hossain *et al.,* 2004, Tanu *et al.,* 2019a). Pearl producing mussel can be cultured with fish in pond, ditches, river, lake with low input and fish farmer can earn additional money from this integrated culture system (Dan *et al.,* 2001). Pearl production and its quality depend on various factors such as mussel species, operation technique, age of mussel, culture environment, water quality, natural food, sunlight penetration to the water body and management techniques (Ram, 2003). Different numbers of mantle tissues transplantation may also affect the production of pearl quality. Considering the above factors this experiment is designed to find out the implantation of suitable numbers of mantle tissue slices in freshwater mussels in order to produce highest numbers of non-nuclei quality pearls in freshwater mussel *L. marginalis*.

Materials and Methods

Mussel collection

Disease free, healthy and young mussels (*L. marginalis*) were collected from different habitats of Mymensingh region and stocked in a pond at Freshwater station, BFRI, Mymensingh. The average length, width and age of stocked mussels were 8.93±0.30 cm, 4.91±0.23 cm and 1-1.5 years, respectively.

Mussel rearing

Collected mussels were reared in pond of which soil was sandy, clean water and pollution free bottom. For plankton production, the pond was fertilized with 5 kg organic manure, 0.125 kg TSP and 0.1 kg urea /dec fortnightly. To maintain the optimum level of pH and calcium, 0.5 kg lime/dec were applied to the ponds fortnightly.

Operation equipment

Mussel cutting knife, Obtuse-headed forceps, Sponge, Glass board, Dropper, Tray, Mantle tissue separation needle, Mussel opener, Stopple, Flat-head needle, Hook-head needle, Operation shelf were used for mussel operation

Operation chemicals

Ajomin solution, 70% alcohol and distilled water were applied during different steps of mussel operation.

Pre-operative conditioning

Before operation, mussels were kept in cistern for seven days without food to remove dirt from intestine and internal organ of the body. Then mussels were brought to laboratory and put in perforated trays for 24 hours, keeping ventral side downwards to remove water.

Mantle tissue transplantation

During operation mussels were divided into two groups; the donor and the receiver mussels. Operation includes two steps; mantle tissue block making from donor and inoculation in the receiver mussel. The whole transplantation process was completed by following flowchart (Fig. 1):

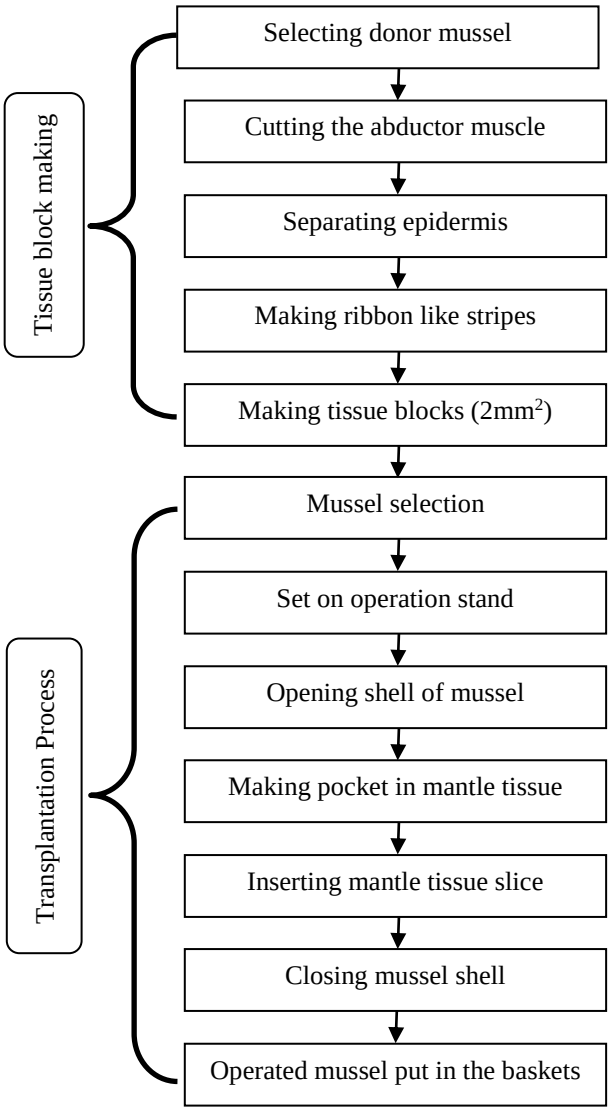

Fig. 1: Flowchart of mantle tissue transplantation

Post-operative conditioning

Post-operative care is a significant phase in pearl culture, which is required for the inoculated mussels to overcome the stressed condition. After operation, mussels were tagged and kept in

nylon bags (diameter 20cm, mesh size 1cm) at the rate of 3 mussels/net bag and put up at

0.2m depth in post-operative care units (ferro-cement cisterns of 5000 L capacity) at a stocking density of 150 mussels/cistern without food for 7 days. The mussels were subsequently fed with natural food for 3 weeks in the cistern. The operated mussels were observed daily; dead mussels were removed.

Culture Method

After post-operative treatment operated mussels were transferred to the previously prepared culture pond for three years (Fig. 2). Five treatments were done using the inserted mantle tissue slices 2,4,6,8 and 10 in t_1, t_2, t_3, t_4 and t_5, respectively. Each treatment included 200 operated mussels. A total of 1000 operated mussels were stocked with a stocking density of 80 mussels/dec. Net bag hanging culture method was practiced for mussel rearing during the experiment. The operated mussels were stocked at 3 mussels/net bag and hanged from a float attached to a rope at 0.30-0.35m depth into pond water. Rope was stretched to hang the net bag across the surface of pond water and the distance between 2 bags and 2 ropes were 0.25-0.30m and 1.5m, respectively.

Fig. 2: Pearl culture pond

Water quality management

The water quality parameters were monitored and data were recorded fortnightly throughout the culture period. Water temperature, dissolved oxygen (DO), pH, alkalinity, ammonia and calcium were measured by Celsius thermometer, digital Oxygen meter (YSI, model 58) and digital pH meter (Jenway, model 3020), Spectrophotometer (DDR-2800), Flame photometer (Buck Scientific FPF-7), Haematocyto meter, respectively. The plankton population was determined by using the following formula (Rahman, 1992)

$$N = \frac{A \times 1000 \times C}{V \times F \times L}$$

Where, N = No. of plankton cells per liter of original water, A = Total no. of plankton counted, C = Volume of final concentrated sample in ml, V = Volume of a field = 1 mm^{-3}, F = No. of fields counted, L = Volume of original water in liter. The numbers of phytoplankton and zooplankton were expressed as cells/l

Statistical analysis

All the collected data were statistically analyzed using SPSS version 17.0 with a significance level of 0.01. Pearson correlation analysis was performed to determine correlations among the treatments.

Results and Discussions

Water quality parameters

The water quality parameters of culture ponds are presented in Table 1. Mean values of temperature were 25.07±2.38°C, DO 5.17±0.85, pH 7.89±0.27, alkalinity 192 ± 24.40, ammonia 0.05±0.03, Ca^{2+}16.29±1.46 and plankton 85.14±7.9. All the water quality parameters were within the suitable ranges for pearl culture (Dan *et al.*, 2001). Temperature, pH, DO, Ca^{2+} and alkalinity ranging from 25.40 to 28.80 °C, 7.1 to 7.9, 5.3 to 6.8 mg/l, 58.90 to 71.20 mg/l and 399.00 to 594.00 mg/l were found during the study period of freshwater mussel (*L. marginalis*) in culture pond water by Natarajan and Susithira (2015). Temperature 29.99±0.20°C, DO 5.63±0.29, pH 8.16±0.12 and phytoplankton 89.817±12.4×10^3cell/l were also assessed by Yulianto *et al.*, (2016) in pearl production. Water quality parameters were also recorded as temperature 25.3±1.55 ^{0}C, pH 6.4±0.21, DO 5.63±0.17 ml/l, Alkalinity 22.44±0.34 mg/l by Rathor (2017) in pearl culture with *L. corrianus* species. The water quality parameters viz, temperature 23.5-36.0^0C, pH 7.5-8.5, dissolve oxygen 9.5-10.85 mg/l, alkalinity 220-275 mg/l, ammonia 0.053-0.065 mg/l were described for optimum growth of mussel by Pandey and Singh (2015). However, water quality parameters were found suitable throughout the current study period.

Table 1: Water quality parameters of trial pond water

Parameter	Average ± mean value	Suitable range for pearl culture (Dan *et al.*, 2001)
Temperature of water (ºC)	25.07±2.38	15-30
pH	7.89±0.27	6.5-8.5
Dissolved oxygen (mg/l)	5.174±0.85	5-8
Total alkalinity (mg/l)	192±24.40	50-300
Ammonia (mg/l)	0.05±0.03	0.03-0.1
Ca^{2+} (mg/l)	16.29±1.46	>10

Phytoplankton (x10^3 cell/l)	85.41±7.9	50-100

Survival rate

After insertion of mantle tissue slices for rice pearl production, higher survival rate was found in t_2 (77%) followed by t_1 (76%) (Fig. 3). More or less similar result was found in t_3 (73%) and t_4 (72%). The lowest survival rate was observed in t_5 (62%). Survival rate was negatively correlated with the inserted number of mantle tissue slices (Table 2). It is noted that Miah *et al.,* (2000) observed 80% survival rate for one month rearing of *L. marginalis* having nucleus implantation. On the otherhand, Hossain *et al.,* (2004) found 100% survival rate for three months culture of *L. marginalis* with mantle tissue. In another study mortality occurred 20% in June then decreased after August in pearl culture with the species of *Parreysia Corrugata* (Suryawanshi and Kulkarni 2015), whereas Fernandez (2013) described 55-95% survival rate in freshwater mussel *Margaritifera falcate*.

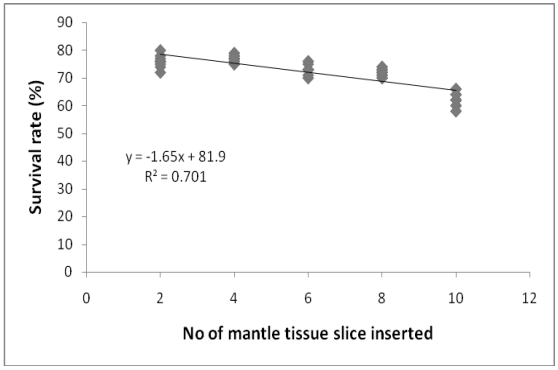

Fig. 3: Survival of operated mussels

Pearl production rate

In the current study, maximum pearl production rate was recorded in t_3 (34%) followed by t_4 (33%), t_5 (30%), t_2 (18%) and t_1 (10%) (Fig. 4). In statistical analysis pearl production was positively correlated with the inserted number of mantle tissue slices (Table 2). In a similar study Ram (1997) stated 60-70% pearl formation in *L. marginalis* and *L. corrianus* after 12 months of culture. Research conducted by Begum *et al.* (1990) on *L. marginalis* revealed 15.1% matured pearl production after inoculation of 2 mm^2 mantle tissue blocks and 1-2 mm ceramic beads.

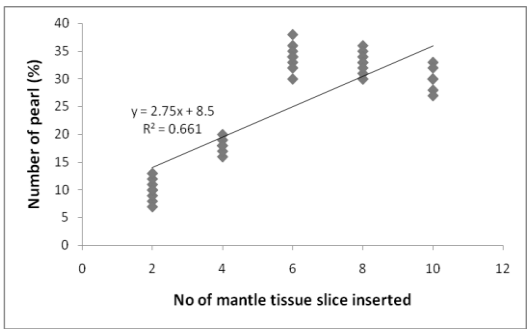

Fig. 4: Pearl producing rate of operated mussel

In the current experiment survival rate of operated mussels was found highest in t_2 (77%) but in case of pearl production t_3 showed the highest (34%) performance. The reason behind it is unknown. Further study is needed along the issue to elucidate the reason.

Pearl quality

The highest nacre layer was observed in t_3 (4.85±0.21 mm) followed by t_1 (4.7±0.27 mm), t_2 (4.6±0.32 mm), t_4 (2.3±0.28 mm) and t_5 (2.12±0.30 mm) in harvested mussels (Fig. 5). Accumulations of nacre layer in mussels were negatively correlated with the inserted number of mantle tissue slices (Table 2). Similar result was also found by Tanu *et al.,* (2019b) while nacre layer accumulated at 4.17-5.19mm with shiny and good luster. In consideration to luster and shape of pearl Rahayu (2013) showed the highest pearl nacre layer thickness of 17 µm from 9 months cultivation of freshwater mussel (*Anodonta woodiana)* after insertion of shell bead nucleus of 10mm diameter. Blay *et al.* (2013) found 0.65-1.24mm pearl nacre deposition from *Pinctata margaritifera* after 18 months of culture. According to Rathor (2017), 3-4mm nacre layer of pearl was found after 9 months of culture of freshwater mussel (*L. corrianus*). In the present study, accumulation of nacre layer was satisfactory in *L. marginalis.*

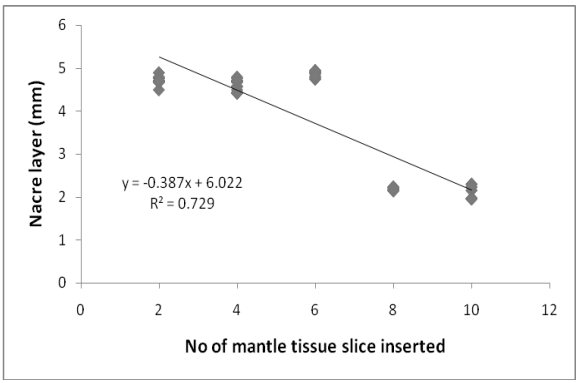

Fig. 5: Accumulation of nacre layer in harvested mussel

Table 2: Pearson's correlation among the treatments of non-nuclei pearl production in freshwater mussels, *L. marginalis*

Correlations	No. of mantle tissue slices inserted	Survival rate (%)	Pearl production rate (%)	Nacre layer (mm)
No. of mantle tissue slice inserted	1	-.838**	.813**	-.854**
Survival rate (%)	-.838**	1	-.503**	.727**
Pearl production rate (%)	.813**	-.503**	1	-.521**

Nacre layer (mm)	-.854**	.727**	-.521**	1

**. Correlation is significant at the 0.01 level (2-tailed).

However, the operated mussels *L. marginalis* having 6 numbers of inserted tissue blocks showed the highest numbers of non-nuclei pearl. Similarly these mussels also showed best performance in nacre secretion, luster, shape and size of pearl. Further research is needed along the line for the refinement of technology.

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