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Original Research Article

Assessment of different freshwater mussel species suitability for image pearl production

Md. Nazmul Hossen[1,*], Mohosena Begum Tanu[3], Mohammad Ferdous Siddique[2], Saymuna Tarin Lupa[3], Md. Harunor Rashid[1] and Sonia Sku[1]

[1] Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh, Bangladesh
[2] Bangladesh Fisheries Research Institute, Riverine Station, Chandpur, Bangladesh
[3] Bangladesh Fisheries Research Institute, Headquarters, Mymensingh, Bangladesh
[*] Corresponding author' E-mail: nazmulpisces@gmail.com

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ABSTRACT

This study was designed to discover the most suitable freshwater mussel species for image pearl production under controlled culture condition. A total of 600 mussels were used for transplantation and for each species 200 mussels were allocated. Three species of freshwater mussel as *Lamellidens marginalis*, *Lamellidens corrianus*, and *Pilsbryoconcha exilis* were collected, acclimatized and then implanted with paraffin-based image nuclei. The operated mussels were cultured in freshwater pond for 11 months maintaining regular water quality monitoring with net-bag hanging method. Fertilizers were applied for plankton production. At the end of the culture period, survival rate of mussels, nacre layer thickness, and luster of pearl were assessed to evaluate the mussels and pearls. The highest survival rate (47%) and superior luster quality were recorded in *Pilsbryoconcha exilis*, followed by *L. corrianus* (42%) and *L. marginalis* (40.5%). Nacre layer thickness was 1.36$\pm$0.12 mm for *L. marginalis*, 0.84$\pm$0.09 mm for *L. corrianus*, and 1.04$\pm$0.09 mm for *P. exilis* among the species. Luster of pearl was 34.89$\pm$0.70 lux for *L. marginalis*, 37.86$\pm$0.67 lux for *L. corrianus*, and 45.33$\pm$1.32 lux for *P. exilis*. While *L. marginalis* deposits the thickest nacre, its inferior luster and lower survival make it less commercially viable; conversely, *P. exilis* achieves the highest survival and brightest luster which is key traits for marketable image pearls demonstrating that pearl quality is not governed by nacre thickness alone, but by a species-specific trade-off that positions *P. exilis* as the most efficient candidate for sustainable, income-generating freshwater image pearl farming.

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INTRODUCTION

Freshwater pearl culture is an emerging aquaculture sector with substantial economic potential, yet its global commercial success remains heavily dependent on a limited number of mussel species, particularly *Cristaria plicata*, *Hyriopsis cumingii*, and *Hyriopsis schlegelii*. Cultured pearl production primarily relies on two groups of molluscs: freshwater mussels and marine pearl oysters (Fiske & Shepherd, 2007; Zhu *et al.*, 2018). Over the centuries, these pearl-producing molluscs have made substantial contributions to cultural heritage, socioeconomic development, and community livelihoods worldwide (Southgate *et al.*, 2011; Bai *et al.*, 2014). Although *H. cumingii* and *H. schlegelii* remain the dominant commercial species, several other members of the families Unionidae and Margaritiferidae have demonstrated considerable potential for pearl culture, with successful applications reported in the United States, Vietnam, Venezuela, India, Brazil, Colombia, Australia, and Mexico (Sweaney & Latendresse, 1984; Bosshart *et al.*, 1993; Lasso *et al.*, 2009, 2018; Gogoi & Mandal, 2011; Pandey & Singh, 2015; Agudo-Padrón, 2015, 2016; Zhu *et al.*, 2018; Saucedo *et al.*, 2018). Among cultured pearl types, image pearls have attracted increasing attention due to their high ornamental and commercial value. An image pearl is produced by inserting a shaped nucleus between the mantle and shell of a living mussel, where successive nacre deposition gradually forms a pearl that retains the design of the implanted nucleus (Southgate *et al*., 2008; Bai *et al*., 2014). The finished product consists of a lustrous nacreous coating over the sculptured nucleus, making image pearls one of the most attractive cultured pearl varieties (Dan *et al*., 2001). Historically, image pearl production originated during the twelfth century, when Buddhist monks produced Buddha-shaped pearls in freshwater mussels (George, 1966). This technique was refined in the thirteenth century through the production of Buddha image pearls on the inner shell surface of *Cristaria plicata* (Abbott, 1972; Alagarswami, 1987; Akamatsu *et al*., 2001). Modern image pearl production typically involves implanting paraffin wax or other shaped nuclei into the extrapallial space of the mussel, where the model becomes progressively coated with nacre over the culture period, ultimately yielding a lustrous pearl preserving the original nucleus shape (Dan *et al*., 2001; Tanu *et al*., 2021a). The composition, size, and physical properties of the implanted nucleus can influence the host mussel's physiological response, affecting survival, nacre secretion, pearl quality, and luster, while recent studies also indicate that culture duration and nucleus characteristics significantly influence nacre thickness and overall pearl quality.

In South Asia, particularly Bangladesh, indigenous freshwater mussels such as *Lamellidens marginalis*, *Lamellidens corrianus, and Pilsbryoconcha exilis* are widely distributed and possess desirable traits for pearl culture. These species differ considerably in shell morphology, mantle structure, and growth characteristics, traits known to influence biomineralisation and nacre secretion. For instance, *L. marginalis* has been shown to express genes involved in biomineralisation including pif, perlucin, calreticulin, calmodulin, chitin synthase, carbonic anhydrase, and tyrosinase key enzymes and matrix proteins that regulate calcium carbonate deposition and nacre formation (Chen *et al*., 2017). Variations in the expression of such genes across species could directly impact nacre thickness, luster, and overall pearl quality. Furthermore, studies on other unionid mussels have demonstrated that species-specific differences in mantle tissue response, survival, and pearl sac formation significantly influence pearl culture success. Despite these biological differences, no comparative study has quantitatively evaluated these three South Asian species specifically for image pearl production under identical controlled conditions particularly regarding survival, nacre deposition, and pearl luster. This gap is economically significant because farmers currently select species based on local availability and anecdotal reports rather than empirical performance data, risking suboptimal survival and pearl quality. Keeping above in view, this study was conducted for the assessment of different freshwater mussel species for their suitability in image pearl production.

MATERIALS AND METHODS

Experimental species and study area

The experiment was conducted using three freshwater mussel species: *Lamellidens marginalis*, *Lamellidens corrianus*, and *Pilsbryoconcha exilis* (Figure 1). Mussels were collected from natural freshwater bodies in Bangladesh and cultured in prepared experimental ponds.

Three ponds with sandy loam, pollution-free soils were selected and prepared following standard aquaculture practices (Figure 2). Initially ponds were drained, dried and treated with lime (1 kg/decimal) and salt (0.25 kg/decimal). After 6–7 days, ponds were filled with water (1-1.5 meter) and fertilized using TSP (0.10 kg/decimal), urea (0.125 kg/decimal), and organic manure (1 kg/decimal) to enhance plankton production (Figure 2).

Figure 1. *Three mussels' species used for pearl production.*

Figure 2. *Preparation of pearl culture pond.*

Mussel collection and conditioning

Collected mussels were sorted by species and acclimatized in stocking ponds for three months. Healthy, disease-free mussels with average (Mean$\pm$SD) length, width and height were 9.93$\pm$0.90 cm, 5.01$\pm$0.20 and 2.82$\pm$0.22. Pre-operative conditioning was performed by keeping mussels without food for 7 days to clear internal organs.

Image preparation and surgical procedure

Paraffin-based images were prepared using paraffin wax. A total of 600 mussels (200 species in each treatment) were operated. Mussels were carefully opened (approximately 8 mm) and a small pocket was created between the shell and mantle tissue. One image was inserted into each mussel, followed by removal of air and water before closing the shell.

Post-operative care and culture

Operated mussels were placed in nylon round net bags (3 mussels per bag) and kept in cisterns for one month with a plankton supply. After post-operative care, mussels were transferred to previously prepared culture ponds and reared for 10 months using the net bag hanging method at a stocking density of 80 mussels/ decimal.

Water quality monitoring

Water temperature, dissolved oxygen, pH, ammonia, and alkalinity were monitored fortnightly using standard instruments (Tanu *et al*., 2021b). Fertilizers and zeolite were applied periodically to maintain optimal water quality.

Data collection and statistical analysis

Survival rate, nacre layer thickness, and pearl luster were recorded after harvest. For comparing the three mussel species, One-way ANOVA was used to analyze survival rate, nacre layer thickness, and luster. After than Tukey's HSD used for post-hoc test to identify which species differ most and significantly difference. Google colab was used for preparing the heatmap.

RESULTS AND DISCUSSION

The quantitative evaluation of freshwater pearl production among *Lamellidens marginalis*, *Lamellidens corrianus*, and *Pilsbryoconcha exilis* revealed distinct variations in host survival rate, nacre deposition thickness, and pearl luster (Table 1). These characteristics of pearls serve as significant indicators of the physiological variation and biomineralization efficiency of each species under experimental conditions.

Table 1. Physiological variation and biomineralization efficiency of each species.

Species name	Survival rate (%)	Nacre layer (mm)	Luster (lux)
Lamellidens marginalis	40.5%	1.36±0.12	34.89 ±0.70
Lamellidens corrianus	42%	0.84±0.09	37.86 ±0.67
Pilsbryoconcha exilis	47%	1.04±0.09	45.33 ±1.32

Survival rate

After operation survival rate is initial determinant of the economic viability of pearl aquaculture, revealing the species-specific immune response and tolerance to surgical stress. *Pilsbryoconcha exilis* showed the highest resilience, demonstrating a survival rate of 47.0%. Conversely, *L. corrianus* and *L. marginalis* showed lower survival rates of 42.0% and 40.5%, respectively. Less number of mortalities in *P. exilis* suggests a more robust physiological recovery post-grafting or a less pronounced inflammatory response to the mantle tissue transplantation compared to the *Lamellidens marginalis* and *L. corrianus*. High mortality following implantation remains a major constraint in freshwater pearl culture, as also reported by Li & Li (2009). The survival rates observed in the present study are comparable to those reported for paraffin image implantation, where a maximum survival rate of 49% was documented for *L. marginalis* (Tanu *et al.*, 2021b). In contrast, significantly lower survival (15%) was recorded when shell images were used (Tanu *et al.*, 2021a). Suryawanshi & Kulkarni (2015) reported a mortality rate of 20% in *Parreysia corrugata*, while Fernandez (2013) documented survival rates ranging from 55% to 95% in Margaritifera falcata. The present findings clearly demonstrate interspecific variation in survival, nacre deposition, and pearl quality among *Lamellidens marginalis*, *Lamellidens corrianus*, and *P. exilis*. Among these, *P. exilis* consistently outperformed the other species in terms of survival rate and pearl luster, suggesting a higher physiological adaptability to the stress associated with surgical implantation. This may be attributed to species-specific differences in immune response, tissue regeneration capacity, and tolerance to handling stress, which are critical determinants of post-operative survival in pearl culture systems.

Nacre deposition rates

Nacre thickness is directly related to the duration of the culture period and the cellular activity of the outer epithelial cells derived from the donor mantle graft. *Lamellidens marginalis* yielded the most robust biomineralization capacity, producing a mean nacre layer thickness of 1.36±0.12 mm. This was substantially higher than the nacre thickness recorded for *P. exilis* (1.04±0.09 mm) and *L. corrianus* (0.84±0.09 mm). The superior nacre accretion in *L. marginalis* points to an accelerated secretion rate of the organic matrix (glycoproteins and chitin) and subsequent calcium carbonate ($CaCO_3$) crystallization, making it a highly favorable candidate for producing structurally durable pearls.In the present study, the nacre layer thickness was substantially higher than values reported in previous studies. For instance, pearls produced using shell images (2×1.5 cm^2) exhibited a nacre thickness of 0.38 ± 0.02 mm (Tanu *et al.*,2021a), while paraffin image implantation resulted in a thickness of 0.47 ± 0.3 mm (Tanu *et al.*,2021b). The enhanced nacre deposition observed in this study suggests that the size and type of implanted image, along with species-specific physiological responses, play a significant role in nacre secretion. Thicker nacre layers are generally associated with improved durability, higher commercial value, and enhanced optical properties of pearls. The superior nacre deposition observed may be linked to the longer culture duration, favorable environmental conditions, or optimal compatibility between host tissue and implanted image.

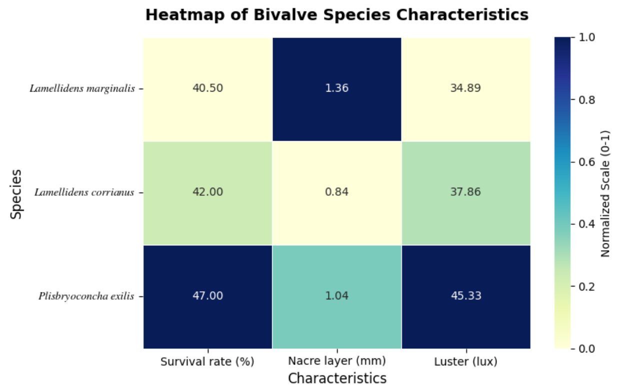

Figure 3. *Comparative study of pearl quality produced from different freshwater mussel.*

Quantitative pearl luster

Luster, measured in lux, evaluates the optical quality of the pearl, which is governed by the regularity, alignment, and thickness of the aragonite platelets within the nacreous layer. Interestingly, an inverse relationship was observed between nacre thickness and luster. *Pilsbryoconcha exilis* produced pearls with the highest optical brilliance (45.33±1.32 lux). This was followed by *L. corrianus* (37.86±0.67 lux), while *L. marginalis* exhibited the lowest luster (34.89±0.70 lux), despite having the thickest nacre. This phenomenon can be scientifically attributed to the microstructural arrangement of the aragonite crystals. The elevated luster in *P. exilis* suggests the deposition of highly uniform, thin, and parallel aragonite laminations, which optimize light reflection and refraction. In contrast, the rapid nacre deposition in *L. marginalis* may have resulted in more microstructural irregularities or thicker, less uniform crystal platelets, thereby scattering light and reducing surface brilliance. Additionally, variations in pearl luster were observed among species, with *P. exilis* producing pearls with superior luster compared to the other species, further supporting its suitability for high-quality image pearl production. The variation in luster among species further emphasizes the importance of host selection in pearl culture. The superior luster observed in *P. exilis* pearls could be due to more uniform nacre layering and better crystallographic arrangement of aragonite platelets, which influence light reflection and surface brilliance. This aligns with the general understanding that pearl quality is not solely dependent on environmental factors but is also strongly influenced by biological characteristics of the host species (Figure 3).

Water quality parameters

Water quality parameters were monitored at 15-day intervals throughout the culture period and remained within acceptable ranges for freshwater pearl culture. The recorded temperature ranged from 18.7 to 29.4°C, pH from 6.5 to 8.5, dissolved oxygen from 3.57 to 7.03 mg L^{-1}, ammonia from 0.01 to 0.04 mg L^{-1}, and alkalinity from 130 to 250 mg L^{-1}. These values are consistent with the optimal ranges reported by (Dan *et al.*,2001), who suggested that suitable conditions for pearl culture include temperatures between 15 and 30°C, pH of 6.5–8.5, dissolved oxygen levels of 5–8 mg L^{-1}, alkalinity of 50–300 mg L^{-1}, and ammonia concentrations of 0.03–0.1 mg L^{-1}. Similarly, Janakiram (1997) reported an optimal pH range of 7.5–8.5 and alkalinity between 75 and 150 mg L^{-1}. Comparable environmental conditions have also been reported in studies on *L. corrianus* (Rathor, 2017) and *L. marginalis* (Natarajan & Susithira, 2015). Although dissolved oxygen levels in the present study occasionally fell slightly below the recommended range, overall water quality conditions were conducive to mussel survival and pearl development. Statistical analysis revealed that nacre layer thickness differed significantly ($p < 0.05$) among the three mussel species, with *Lamellidens marginalis* producing the thickest nacre. However, survival rate and luster quality did not show significant differences ($p > 0.05$). Despite the lack of statistical significance, *Pilsbryoconcha exilis* demonstrated relatively higher survival and better luster performance. Therefore, all three species are suitable for image pearl production, with *Pilsbryoconcha exilis* being comparatively more favorable.

Conclusion

This study demonstrated that freshwater mussel species, implant size, and water quality were key factors influencing successful image pearl production. Among the three species evaluated, *Pilsbryoconcha exilis* showed the highest survival rate (47%) and produced pearls with the greatest luster, indicating their superior potential for image pearl culture. In contrast, *Lamellidens marginalis* produced the thickest nacre layer (1.36 ± 0.12 mm), suggesting a greater capacity for nacre deposition despite its lower survival and luster. The results further indicate that implant size and type significantly affect nacre thickness and pearl quality, while maintaining suitable water quality throughout the culture period supports mussel survival and pearl formation. Overall, the findings suggest that selecting an appropriate mussel species, optimizing implant characteristics, and maintaining favorable environmental conditions are essential for improving survival, nacre deposition, and the commercial quality of image pearls.

DECLARATIONS

Author contribution statement: Conceptualization: M. B. T. and M.N.H.; Methodology: M.N.H. and S. S.; Software and validation: M. N. H., S.T.L. and M. F. S.; Formal analysis and investigation: M.N.H. and S. S.; Resources: M.N.H. and S. S.; Data curation: M.N. H. Writing—original draft preparation: M.N.H. and S. S.; Writing—review and editing: M.N.H., M. B, T. and S. S.; Visualization: M. N. H..; Supervision: M. B. T. and M.F.S.; Project administration: M.N. H.; Funding acquisition: M. N. H. All authors have read and agreed to the published version of the manuscript.

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