*J Bangladesh Agril Univ 17(3): 424–429, 2019* https://doi.org/10.3329/jbau.v17i3.43226

ISSN 1810-3030 (Print) 2408-8684 (Online)

Journal of Bangladesh Agricultural University

Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

# Production and quality of mantle tissue transplanted pearls in *Lamellidens marginalis* cultured in different locations of Mymensingh district, Bangladesh

**Mohosena Begum Tanu ✉, Arun Chandra Barman, Mohammad Ferdous Siddique, Sonia Sku, Yahia Mahmud**

Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh

**ARTICLE INFO**

Article history:
Received: 13 January 2019
Accepted: 01 September 2019
Published: 30 September 2019

Keywords:
Pearl,
*Lamellidens marginalis*,
Water quality management,
Mantle transplantation,
Freshwater

Correspondence:
Mohosena Begum Tanu
✉: tanubfri@yahoo.com

**ABSTRACT**

A study was conducted from July 2013 to June 2016 on production rate, quality, and color of pearl, cultured at Trishal, Fulbaria and BFRI (Bangladesh Fisheries Research Institute) of Mymensingh region. Freshwater pearl producing mussel, *Lamellidens marginalis* was used in this experiment. Eighty mussels per decimal were stocked in integration with fish culture. Three ponds from three locations were used. The area of each pond was 40 decimal having water depth of 1.5m in Trishal, Fulbaria and BFRI respectively. Water temperature, pH, Ammonia, Dissolve oxygen, Alkalinity, $Ca^{2+}$ and Phytoplankton ($\times 10^3$) ranged from 25.37-28.73$^0$C, 6.2-7.92, 0.02-0.3mg/l, 5.09-6.08mg/l, 100.17-191.50, 15.31-25.87and 48.99-63.45, respectively. Survival rate of the operated mussels were 30.62%, 34.62% and 40.25%, respectively. After 3 years of rearing, 53%, 76% and 93% pearls containing mussels were harvested from Trishal, Fulbaria and BFRI respectively. After final harvesting total pearl production, its quality and color were observed and found better at the location of BFRI, followed by Trishal and Fulbaria. Research study concluded that, the quality of pearl depends on intensive care, proper management, suitable water quality parameter, sunlight penetration, food availability and soil quality of the culture pond.



## Introduction

Pearl is one of the most attractive objects considered as symbol of beauty and known as "Queen of Gems". In ancient time, pearls were also known as a symbol of royalty, wealthy and powerful people (Pandey and Singh, 2015). Naturally a pearl is produced from a piece of sand or parasite when accidentally enter into the particular species of mussels and cannot be excluded. The mussels secrete objects, called nacre to cover the external substance as a protection mechanism. Gradually the layer of this covering is deposited on the aggravation, resulting in a shining and glittering creation of a precious stone (Pandey and Singh, 2015). Cultured pearls are human creations formed by inserting a tissue graft from a donor mussel, upon which a pearl sac forms, and the inner side precipitates calcium carbonate, in the form of nacre or "mother-of-pearl" (Misra *et al.*, 2009). China is the first largest pearl producing country and 95% freshwater pearl in the world market comes from China. China is producing an estimated of 800 to 1000 metric tons of freshwater cultured pearls annually (Dan *et al.*, 2001).

Japan is the 2nd largest freshwater pearl ('Biwa pearl') producing country. Bangladesh was famous for natural pink pearls, locally known as "Mukta". They were collected from a species of freshwater mussels which are abundant in inland water bodies such as lakes, rivers, ponds and dams (Pagcatipunan, 1986). Pearl culture can be done with fish culture. Bangladesh has large inland water bodies with area of 4760894 ha knowing the potentiality; BFRI has been started pearl culture from 1999 and successfully produced pearl in pearl producing native mussel such as *Lamellidens marginalis*, *L. corrianus*, *L. phenchooganjensis* and *L. jenkinsianus*. Among them *L. marginalis* is the best species for pearl production (Hossain *et al.*, 2004). Culture environment and management techniques are very important because growth, luster, nacre secretion, color of pearl mainly depends on culture environment and management

**Cite this article**

Tanu, M.B., Barman, A.C., Siddique, M.F., Sku, S. and Mahmud, Y. 2019. Production and quality of mantle tissue transplanted pearls in *Lamellidens marginalis* cultured in different locations of Mymensingh district, Bangladesh. *Journal of Bangladesh Agricultural University*, 17(3): 424–429. https://doi.org/10.3329/jbau.v17i3.43226

techniques. Pearl production and its quality can be varied from place to place on the basis of different culture environment such as water depth, water quality, sunlight penetration and proper management. The present study was conducted at three locations of Mymensingh district to observe the quantity and quality of pearl in *L. marginalis*.

## Materials and Methods

### *Mussel collection*

Mussels (*Lamellidens marginalis*) were collected from different locations of Mymensingh region. *L. marginalis* was selected because it was identified as the best pearl producing mussel species for mantle transplantation in Bangladesh (Hossain *et al.*, 2004). At the sorting process, emphasis was given on the health, size, length, age and external color of the mussels. The average length, width and age of sorted mussels were 9.0 cm, 5.0 cm and 1-1.5 years respectively.

### *Mussel rearing*

The ponds having sandy soil, clean water and pollution free bottom were selected for the rearing of mussels. To stimulate the growth of plankton, organic and inorganic fertilizers applied fortnightly. The doses of organic and inorganic fertilizers were 5 kg organic manure, 0.125 kg T. S. P. and 0.1 kg urea per decimal. Lime was applied at 0.5 kg/decimal fortnightly to keep the pH in optimum range. Sorted mussels were reared in pond until transplantation process. Different types of tools used for mantle tissue grafting are given in Table 1.

### *Pre-conditioning*

Before transplantation, mussels were kept without food for 7 days to remove the inner mud and dirt from the body. Then the mussels were brought to laboratory and kept them in downward in a porous basket to remove the water from its body.

### *Inoculation and culture of grafted mussels*

The mussels were divided into two groups; the donor and the recipient mussels. Then the inoculation was done following the steps as described in Figure 1.

### *Stocking and management*

After transplantation mussels were transferred in the culture ponds. Three rearing ponds having 40 decimal areas were set in three different areas. 10 decimal area were separated by bamboo fence (BANA) from each pond. The areas were Trishal (Uzanpara, Darirampur), Fulbaria (Shaon Fisheries, Boruka) and BFRI (Bangladesh Fisheries Research Institute) (Figure 1). Stocking density of mussels was 80/decimal. A total of 800 operated mussels were cultured in each experimental pond. Transplanted mussels were cultured in net bag hanging method for three years. Three transplanted mussels were stocked in a net bag and hanged from a rope at 30-35cm depth with float. The rope stretched across the pond in the surface of water. The distance between adjacent two bags were 25-30 cm and two hanging rope were 1.5m. Organic and inorganic fertilizers were given 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.

Table 1. Tools and Chemicals used in the grafting of mussels

| Name of tools | Application |
|---|---|
| Mussel dissecting knife | Used for cutting the abductor muscle |
| Needle | Used to separate the mantle tissue from mussel body |
| Forceps | Used to separate the mantle tissue and to transfer the tissue strip on glass board |
| Sponge | Used to assist the forceps for separating mantle tissue |
| Glass board: | Used to keep the mantle tissue pieces |
| Tissue cutting knife | Used for cutting the mantle tissue into 2 $mm^2$ |
| Mussel opener | Used for opening the shell of mussel which used to insertion of mantle tissue |
| Stopple | Used to keep two valves of mussel open |
| Flat head needle | For making pocket into the muscle and transferring the tissue pieces into the pocket |
| Hook head needle | Used to assist the flat head needle |
| Operating stand | Used for keeping the mussel during operation |
| Tray | Used to hold the operated mussel and using tools |
| Dropper | Used to remove mud from mussel body |
| Ajumin, | To keep the mantle tissue fresh |
| 70% Alcohol | To disinfect the tools |

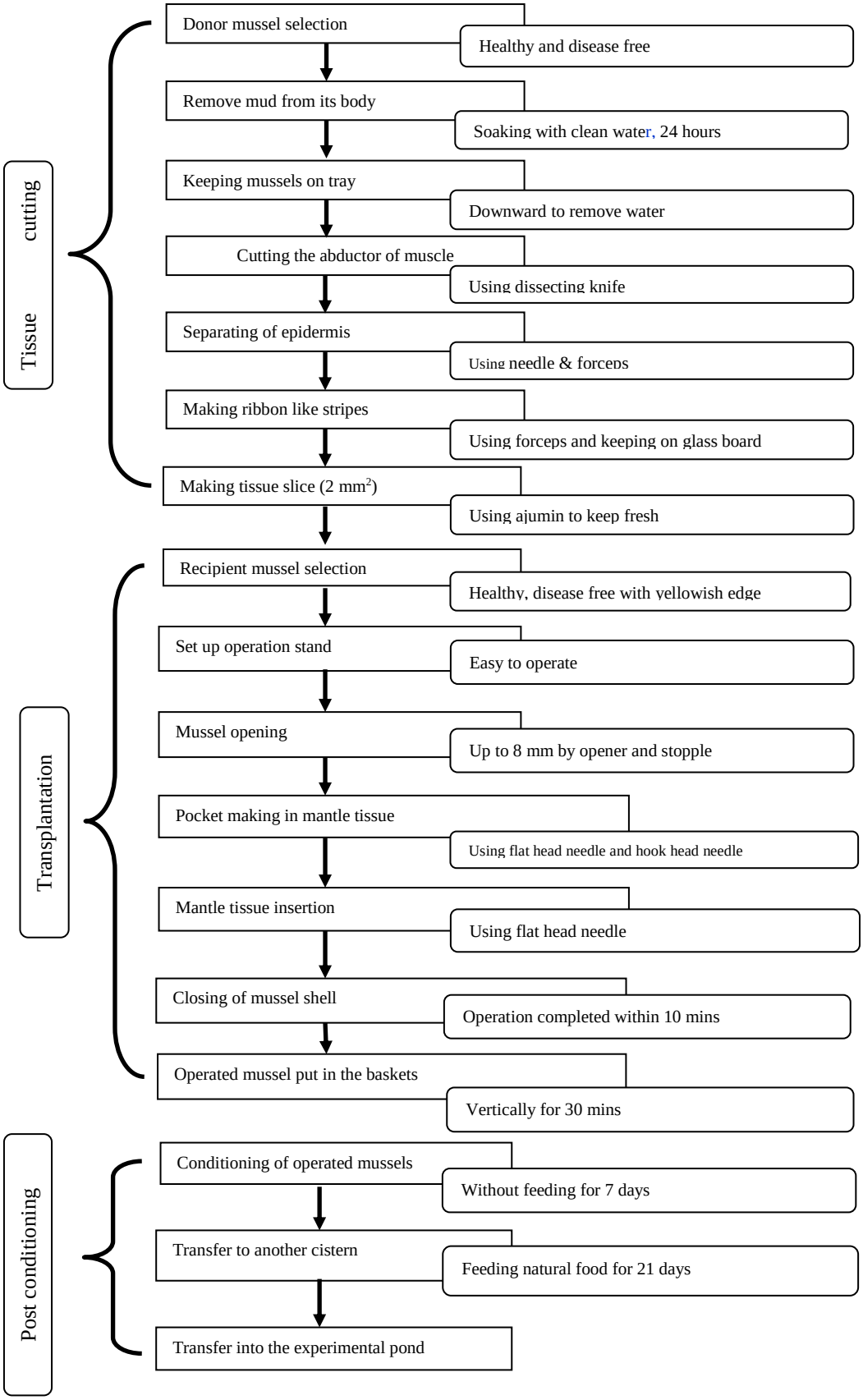


Fig. 1 Steps followed for inoculation of grafted mussels

Survival and growth of pearl were monitored once in a month. Water temperature, pH, plankton growth, $NH_4$-N, DO and $Ca^{2+}$ were monitored fortnightly. The experimental duration was three years and remained same for all the locations.

*Monitoring of water quality parameters*

Water temperature, dissolved oxygen and pH were monitored with the help of Celsius thermometer, a digital oxygen meter (YSI, model 58) and pH meter (Jenway, model 3020). Flame photometer (Buck Scientific FPF-7), Haemato-cytometer and HACH test kit (FF-3 model) were used for $Ca^{2+}$, plankton growth and $NH_4$-N measurement respectively.

*Statistical analysis*

All the collected data were analyzed statistically and expressed as mean (±SD) and standard deviation using SPSS package 23.0 statistical analysis software.

**Result and Discussion**

Maximum survival rate (40.25%) was recorded for tissue transplanted mussel in BFRI pond. On the other hand the transplanted mussel survival rate was recorded 30.62% and 34.62% in Trishal and Fulbaria respectively (Table 2). Miah *et al.* (2000) and Hossain (1983) found 80% survival for the nucleus (sand, stone, fish eye) inserted mussel in case of *L. marginalis* for one month rearing. But the mantle tissue implantation either with nuclei or without nuclei was not mentioned. At least 1 year to 14 month is needed for pearl formation. Liu *et al.* (2013) found 94.7% survival rate after three months of rearing after inserting mantle saibo (tissue slice).

Dan (2005) found 65-80% survival rate after 10 month of rearing of non-nucleated tissue insertion. At BFRI pond 93% pearl containing mussel was found among the survived mussels. Hossain *et al.* (2004) found similar pearl containing mussel among the *L. marginalis* after three months of rearing. In case of Trishal and Fulbaria, pearl containing mussels were 53% and 76% respectively. Different color (ash, silvery, pinkish, orange and white), shape (oval, round) and good luster with shiny appearance of pearl were found in BFRI pond (Figure 2). On the other hand, less variable color as ash, white, orange), different shapes (oval, button, not round), moderate and slightly shiny luster, few and less shiny luster were found in the pond of Trishal and Fulbaria respectively (Table 3). Dan *et al.* (2001) noticed that pearl color varying from silver, white to gold, pink, purple, black etc. The luster and different shape of pearl were judged by pearl appearance. On the other hand, Ram (1997) found golden to silvery or yellow and pinkish to whitish color of half round and irregular pearl from *L. marginalis* and *L. corrianus*. Jiale and Yingshen (2009) carried out research on pearl colour obtained from *Hyriopsis cumingii* and stated different colour as orange, red, green, black and purple.

Table 2. Pearl production in operated mussels reared at Trishal, Fulbaria and BFRI

| Location | Operated mussel | Survival rate (%) | Mussel containing pearl (%) |
|---|---|---|---|
| Trishal | 800 | 30.62 | 53 |
| Fulbaria | 800 | 34.62 | 76 |
| BFRI | 800 | 40.25 | 93 |

Table 3. Pearl colour, shape, luster and nacre layer in mussels reared in different locations

| Location | Pearl colour | Shape or size | Luster | Nacre layer (mm) |
|---|---|---|---|---|
| Trishal | white, ash, orange | oval, round, button | moderate luster, slight shiny | 2.39-2.40 |
| Fulbaria | white, orange | oval, button | fewer lusters, less shiny | 2.67-3.13 |
| BFRI | Ash, silvery, pinkish, orange, white | oval, round | good lusters, very shiny | 4.17-5.19 |

Table 4. Water quality parameters of different mussels culture locations

| Location | Temperature (°C) | DO (mg/l) | pH | Alkalinity (mg/l) | Ammonia (mg/l) | $Ca^{2+}$ | Phytoplankton (× 1000) cells |
|---|---|---|---|---|---|---|---|
| Ttrishal | 25.37 | 3.09 | 5.2 | 100.17 | 0.2 | 15.31 | 45.23 |
| Fulbaria | 24.68 | 3.89 | 6.32 | 140.42 | 0.3 | 17.92 | 49.99 |
| BFRI | 28.73 | 6.08 | 7.92 | 191.67 | 0.004 | 25.87 | 63.45 |

(a) Trishal (b) Fulbaria (c) BFRI

Fig. 2 Pearls produced from different study locations

Researchers also opined that pearl color depend on the host mussel, the cell slices inserted and the minerals in the water and sunlight penetration. By the insertion of mantle tissue into the mantle cavity of the studied mussel, the nacre layer of pearl 4.17-5.19 mm was found in BFRI pond after 3 years of culture, where nacre layer 2.67-3.13 mm and 2.39-2.40 mm were found in Fulbaria and Trishal respectively. The result is differed from Pandey and Singh (2015) where they found 0.35 and 0.20 mm of nacre layer on pearl by the insertion of nuclei into the mantle cavity of *L .marginalis* and *Parreysia corrugata*. Rahayu *et al.* (2013) implanted shells nucleus of 10 mm diameter and observed the highest layer thickness of 17 µm after 9 months cultivation of freshwater mussel *Anodonta woodiana*. In the present study, no nucleus but only mantle tissue had been used as foreign particles. So the harvested pearls were full of nacre accumulation which increased the thickness of nacre layer. The best quality pearl were found in BFRI pond than the other region. BFRI pond had given best result might be for regular monitoring, close observation, water quality management and overall for good management system. Similar statement was found by Dan *et al.* (2001). He stated that Management is very important during culture period, which affects the pearl qualitatively and quantitatively. He also added that Pearl quality depends on proper sunlight penetration, suitable site selection, presence of available plankton and regular water quality monitoring as in the present study fulfilled by BFRI area only. That's why the result at BFRI area was found better than the other areas of Mymensingh district.

The water quality parameter of different habitat with mean value is presented in Table 4. Water temperature, Dissolve oxygen, pH, Alkalinity, Ammonia, $Ca^{2+}$ and phytoplankton were found suitable at BFRI (Bangladesh Fisheries Research Institute) pond**.** After measurement it was found that temperature ranged from 17.12-26.8 °C, 18.3-27.10 °C and 25.12-30.30 °C, dissolve oxygen ranged from 2.63-4.22 mg/l, 3.67-4.5 mg/l and 5.3-6.8 mg/l , Alkalinity ranged from 100-240, 120-230 and 150-220, Ammonia ranged from 0.1-0.4, 0.3-0.4 and 0.002-0.07 and pH ranged from 5.5-9.1, 5.7-9.9 and 7.0-8.13, $Ca^{2+}$ ranged from 9.75-15.19 mg/l, 15.91-23.48 mg/l and 25.63-34.57 mg/l, phytoplankton ranged from 30.43-60.16 $\times 10^3$ cells/l, 30.09-55.39 $\times 10^3$ cells/l and 60.12-80.51 $\times 10^3$ cells/l in Trishal, Fulbaria and BFRI, respectively. Mean values of temperature 25.37±0.97 °C, 24.68±0.97 °C, 28.73±0.96 °C, DO 3.09±0.35, 3.89±0.39, 6.08±0.26 and pH 5.2±0.11, 6.32±0.26 7.92±0.07 and alkalinity 100.17±11.18, 140.42±11.24, 191.67±6.13 and ammonia 0.2±0.00, 0.3±0.01, 0.004±0.01 and $Ca^{2+}$ 15.31±1.32, 17.92±2.01, 30.87±1.53 and phytoplankton ($\times 10^3$) 45.23±19.9, 49.99±10.4, 63.45±13.5, in Trishal, Fulbaria and BFRI, respectively.

Natarajan and Susithira (2015) found temperature, pH, dissolve oxygen, $Ca^{2+}$ and alkalinity ranging from 25.40 °C to 28.80 °C, 7.1 to 7.9, 5.3 mg/l to 6.8 mg/l, 58.90 mg/l to 71.20 mg/l and 399.00 mg/l to 594.00 mg/l during the study period of freshwater mussel (*L. marginalis*) in pond water. Dan *et al.* (2001) showed that the appropriate temperature15-30 °C, and dissolve oxygen 5-8 mg/l, pH 6.5-8.5, ammonia 0.03-0.1 mg/l, alkalinity 50-300 mg/l and $Ca^{2+}$ >10 mg/l required for freshwater pearl culture. Ram (1997) found pH 7.5-8.5 and alkalinity 75-150 mg/l. Yulianto *et al.* (2016) assessed the water suitability (temperature 29.99±0.20 $^0$C, dissolve oxygen 5.63±0.29mg/l, pH 8.16±0.12, phytoplankton 89.817±12.4$\times 10^3$ cells/l) for the aquaculture area of pearl production. Pearl production and its quality depend on different environmental factors like, proper sunlight penetration, suitable water depth, soil quality, good water quality maintenance and no vegetation was found in BFRI pond which is suitable for pearl culture. In case of temperature increased or decreased, the transplanted mussels were replaced in deep water or brought to the low level of water with net bag when necessary in BFRI pond. Significant steps were taken to solve the water quality problem during water quality monitoring in BFRI pond. Regular application of organic and inorganic fertilizer increased the availability of natural food for mussel. The mussel mainly grazes on plankton for food; the more plankton in the water, the more rapid growth of the mussel and production of better freshwater pearls. So, pearl ponds need to be fertilized to increase plankton blooms Jiale and Yingshen (2009). All important steps were taken

in BFRI pond which needed for pearl production. But those facilities couldn't be maintained in other ponds. Vegetation was high in some ponds, water depth and quality didn't maintain when water quality parameter was fluctuated. High stocking density of fish causes water pollution and scarcity of food. Overall management of pond in Fulbaria and Trishal was not maintained properly. Because of poor management the pearl production and its quality was found lower than the BFRI pond. For all this reason pearl production, survival rate and pearl quality was lower than the BFRI pond. Several factors affecting the success or failure of pearl farming activities including physical factors, water quality, biology and anthropology factor Suryanto *et al.* (2005). Submerged and floating plants should not be allowed to grow in abundance as they will impede penetration of the light thereby diminishing the plankton content of the water Pagcatipunan (1986). The success of pearl culture is strongly influenced by the food presence.

## Conclusion

The present study concluded that pearl production from BFRI pond area was better than Trishal and Fulbaria. It is clear that the environment, water quality parameters and management techniques play a vital role in pearl production and its quality. Not only the careful operation procedure but also the regular monitoring, close observation of operated mussels and water quality management can give better result in pearl production. So, good management of pond can produce better quality pearl with higher survival rate and high production of pearl.

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