International Journal of Natural and Social Sciences
Print: 2617-6637 < ISSN > Online: 2313-4461

Maximizing pearl production in freshwater mussel (*Lamellidens marginalis*) against tissue slices

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ARTICLE INFO

Article history

Received: 15 June 2019
Accepted: 17 July 2019

Keyword

Pearl, *Lamellidens marginalis*, Mantle tissue, Mussel, Nacre

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ABSTRACT

In order to establish appropriate culture techniques for non-nuclei (rice) pearl production, a study was conducted from July 2014 to June 2017 with net bag hanging method and grazing method at Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. Pear was produced by inserting certain number of tissue slices in freshwater mussel *Lamellidens marginalis*. Earthen pond was used for stocking operated mussels (80/decimal). Different water quality parameters *viz.*, Temperature, Dissolve oxygen, pH, Ammonia, Alkalinity, Calcium, Phytoplankton and Zooplankton were monitored and observed within normal range. After three years of culturing, survival rate of operated mussels in grazing method was found higher (42.1%) than in net bag hanging method (40%) but pearl production rate was higher in net bag hanging method (43%) than in grazing method (39%). Accumulation of nacre layer of produced pearl was found 3.14±0.2mm with high and medium shiny luster in net bag hanging method whereas, in grazing method, nacre layer was 3.76± 0.2mm with medium and low shiny luster. The study showed that both in view of pearl production rate and shining of luster of produced pearl, net bag hanging method gave better result than grazing method.

INTRODUCTION

Pearl is a natural gem, which is formed by number of molluscan species including freshwater mussels. In nature, a pearl is created by the deposition of a natural secretion called 'nacre' over a foreign particle (sand, parasite etc.) that enters the molluscan body accidently. The natural process has been exploited to produce a wide range of natural pearls under captivity across the world, by introducing mantle tissue inside the body of the mussels by various surgical procedures. The finest quality natural pearls have been highly valued as gemstones and objects of beauty for many centuries. Pearl culture technology is a developed sector in countries like China and Japan. China has made tremendous progress in culturing freshwater pearls in triangular mussel *Hyriopsis cumingii* (Yan et al., 2009), through which pink-to-purplish coloured quality pearls are produced. Realizing the potential, several other countries have taken up this practice. However, the base technology to produce cultured pearls has been standardized (Janakiram, 2003) and more attention is being paid to improve the implantation technique. The Bangladeshi freshwater pearl producing mussel (*Lamellidens marginalis*) is widely distributed throughout the country in majority of the freshwater bodies. Pearl culture technologies involving different implantation methods have been developed with different mussel species (Janakiram, 1989; Janakiram and Tripathi, 1992; Janakiram et al., 1994; Sakpal and Singh, 2000). Barman et al., (2018) reported availability of *L. marginalis L. corrianus, L. jenkensianus* and *L. phenchooganjensis* in natural waters of Bangladesh and their potential for pearl culture. Pearl luster, quality and deposition of nacre layer may depend on culture environment and culture process. The operated mussels can be cultivated in various ways such as releasing it directly in the water body or by hanging it in a bag or by creating a specific area with bamboo fence (*bana*). Most pearl producing countries like China, Japan, Philippines, etc. follow the net bag hanging method. In this context, fresh water mussels, *Lamellidens marginalis*, collected from natural water bodies were cultured following different methods to find out the suitable culture method of freshwater pearl production in Bangladesh.

How to cite this article: Barman AC, Tanu MB, Siddique MF, Sku S, Rayhan A and Mahmud Y (2019). Maximizing pearl production in freshwater mussel (*Lamellidens marginalis*) against tissue slices. International Journal of Natural and Social Sciences, 6(1): 70-77.

MATERIALS AND METHOD

Pond preparation

A pond having 30 decimal areas was taken for stocking collected mussels. Another pond having 40 decimal areas was divided into two parts by bamboo fencing each having 20 decimal (*bana*) was used for culturing the operated mussels. Pollution free pond bottom containing sandy soil and clean water was selected. The ponds were prepared following standard procedure. Water from the ponds were totally drained out and dried. After drying, lime and salt were applied at the rate of 1kg/decimal to remove the insects and earthworms. After 6-7 days of liming, freshwater was supplied to the ponds.

Mussel collection, selection, rearing and operation

After pond preparation, mussels were collected from different fish farms of Trisal upazila of Mymensingh district and stocked in previously prepared rearing ponds. Healthy, disease-free mussels having a yellow edge on their outer part of the shell were collected from different places of the country. From the collected species, *Lamellidens marginalis* was selected for operation for image pearl production. The average length and width of the selected stocked mussels for operation were 9-10 cm and 4-5 cm, respectively. Based on survival rate and state of pearl production, *L. marginalis* was identified as the suitable species (Hossain et al., 2004). *L. marginalis* species was used for image pearl culture due to its size, availability, and suitability to operate. After selection, mussels were stocked in a prepared pond, reared, and nourished to make healthy and eligible for the operation.

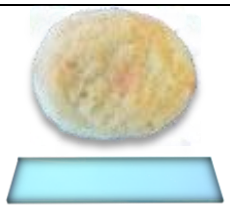
Operation Method

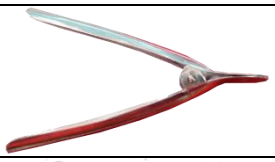
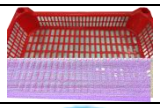
Pre-conditioning

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

Operation tools and chemicals

Table 1: Operation tools and chemicals

Operation shelf Operation shelf was used to hold the mussel during the operation process.	
Mussel cutting knife It was used for cutting adductor muscle of mussel to open it.	
Mantle tissue separating needle Two layers of mantle tissue were separated for mantle tissue slice making.	
Obtuse-headed forceps Obtuse-headed forceps were used for separating mantle tissue.	
Sponge and glass board Sponge was used for pressing mantle tissue and helps to separate tissue strip from the mussel body and cleaning the mussel mucus during operation process. Glass board was used for mantle tissue slice making.	
Mantle tissue cutter Cutter was used for mantle tissue slice making.	
Curve-head needle It was used for creating a pocket in mussel mantle tissue for slice transplantation.	

Slice transfer needle It is with sharp and flat head, used for transfer the mantle tissue slice into the pocket.	
Gill adjusting oar It is a spatula like apparatus which was used as tongue depressor; adjust the gill and visceral mass at proper place.	
Mussel opener It was used for opening the mussel in proper distance to prepare for operation.	
Stopple Stopple was used to keep two valves of the mussel open, material of which can be wood or steel.	
Porous tray Tray was used for holding the mussel and tools.	
Ajumin Ajumin was used for disinfecting and keeping alive the mantle tissue slice.	
Alcohol The operation tools were washed by using 70% alcohol.	

Mantle tissue slice making

Operation included two steps *i.e.* mantle tissue slice making and transplantation. Healthy, disease free and strong and stout mussels were selected for slice making. Mussels were opened and mantle tissue strip was separated along pallial line from the mussel. Then the separated tissue strip were transferred on a glass board and cut into small pieces (2 mm×2mm) and transplanted into another live mussel to produce pearl by transplantation (Figure 1).

Transplantation

Mussels were washed by distilled water to remove inner dirt. Live mussel was opened about 8-10mm (depending on the size) with the help of mussel opener. A pocket was made by curve-head needle in the mantle tissue. Then the mantle tissue slice (2 mm×2 mm) was inserted into the pocket and gently removes the needle and closed the operated mussel softly with the help of mussel opener.

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 1 cm) at the rate of 3 mussels/net bag and put up at 0.2m depth in post operative care units (ferro-cemented cistern of 5000L capacity) at a stocking density of 150 mussels/cistern without food for 7 days. The mussels were fed with natural food for following 21 days in the cistern and observed daily to remove dead mussels. After one month of post operative care, the mussels were transferred to the ponds.

Experimental design

Mussels were inoculated with 6 pieces of mantle tissue slices and cultured with two different methods (Net bag hanging method and Grazing method) and experiment was set for 3 years. Total 1600 mussels were operated and among them 800 operated mussels were used for net bag hanging method (Figure 2) and another 800 mussels used for grazing method (Figure 3).

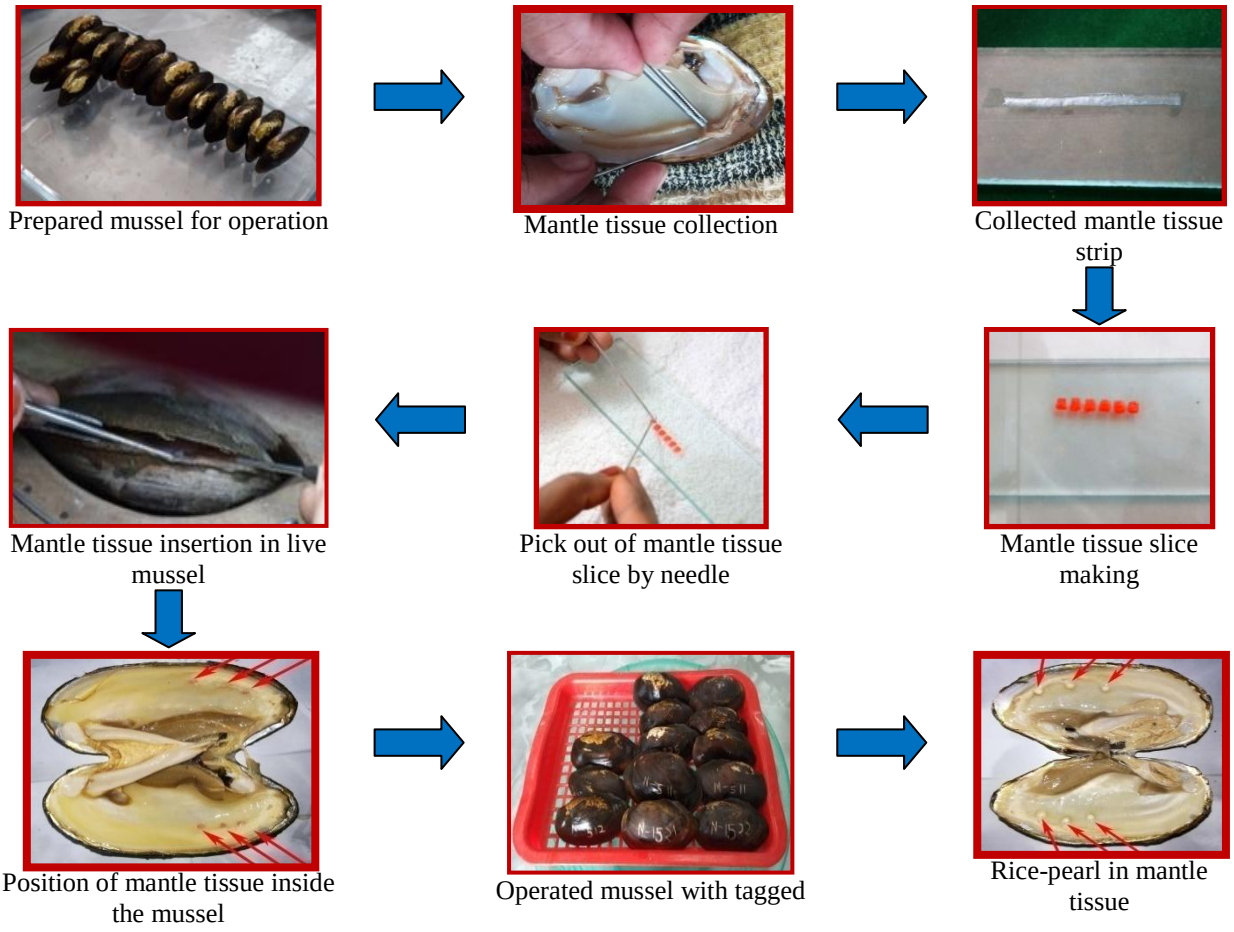


Figure 1: Operation method for non-nuclei (rice) pearl production

Table 1: Design of the experiment for culture method

Culture method	No. of tissue slice /mussel	Number of operated mussel	Mussel used for pearl production
Hanging in net bag	6	800	*Lamellidens marginalis*
Stocking in open pond (Grazing)	6	800	

Culture method

The operated mussels having 6 pieces of inserted mantle tissue slice were cultured in net-bag hanging method (Figure 3) and grazing method (Figure 4) in previously prepared culture ponds for 3 years. The stocking density of mussels and fish was 80 mussels/decimal and 30 fish/decimal, respectively. Organic and inorganic fertilizers were applied fortnightly to the pond at the rate of 5 kg organic manure, 0.125 kg T.S.P. and 0.1 kg urea per decimal. The operated mussels were checked in the ponds for survival once a month. Water temperature, pH, plankton growth, NH_4-N, DO, and Ca^{2+} parameters were recorded fortnightly.

Net bag hanging method

Net Bag hanging method is a method where a square or round shaped bagsmade with nylon net, hanged from a rope with float containing the operated mussels. In this experiment, 800 operated mussels were stocked in round shaped net bags and hanged by the rope till 30-35 cm depth with floats. The rope stretched across the pond on the surface of the water. The distance between two bags was 25-30cm and between two ropes was 1.5m.

Figure 2: Net Bag Hanging Method

Grazing Method: Grazing method is a method where the operated mussels released in open water. Here 800 operated mussels were released to the pond bottom.

Figure 3: Grazing Method

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 determine (Buck Scientific FPF-7), haemacytometer, respectively. The plankton population was determined by using the following formula (Rahman, 1992)

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

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=$1mm^{-3,}$ F= No. of fields counted, L=Volume of original water in liter. The numbers of phytoplankton and zooplankton were expressed as cells/l.

RESULTS AND DISCUSSION

Survival rate

After three years of rearing in pond pearl was harvested. In net-bag hanging method and grazing method, a total of 1600 mussels were operated where each mussel inoculated with 6 pieces of mantle tissue slices. Survival rate of mussels were found 40% for net bag hanging method and 42.1% for grazing method (Table 2). On the other hand, 80% survival rate was reported for the nucleus (without any mantle tissue) inserted mussel in *L. marginalis* for one month rearing (Miah et al., 2000). However, they didn't mention whether they had attachedany mantle tissue with the nucleus as they inserted only sand, stone, fish eye as nuclei and recorded highest pearl production in stone and lowest in the sand. Survival rate was found 100% after three months of transplantation of mantle tissue in *L. marginalis* (Hossain et al., 2004). Butsuch a short duration study is not enough for pearl creation as it requires at least 1year to 14 months for creation of desired pearl. Mortality occurred 20% in June, then decreased after August for pearl culture in *Parreysia corrugate* (Suryawanshi and Kulkarni, 2015). Survival rate observed 55-95% on pearl shell freshwater mussel (*Margaritifera falcate*) (Fernandez, 2013).

Pearl production and quality

Pearl producing rate in net bag hanging method was 43% and in grazing method 39%. Deposition of nacre layer on produced pearl was found higher in grazing method (3.76±0.2mm with medium and low shiny luster) but luster shining was found lower than net bag hanging method (3.14±0.2mm with high and medium shiny luster). Pandey and Singh (2015) found 0.35 and 0.20 mm of nacre layer from the insertion of mantle cavity in *L. marginalis* and *P. corrugata*. Rahayu et al., (2013) showed the pearl nacre layer thickness of 17 µm from 9 months cultivation of freshwater mussel *Anodonta woodiana* after the insertion of shell bead nucleus of 10mm diameter. Blay et al., (2014) found 0.65-1.24mm pearl nacre deposition from *Pinctata margaritifera* after 18 months of culture. Rathor (2017) found 3mm-4mm nacre layer of pearl after 9 months of culture in freshwater mussel *Lamellidens corrianus.*

Sunlight penetration is a major factor for shining of luster at pearl. Net bags mussels received enough sunlight than grazing mussels (Figure 4) , might be the reason for getting more shiny pearls from net bag hanging method than from the grazing method.

Table 2: Pearl production against culture technique

Culture method	Inserted mantle tissue	No. of mussel operated	Survival rate (%)	Pearl production rate (%)	Nacre layer	luster
Net bag hanging	6	800	40	43	3.14±0.2	Medium shiny (13%) High shiny (30%)
Grazing	6	800	42.1	39	3.76±0.2	Medium shiny (14%) Low shiny (25%)

Grazing method Net bag hanging method

Figure 4: Produced rice pearl through culture methods

Table 3: Water quality parameters of pond

Parameters	Year 1	Year 2	Year 3
Water temperature (^oC)	29.95 ± 1.3	23.49±1.9	24.36±2.1
Dissolved oxygen (mg/ l)	4.41 ± 0.6	4.41±0.7	6.16±0.4
Total alkalinity (mg/ l)	150 ± 11	120±13	190±10
pH (mg/ l)	7.55 ± 0.2	7.55±0.3	8.28±0.6
NH_{4-}-N (mg/ l)	0.03± 0.01	0.04±0.01	0.03±0.01
Ca^{2+} (mg/l)	17.44 ± 1.4	17.10±2.0	19.30±1.1
Phytoplankton (x10^3cells/L)	23.49± 5.3	50.53±4.9	53.20±3.2
Zooplankton (x10^3cells/L)	13.0± 2.2	10.07±2.5	5.71±2.1

Water quality parameter

Water quality parameters of the experimental ponds were recorded fortnightly. The results with mean values as presented in Table 3 were found within the suitable range for pearl culture. Temperature, dissolve oxygen, alkalinity, pH, ammonia, Ca^{+2}, Phytoplankton and Zooplankton ranged from 23.49±1.9°C to 29.95 ± 1.3°C, 4.41± 0.6 to 6.16±0.4, 120±13 to 190 ±10mg/l, 7.55± 0.2 to 8.28±0.6, 0.03± 0.01to 0.04 ±0.01mg/l, 17.10±2.0 to 19.30±1.1 mg/l, 23.49± 5.3 to 53.20±3.2 (×10^3cell/L) and 5.71 to 13(×10^3cell/L), respectively. According to Dan et al., 2001, the ideal range of water quality parameters were temperature 15-30^{o}C, DO 6.5-8.5 mg/l, pH 5-8, ammonia0.03-0.01mg/l, alkalinity 50-300 mg/l, Ca^{+2}>10 mg/l and phytoplankton (x 10^3cells/L) 50-100 mg/l. Many other authors recorded water quality parameters in the freshwater pearl culture ponds within normal levels ranging for temperature 23-36^{o}C, dissolve oxygen 4.41-8.5 mg/l, pH 6.4-8.5, ammonia 0.03-0.065 mg/l, Ca^{+2} 17.10-71.20 mg/l, alkalinity 22-594 mg/l, and Phytoplankton 23.49-89.817×10^3cell/L (Natarajan

and Sushitira, 2015; Janakiram, 1997; Yulianto *et al.*, 2016; Rathor, 2017; Pandey and Singh, 2015).

During monitoring the water quality parameters, plankton population was also monitored under microscope and different groups of phytoplankton and zooplankton were identified. Among them *Cyanophycae, Bacillariophycae, Euglenophycae, Chlorophycae, Chrysophyceae* were identified among the phytoplankton, while *Moina, Daphnia, Nauplius, Brachionus* identified among the zooplankton groups during the culture period

CONCLUSION

By cultivating pearls in two culture methods, it was observed that pearl production is possible in both two methods. However, the quality of pearl produced in the net bag hanging method is better than the grazing method. From this experiment, it can be concluded that, superior quality, higher production rate with better shiny luster pearl can be produced from the net bag hanging method after 6 pieces of mantle tissue slice inoculation in freshwater mussel *L. marginalis.*

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