Impact of Culture Period on Quality of Image Pearls Produced by the Freshwater Mussel, Lamellidens marginalis, in Bangladesh

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IMPACT OF CULTURE PERIOD ON QUALITY OF IMAGE PEARLS PRODUCED BY THE FRESHWATER MUSSEL, *LAMELLIDENS MARGINALIS*, IN BANGLADESH

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ABSTRACT Pearls are gems produced by biological processes within the living tissues of molluscs. Image pearls are a form of blister pearl that portray a design or image such as a bird, fish, flower, goddess, etc. To achieve this, a paraffin wax mold is inserted between the shell and the mantle of a freshwater mussel and subsequent nacre coverage produces an image pearl after an appropriate culture period for the mussel. The optimum culture period has not yet been determined to maximize the quality of image pearls produced in Bangladesh, and this knowledge gap was addressed in this study. Image pearl production using the freshwater mussel *Lamellidens marginalis* was conducted over three culture periods (T_1 = 7 mo, T_2 = 9 mo, and T_3 = 11 mo). A total of 3,150 mussels were implanted with 2.5×1.5 cm^2 paraffin wax molds, with 1,050 mussels allocated to each treatment, which had five replicates. Survival and pearl production rate of mussels were negatively correlated with culture period. Survival and pearl production rate were 14.7%, 12.3%, and 11.9% for mussels cultured for 7, 9, and 11 mo, respectively. The thicknesses of the nacre making up the image pearl and pearl luster were both improved with longer culture periods. The highest quality pearls with a mean nacre thickness of 0.71 mm were produced after 11 mo. Pearls produced after 11 mo also had the highest luster (40.05 lux) compared with pearls cultured for 7 mo (12.85 lux) or 9 mo (30.2 lux). There were statistically significant differences at the $P < 0.05$ level in survival rate F (2, 12) = 9.40, $P = 0.004$ and nacre layer thickness F (2, 12) = 13.30, $P = 0.001$ between T_1 and T_3. The results indicated that image pearls with high luster and improved quality are produced after longer culture durations and confirms the influence of culture period on image pearl production and quality. Further research is required to improve image pearl production methods and pearl yield.

KEY WORDS: image pearl, culture period, nacre layer, luster, *Lamellidens marginalis*, Bangladesh

INTRODUCTION

Pearls are considered symbols of beauty, love, purity, and aristocracy, and are strongly associated with affluence, style, and fashion (Dirlam et al. 1985, Pandey & Singh 2015). Cultured pearls are formed by living molluscs including marine pearl oysters, freshwater mussels, abalone, and other gastropods (Acosta-Salmon & Davis 2007, Strack et al. 2019, Zhu et al. 2019, Southgate 2021). Commercial cultured pearl production generally utilizes species with the ability to produce nacreous [mother-of-pearl (MOP)] pearls and the majority of global cultured pearl production originates from marine pearl oysters (Pteriidae) and freshwater mussels (Unionidae) (Zhu et al. 2019).

Cultured pearls can be divided into two main categories: bead-nucleated pearls are formed within oyster tissues after nucleus implantation, whereas blister pearls are formed by the insertion of an object, as a substrate for nacre deposition, between the inner shell surface and the mantle tissue of the mollusc (Taylor & Strack 2008). The latter includes mabé pearls or "half-pearls" produced by marine pearl oysters (e.g., Kishore et al. 2015, Gordon et al. 2019), and image pearls produced by freshwater mussels, which are made in the form of a design or image such as a bird, fish, flower, goddess, etc. The substrate or image mold used to make image pearls does not require gluing to the inner surface of the shell but is placed on the concave inner shell surface of the mussel shell, without glue, where it retains position. Image pearls reflect the original sculpture of the image mold which, during subsequent mussel culture, is covered by nacre before being cut from the shell for value-adding (Dan et al. 2001). The image mold is usually made from paraffin wax but can also be made from carved mussel shell. One of the first records of image pearls dates from the 12th century and recounts Buddhist monks who produced MOP Buddha figures using freshwater mussels (George 1966). In the late 13th century (Ming Dynasty), image pearl culture was used to produce pearl Buddhas (image pearl of Buddhas) (Abbott 1972, Alagarswami 1987) where image molds of Buddha figures were set on the inner shell surfaces of the freshwater mussels (Cristaria plicata), to be coated with nacre (Akamatsu et al. 2001). In modern times, image pearls are used as jewelry, for personal adornment and as decoration pieces following value-adding.

Cultured marine pearl production has relatively high risk, requires high capital inputs, has high operational costs, and a relatively long period before profitability (Johnston et al. 2019, 2020). Freshwater image pearl production, in contrast, has much lower capital and operational costs and is less resource demanding with pearl formation requiring less than 1 y (Rachman & Maskur 2006). Image pearl culture can be conducted in a variety of water bodies including ponds, lakes, rivers, reservoirs, etc., and is appropriate for extension in rural areas with low input and high output (Hossain et al. 2004). Factors influencing the production rate and quality of image pearls include the species of mussel used, mussel size, operation technique, culture environment, and the length of culture period. This experiment was conducted to examine the impact of culture period on resulting production and quality of image pearls produced by *Lamellidens marginalis*.

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MATERIALS AND METHOD

Cistern and Pond Preparation

A disinfected cistern (2.42 m × 1.88 m × 1 m) with a water exchange facility was used for the post- and preoperative treatment of the mussels. Ponds were prepared according to regular pond preparation procedure. Organic and inorganic fertilizers were applied to the ponds viz. organic manure applied at the rate of 5 kg/40.5 $m^{2,}$ triple super phosphate (T.S.P.) at a rate of 0.125 kg/40.5 m^2, and urea at a rate of 0.1 kg/40.5 m^2. Lime was applied at a rate of 0.5 kg/40.5 m^2.

Collection, Selection, and Rearing of Mussels for Operation

Healthy, disease-free mussels (*Lamellidens marginalis*) with a yellow shell margin were collected from different locations within the Mymensingh region of Bangladesh. This species was chosen from other available pearl producing mussels found in Bangladesh because of its optimum size, availability, improved survival, relatively high pearl producing rate, and suitability to operate for image pearl production (Hossain et al. 2004). The average shell length and width of the mussels selected for this experiment were 10 cm and 5 cm, respectively (Fig. 1). After selection, mussels were stocked into previously prepared ponds before their operation for image pearl production.

Preconditioning of Selected Mussel

Selected mussels were preconditioned without feeding in a disinfected cistern for 7 days to prepare for the image pearl operation. Water was changed to maintain water quality and any adherent clay was removed from the outer shell and inner soft tissues of the mussel. Before operation, mussels were brought to the laboratory and held in a downward position (i.e., hinge uppermost) in a porous basket for 2 h to allow water to drain from the internal organ of the mussel. The basic tools used for image pearl production, and their functions, are outlined in Table 1.

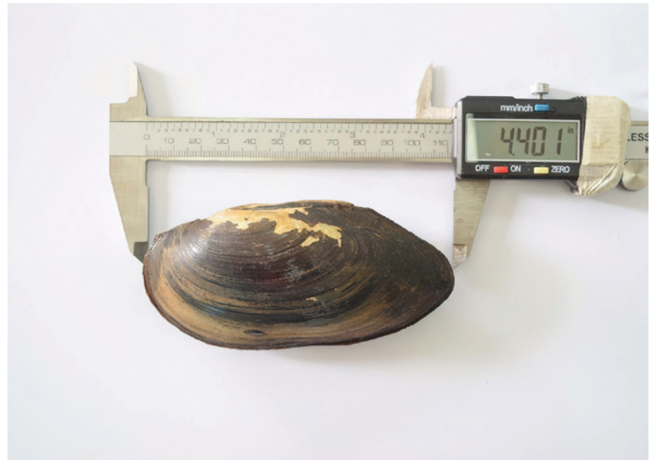

Figure 1. Mussel (*Lamellidens marginalis*) size measurement by slide calipers.

Paraffin Image Preparation

Shells of a dead mussel, soybean oil, paraffin, a heater, and needle were used to prepare paraffin wax image molds. Soybean oil was applied to the concave side of the dead mussel shell to ease separation of the paraffin image from the shell. Liquid wax was then poured onto the oil layer within the concave side of the shell and agitated to form a thin layer of wax about 1.5–3.5 mm thick. After that, an image or sculpture is drawn into the wax with a needle, before the complete paraffin wax image mold is removed for use (Fig. 2).

TABLE 1.

Basic tools used for image pearl production and their function.

Tool	Function
Wooden stand	To hold the mussel in a fixed position so that the operator can handle it easily during image mold placement operation.
Spatula	Gill adjusting oar used as tongue depressor, which can adjust the gill and visceral mass into an appropriate position during operation
Mussel opener	Mussel opener is used to open the mussel to the required gape in preparation for the operation
Stopple	Used to hold the two shell valves apart after opening. Can be made of wood or metal.
Obtuse headed forceps	Forceps used for transferring paraffin wax image mold into position within the mussel.
Trays for holding	A tray is used for holding operated mussels and operation tools and a porous tray is used for draining out water remaining in mussel body.
Mussel shell and prepared paraffin image	Dried dead mussel shells are used as a substrate to make paraffin wax images of appropriate shape. Paraffin wax is shaped and sculpted to generate an image mold used for image pearl production (Fig. 2).
Dropper bottle, paraffin wax, beaker, and heater	Dropper bottle used for cleaning the dirt, and paraffin wax, beaker, and heater used to produce image or design.

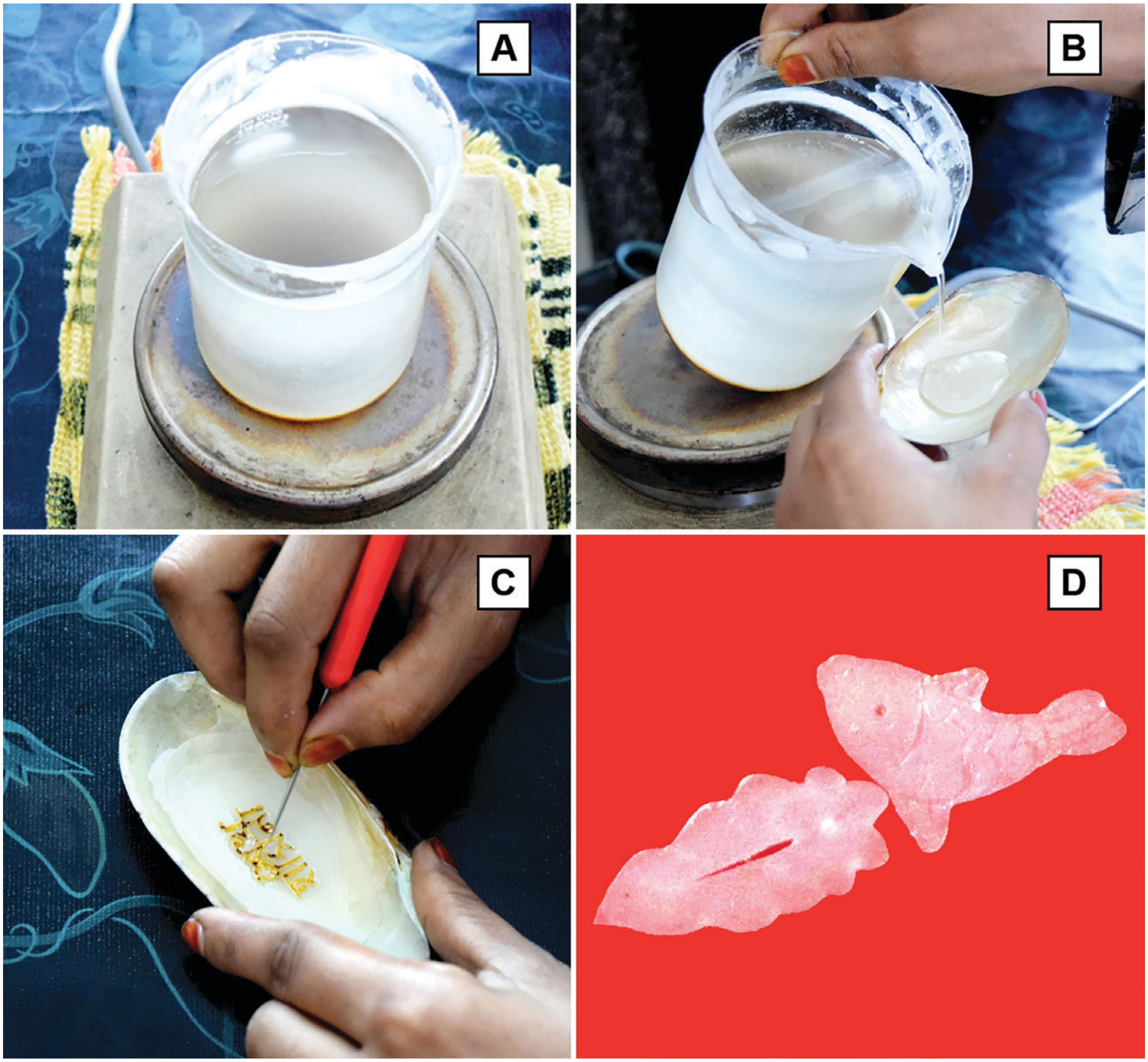

Figure 2. The procedure used to prepare paraffin wax images (molds) used for image pearl production, showing: (A) paraffin wax is melted using a heater, (B) liquid paraffin is poured into the mussel shell, (C) drawing of an image into the wax using a needle, and (D) prepared paraffin image molds ready for insertion into recipient mussels.

Operation Method

Before the paraffin wax image mold is inserted into the receiving mussel, it is washed with distilled water to remove any dirt and soaked in water to make it slippery. The selected live mussel to receive the image mold is opened to a gape of about 8 mm using the mussel opener. A small area of the mussel mantle is gently moved away from the inner shell surface using a spatula and the image mold is inserted into the resulting cavity between the shell and mantle of the mussel without glue. After image mold insertion, its position may be adjusted within the cavity and air from the cavity area is gently removed. Finally, operated mussels are held in an upward position (hinge down) in the tray until transfer to the cistern, so that the implanted image molds cannot dislodge (Fig. 3). The operation procedure followed that of Tanu et al. (2021).

Postconditioning of Operated Mussel

After image mold insertion, mussels were conditioned in a cistern, with regular water change, for 7 days without feeding. For the next 21 days in the cistern, operated mussels were fed with pond collected plankton at a density of 60×10^3 cell/L. Operated mussels were then transferred from the cistern to culture ponds for a 7-, 9-, or 11-mo culture period to produce image pearls.

Figure 3. Stepwise process of image pearl production: (A) prepared mussels ready for operation, (B) soaking of paraffin wax image molds in water, (C) mussel in open condition with stopple (8–10 mm) to hold valves apart, (D) removing clay and dirt, (E) separation of mantle from shell using spatula, (F) insertion of image into live mussel, (G) placing image mold on inner surface of shell, (H) internal view of image insertion using dissected mussel, (I) tagging of operated mussel, and (J) final product of image pearl.

Stocking and Culture Management of Mussels

Operated mussels were stocked into the prepared culture pond at a density of 80 mussels/40.5 m^2 using hanging net bags. A total of 3,150 operated mussels were cultured in an experimental pond using the net bag hanging method reported to be superior for image pearl production (Siddque et al. 2020). Six operated mussels were stocked into each rectangular net bag, within individual pockets, and each net was hung by rope at a depth of 30–35 cm with a float (Fig. 4). The header ropes, to which nets were attached, stretched across the pond surface. The distance between two bags was 25–30 cm and the distance between header ropes was 1.5 m throughout the culture period. Organic and inorganic fertilizers were applied to the pond

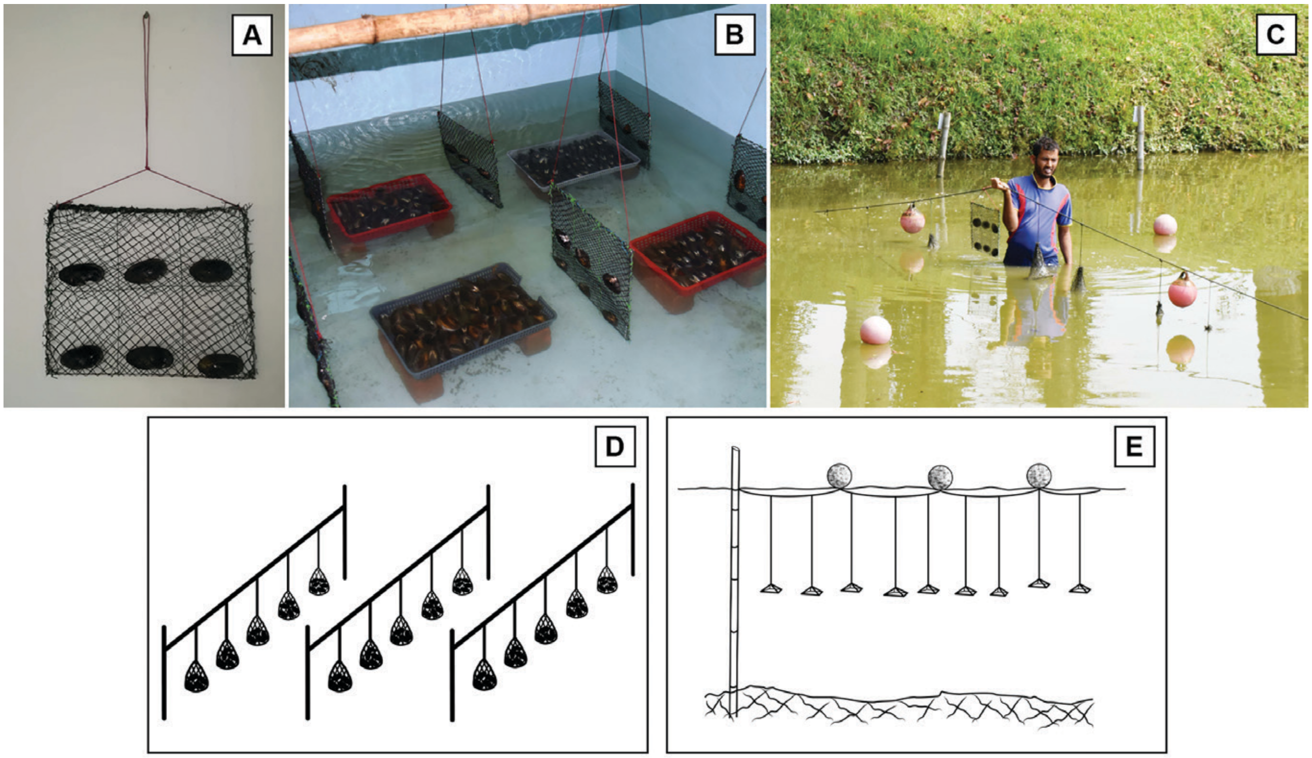

Figure 4. Net bag hanging culture method in cistern and pond showing: (A) six operated mussels in pockets within nets, (B) operated mussels in nets and trays held before transfer to ponds, (C) pond for mussel culture showing header ropes and floats, (D and E) diagram showing arrangement of header ropes and nets within the pond culture system.

fortnightly at the rate of 5 kg organic manure, 0.125 kg T.S.P., and 0.1 kg urea per 40.5 m^2 pond areas. Operated mussels were checked monthly to assess survival. Water temperature, pH, plankton growth, ammonia, dissolved oxygen (DO), and Ca^{+2} levels within pond culture water were recorded fortnightly.

Water Quality Parameter Measurement

Water temperature was measured using a thermometer and DO, pH, ammonia, and calcium ion levels were measured by using DO meter (YSI, model 58, YSI Incorporated, Yellow Springs, OH), pH meter (Jenway, model 3020, Cole-Parmer, Stone, Staffordshire, UK), ammonia test kit (HACH test kit; FF-2 model, Mentor, OH), and spectrophotometer (HACH-DR1900, Mentor, OH), respectively. Plankton was collected using a plankton net and plankton density was estimated by counts using a light microscope (NOVEX Holland, Lens no.: 10/0.25) and SR cell, and subsequent application of the following formula (Stirling 1985, Rahman 1992):

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

Experimental Design

A total of 3,150 mussels were operated with paraffin image of the same size (2.5 × 1.5 cm^2). This study assessed three culture duration treatments: 7 mo (T_1), 9 mo (T_2), and 11 mo (T_3). In each treatment, 1,050 mussels were operated with five replications per treatment among five different ponds. After the respective image pearl culture periods of 7, 9, and 11 mo, mussels were harvested and resulting image pearls were analyzed.

Luster and Nacre Layer Assessment

Luster was assessed using a light meter (model: Lutron LX-101AS, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) and human observation based on standard grading criteria (Matlins 1996). The distance between the image pearl and the light meter sensor was 1 inch during luster measurement. The thickness of the nacre layer of resulting image pearls was determined with Gauge calipers.

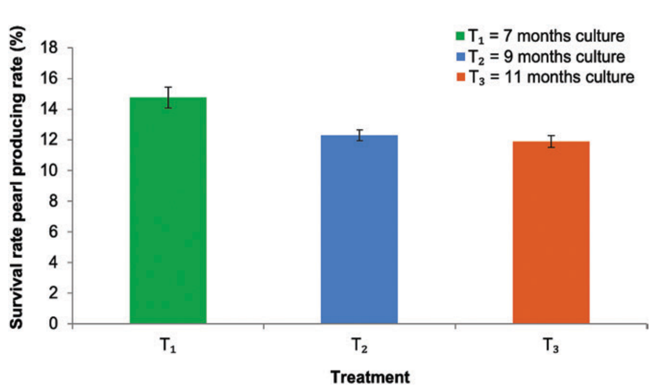

Figure 5. Mean (±SE) survival (%) of mussels (*Lamellidens marginalis*) cultured for image pearl production for 7, 9, or 11 mo.

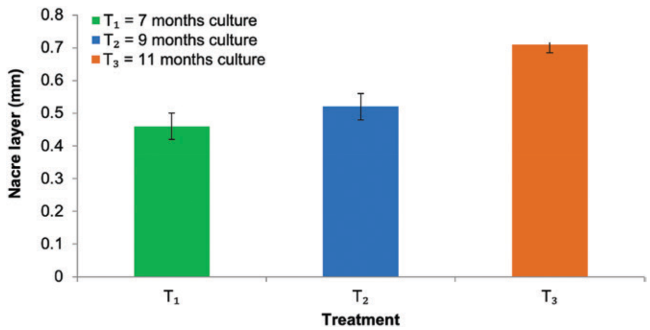

Figure 6 Mean (±SE) nacre layer thickness of image pearls from mussels (*Lamellidens marginalis*) cultured for 7, 9, or 11 mo.

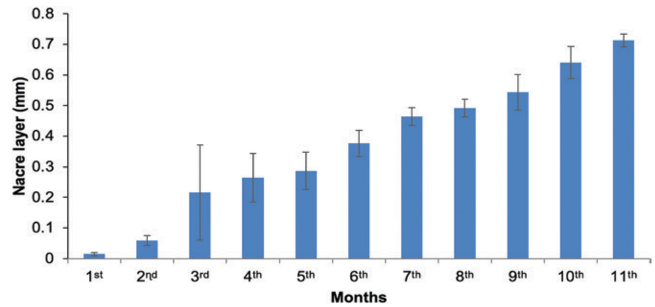

Figure 8. Changes in the mean (±SE) nacre layer thickness of image pearls over the 11-mo study period.

Statistical Analysis

All data are expressed as mean and (±) standard deviation and analyses were conducted using IBM SPSS Statistics, package version 25.0 software, document number: 589145 Modified date: 21 May 2021, Developer: Norman H. Nie, Dale H. Bent, C. Hadlai Hull.

RESULTS

All mussels that survived to harvest produced an image pearl and, on this basis, survival and image pearl production rate are represented by the same values: 14.7%, 12.3%, and 11.9% for T_1, T_2, and T_3 treatments, respectively (Fig. 5). Mean nacre thicknesses of image pearls produced by mussels in treatments T_1, T_2, and T_3 were 0.46 ± 0.1 mm, 0.52 ± 0.01 mm, and 0.71 ± 0.01 mm, respectively (Fig. 6). There were statistically significant differences at the $P < 0.05$ level in survival rate $F\,(2, 12) = 9.40$, $P = 0.004$ and nacre layer thickness $F\,(2, 12) = 13.30$, $P = 0.001$ between T_1 and T_3. Average values for image pearl luster varied from 12.85 lux in T_1 to 30.2 lux in T_2, and was maximum at 40.05 lux in T_3 (Fig. 7). Image pearls produced in treatment T_3 had the thickest nacre layer (0.71 ± 0.01 mm) and the highest luster (40.05 lux) of all treatments, but mussels in this treatment had reduced survival (and lower pearl production) when compared with those in treatments T_1 and T_2. Highest survival was shown by mussels in treatment T_1,which also recorded the lowest nacre thickness and lowest luster among the treatments tested. Of the harvested image pearls from treatment T_1, 7.2% had medium luster and 7.5% had low luster. In treatment T_2, 6% of pearls had medium luster whereas 6.3% had low luster. In contrast, image pearls cultured for the longest (11 mo) culture period (T_3) showed improved pearl luster with 8% of pearls showing high luster and 3.9% pearls with medium luster. Changes in the mean (±SE) nacre layer thickness of image pearls during the experiment from month 1 (0.015 ± 0.005 mm) to month 11 (0.713 ± 0.02 mm) are shown in Figure 8.

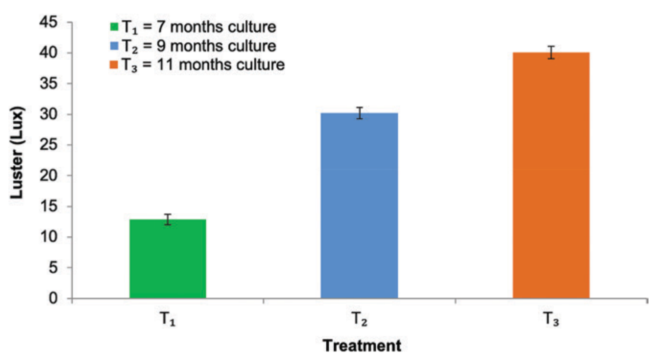

Figure 7. Mean (±SE) luster (Lux) of image pearls from mussels (*Lamellidens marginalis*) cultured for 7, 9, or 11 mo.

Water Quality Parameters

Physicochemical parameters within pearl culture ponds were monitored at 15 days interval during the 11-mo study period. Water temperature ranged from 20.3°C to 29.5°C, DO from 4.14 to 8.18 mg/L, alkalinity from 140 to 200 mg/L, pH from 5.89 to 8.25, ammonia from 0.01 to 0.125 mg/L, and Ca^{+2} from 9.31 to 13.13 mg/L, phytoplankton from 50.1 to 59.8 × 10^3 cell/L, and zooplankton from 35.5 to 55.8 × 10^3 cell/L. All water quality parameters were within an acceptable range throughout the study (Dan et al. 2001).

DISCUSSION

Image Pearl Production and Quality

A total of 3,150 operated mussels (*Lamellidens marginalis*) cultured for 7, 9, or 11 mo had survival rates of 14.7%, 12.3%, and 11.9%, respectively. Every surviving mussel produced a pearl. There were statistically significant differences at the $P < 0.05$ level in survival $F\,(2, 12) = 9.40$, $P = 0.004$ between T_1 and T_3. These rates of survival are relatively low when compared with the results of similar studies of image pearl production using *L. marginalis* and other species (Table 2). For example, it is common for survival of *L. marginalis* to exceed 50% over an image pearl culture period of 12–36 mo, which is much longer than the maximum culture period of 11 mo used in the present study (Janakiram 1997, Tanu et al. 2019a, 2019b) (Table 2). Similar investigations of image pearl culture using other species of freshwater mussel have also reported relatively high survival compared with the present study with, for example, 55%–95% survival of *Margaritifera falcata* over a 19-mo culture period (Fernandez 2013), 80% survival of *Parreysia corrugata* over a 12-mo culture period (Suryawanshi & Kulkarni 2015), and 55% survival of *Lamellidens corrianus* over a 9-mo culture period (Rathor 2017) (Table 2). The reason for the apparently low rate of survival in the present study is unclear although it is notable that survival decreased with

TABLE 2.

Nacre thickness and survival reported in prior studies of image pearl production using freshwater mussels.

Species	Culture duration (months)	Survival (%)	Nacre thickness (mm)	Author(s)
Margaritifera falcata	19	55–95	N/A	Fernandez (2013)
Lamellidens marginalis, L. corrianus, Parreysia corrugata	12	60–70	N/A	Janakiram (1997)
Lamellidens marginalis	1	80	N/A	Miah et al. (2000)
Parreysia corrugata	12	80	N/A	Suryawanshi and Kulkarni (2015)
Lamellidens marginalis	36	62–77	2.12–4.85	Tanu et al. (2019a)
Lamellidens marginalis	36	53–93	4.17–5.19	Tanu et al. (2019b)
Lamellidens corrianus	9	55.31	–	Rathor (2017)
Anodonta woodonia	9	N/A	17 μm	Rahayu et al. (2013)
Lamellidens marginalis *Parreysia corrugata*	12	N/A	0.20–0.35	Pandey and Singh (2015)
Lamellidens marginalis	7	14.7	0.46 ± 0.1	This study
	9	12.3	0.52 ± 0.01	
	11	11.9	0.71 ± 0.01	

increasing culture period. Image pearl nucleation and pond-based culture methods followed standard procedures (Tanu et al. 2021) and water quality parameters in culture ponds were within recommended levels (Dan et al. 2001). Further research is required to fine-tune mussel handling and image pearl production and culture methods to improve mussel survival and pearl yield.

Although survival and image pearl production rate decreased with increasing culture period in the present study, the thickness of the nacre layer of resulting image pearls and their luster were found improved with longer culture time, with those cultured for 11 mo having both the thickest nacre layer and the best luster. Evaluation of pearl quality depends mainly on nacre thickness (Matlins 1996). In a similar study to this, a nacreous layer of 0.20–0.35 mm (maximum and minimum) was reported after 12 mo of image pearl culture using *Lamellidens marginalis* (Pandey & Singh 2015) while, in the present study, using the same species, greater nacre layer thicknesses of 0.46 mm, 0.52 mm, and 0.71 mm were recorded for image pearls cultured for shorter period of 7, 9, and 11 mo, respectively, with that of image pearl cultured for 11 mo (0.71 mm) being twice that reported for image pearls cultured for a 12-mo period, using the same mussel species, by Pandey and Singh (2015) (Table 2).

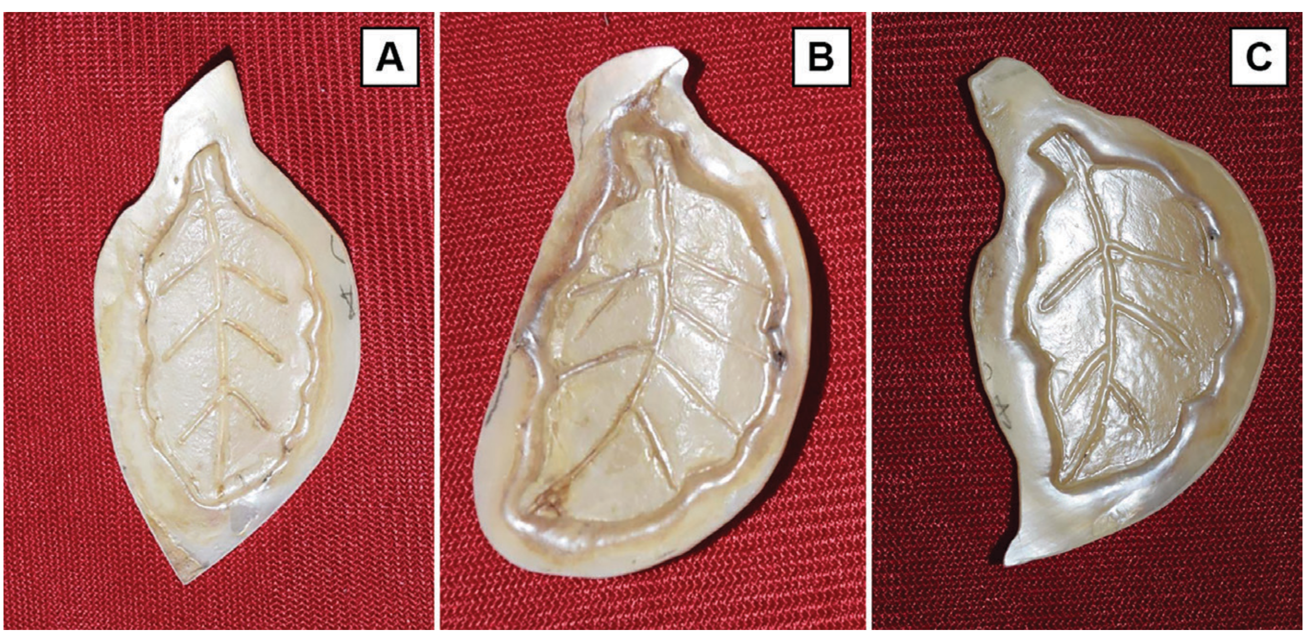

Figure 9. Image pearls resulting from the three culture periods tested in this study (A) image pearl of 7 mo, (B) image pearl of 9 mo, and (C) image pearl of 11 mo.

TABLE 3.

A grading system for cultured pearls based on Ruiz-Rubio et al. (2006) and illustrated by image pearls produced during this study.

Grade	Remarks	Pictorial view
AAA	Excellent luster, no surface flaws, and good symmetry characterize the highest grade with superb attributes	
AA	Good quality, good luster, homogeneous coloration with a few surface defects	
A	Medium quality, good luster, nonuniformity in coloration with some surface imperfections and poor symmetry	
B	Good luster with irregular surface and coloration, as well as a few surface flaws	
NC	No economic value, the shine is low, the nacre layer is weak, and serious flaws on the surface	

Of the five "virtues" influencing the quality, grading, and value of cultured pearls (size, surface, shape, color, and luster; Southgate 2021), luster, defined as "shine with depth" (Matlins 1996), is key. Luster is assessed by the sharpness of the reflection of a light source from the surface of a pearl and may range from dull to very bright (CIBJO 2021). A grading system for cultured pearls, based on quality assessment, is described in Table 3 (Ruiz-Rubio et al. 2006) and illustrated by image pearls produced during this study.

Water Quality

Factors such as water temperature and food availability are key ecological parameters affecting the physiology, metabolism, and growth rate of pearl producing molluscs (Lucas 2008) which, in turn, are correlated to pearl quality (Wada 1973). Ideal water quality parameters for freshwater mussel culture are reported to be a water temperature range of 15°C–30°C, pH range of 6.5–8.5, DO 5–8 mg/L, alkalinity 50–300 mg/L, ammonia 0.03–0.1 mg/L, phytoplankton and zooplankton $50–100 \times 10^3$ cell/L, and calcium over 10 mg/L (Dan et al. 2001). Janakiram (1997) reported pH from 7.5 to 8.5 and alkalinity of 75–150 for pearl culture. The water quality parameters reported by Rathor (2017) (water temperature 25.3 ± 1.55°C, pH 6.4 ± 0.21, DO 5.63 ± 0.17 mL/L, alkalinity 22.44 ± 0.34 mg/L), during *Lamellidens corrianus* culture for pearl production, were more or less similar to those for the same parameters in the present study. Natarajan and Susithira (2015) reported ranges for water temperature, pH, DO, Ca2+, and alkalinity ranged from 25.40°C to 28.80°C, 7.1–7.9, 5.3–6.8 mg/L, 58.90–71.20 mg/L, and 399.00–594.00 mg/L for pond culture of *Lamellidens marginalis*.

In summary, the results of this study show that culture period has a significant effect on production (yield) and quality of image pearls produced by the freshwater mussel *Lamellidens marginalis*. Thickness of the nacre layer and image pearl luster are two important factors for grading pearls. Both were improved by a longer period of pearl culture (11 mo) compared with shorter culture periods of 7 and 9 mo (Fig. 9). Despite this, the longer culture period resulted in reduced pearl yield, with survival to harvest relatively low compared with that reported in prior studies. Further research is required to improve image pearl production method and pearl yield.

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